Western Regional Meeting Abstracts

WAFMR, WSCI, WAP and WSPR
Joint Plenary Session II
1:45 PM
Thursday, January 31, 2008

1 EXPRESSION AND FUNCTION OF ERYTHROPOIETIN AND VASCULAR ENDOTHELIAL GROWTH FACTOR INCREASED IN OXYGEN INDUCED RETINOPATHY
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Purpose of Study: Retinopathy of prematurity (ROP) is a prevalent morbidity among preterm infants and is characterized by retinal neovascularization. Recent research in diabetic retinopathy, thought to have similar pathogenesis to ROP, has shown that erythropoietin (Epo) and vascular endothelial growth factor (VEGF) are involved in neovascular angiogenesis. It is not known whether and how these growth factors are involved in ROP. We hypothesized that Epo and VEGF mRNA expression is up-regulated in oxygen induced retinopathy (OIR) and these proteins are involved in endothelial cell proliferation, migration and permeability.

Methods Used: After exposure to hyperoxic conditioning, murine vitreous samples were obtained at postnatal day 17, retina isolated and RNA extracted for Epo and VEGF mRNA determination. This procedure was repeated in control mice. Migration and proliferation assays were performed according to manufacturer’s instruction (Promo-cell, Germany; Cambrex, Walkersville) on in vivo endothelial cells using increasing concentrations of Epo and VEGF. Cell growth and migration was measured manually and using Cell Counting Kit 8 (Dojindo Molecular Technologies, Gaithersburg). Finally, Epo was injected into the vitreous of murine retina, followed by intravenous injection of Evan’s blue dye 6 hours later. Retinal vascular permeability was assessed by measuring Evan’s blue leakage into vitreous cavity.

Summary of Results: Both Epo and VEGF mRNA levels were significantly greater in hyperoxia exposed mice (p < 0.001, n = 4). However, Epo levels were significantly higher than VEGF levels (15-fold increase vs. 2.5 fold increase, p < 0.05). In addition, both Epo and VEGF stimulated endothelial cell migration and proliferation in a dose dependent manner (p < 0.05, n = 4). Finally, intravitreal Epo injection increased retinal vascular permeability (p < 0.05, n = 5).

Conclusions: Retinal Epo and VEGF mRNA are upregulated in the murine model of OIR. Epo levels were significantly higher than VEGF, and both proteins stimulate endothelial cell proliferation and migration. Like VEGF, Epo increases endothelial cell permeability. We speculate that Epo and VEGF play an important role in OIR.

2 NEUTRALIZATION OF IL-17 WITH POLYCLONAL ANTI-IL-17 ANTIBODY ATTENUATES DEXTRAN SULFATE SODIUM-INDUCED COLITIS IN MICE
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Purpose of Study: Inflammatory bowel disease (IBD), mainly consisting of Crohn’s disease and ulcerative colitis, is a gastrointestinal disorder characterized by chronic and relapsing inflammation. The pathogenesis of IBD is mediated by an aberrant immune response to gut bacterial flora. Our previous study demonstrated that TH17 cells, a new and distinct T-helper cell subset, are up-regulated in dextran sulfate sodium (DSS)-induced colitis, a widely used rodent model for IBD research. TH17 cells produce the cytokine interleukin-17 (IL-17), which has been shown to upregulate the inflammatory cytokines, IL-1β, IL-6 and TNF-α in numerous autoimmune diseases, including IBD. One previous study reported that monoclonal anti-IL-17 antibody (Ab) exaggerated DSS-induced colitis. Thus, the role of IL-17 in the colitis remains to be fully elucidated. This study investigates whether inhibiting IL-17 with anti-IL-17 polyclonal Ab (pAb) attenuates the DSS-induced colitis.

Methods Used: Seven week old Balb/c were provided either 4% DSS or vehicle in their drinking water for 10 days to induce colitis. One group of wild-type (Wt) mice received an intraperitoneal (ip) injection of anti-IL-17 pAb on days 3 and 7. The mice were checked for rectal bleeding and weighed daily to evaluate the extent of colitis. On day 10, mice were sacrificed and spleen and colonic tissues were collected for evaluation. ELISA was performed to evaluate the concentration of TNF-α, a downstream mediator of IL-17.

Summary of Results: It was found that anti-IL-17 pAb was protective, with control DSS-mice losing significantly more weight over the period of 10 days than the DSS-mice receiving the pAb. Further, mice receiving antibody experienced less rectal bleeding. In addition, mice receiving anti-IL-17 pAb expressed lower levels of TNF-α, a downstream mediator of IL-17.

Conclusions: Our study shows that IL-17 is an active player in DSS-induced colitis, and highlights the value of further exploration into IL-17-targeting therapy in the management of IBD.
and HA tags respectively. Viral supernatants from 293T cells expressing these viral proteins were ultracentrifuged to purify viral like particles (VLP’s), and these and their corresponding cell lysates were analyzed by Western blotting. Lipid rafts were collected by sucrose gradient fractionation and precipitated. Cell surface expressions were quantified by flow cytometry.

Summary of Results: The Matrix (M) protein was shown to localize to the most lipid rafts and to bud at the highest levels followed by NiV-F and NiV-G, respectively. Interestingly, the NiV-F glycoprotein was found to bud autonomously, something that has been recorded for very few paramyxoviruses. Additionally, the YHQL motif on NiV-F was shown to be important for NiV-F budding and localization in lipid rafts. Furthermore, when NiV-M was co-expressed with the envelope glycoproteins, it increased NiV-F and NiV-G localization into lipid rafts and their budding efficiencies. NiV-G had a similar effect on NiV-G but did not affect NiV-M.

Conclusions: In toto, these results revealed important determinants for NiV assembly and implicate lipid rafts in this process. In addition, they comprise an initial understanding of the assembly process for the newly emerging henipavirus genus, and may aid in the elucidation of general parvoviral assembly. Further investigation may prove helpful for the development of assembly inhibitors for NiV.

4 VISFATIN: A GENETIC DETERMINANT OF INSULIN SENSITIVITY

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Purpose of Study: Adipose tissue is no longer considered a simple depot for triglyceride storage. On the contrary, adipose tissue secretes a variety of adipokines into the circulation that can influence insulin sensitivity in distant sites as well as in adipose tissue itself. Adipokines have increasingly been implicated as mediators of obesity-related insulin resistance and the increased risk of type 2 diabetes associated with obesity. Visfatin, also known as pre-B cell colony-enhancing factor (PBEF1), is highly enriched in visceral fat of mice and humans; circulating visfatin levels are increased in obesity and correlate with measures of visceral adiposity. However, its role in human obesity and insulin resistance remains unknown. In this study, we sought to determine whether visfatin is a genetic determinant of metabolic traits.

Methods Used: We genotyped 5 single nucleotide polymorphisms (SNPs) in PBEF1 in 975 Mexican Americans from 190 families. These SNPs were selected using HapMap data to tag the majority of the variation (SNPs) in PBEF1 in 975 Mexican Americans from 190 families. These analyses were conducted using generalized estimating equations (GEE1). Two linked SNPs were associated with improved insulin sensitivity and lower free fatty acid (FFA) levels (see Table). A third SNP was associated with lower FFA. Haplotype analysis did not provide additional information.

Conclusions: These results implicate visfatin as an inherited factor mediating the relationship between FFA and insulin sensitivity. Considering its high expression in visceral fat, visfatin may regulate lipolysis and/or FFA release from adipose tissue; in turn, these FFA may enter the circulation and impact on whole-body insulin sensitivity.

5 ATTITUDES TOWARDS COMPLEMENTARY AND ALTERNATIVE MEDICINE IN A PEDIATRIC HOSPITAL

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Purpose of Study: Complementary and Alternative Medicine (CAM) has been an area of growing interest in pediatric care. In this study, we aim to assess the attitudes of physicians in a pediatric hospital in Washington State towards CAM, with a special emphasis on patterns for recommending CAM, level of trust for CAM providers, and concerns for development of CAM in a hospital setting.

Methods Used: An anonymous electronic survey with 25 items was sent to all physicians affiliated with Children’s Hospital and Regional Medical Center. Two reminder emails were sent.

Summary of Results: Response rate was 31.3% (266 of 851). Of the 67% of providers who recommended some form of CAM therapy to patients in the last six months, providers were most likely to recommend biofeedback (42.0%) and acupuncture (32.8%). Physicians who use CAM (71.0%) were more likely to recommend CAM therapies to their patients than those who do not use CAM (75.8% vs. 45.2%, p value 0.0001). Major concerns related to CAM therapies include lack of safety data (63.6%), lack of education about CAM (44.7%), adverse interactions with current therapies (41.3%) side effects related to CAM therapies (38.6%) and qualifications of CAM providers (39.8%). MD CAM providers were perceived to be most trustworthy followed by licensed massage therapists, allied health care providers with CAM training, and licensed acupuncturists. Naturopaths and chiropractors inspired the least trust in this survey. Providers indicated they would like more education in the areas of efficacy (88%), impact on CAM on health outcomes and safety (78%), interactions of CAM therapies with conventional therapies and indications for use of various CAM therapies (74%).

Conclusions: This study indicated a strong positive correlation between personal use of CAM by physicians and recommendation of CAM to their patients. Qualifications of CAM providers and limited education relating to CAM therapies negatively impact recommendations of CAM therapies by physicians. Rigorous licensing processes and more CME courses regarding CAM therapies may improve the acceptance and integration of CAM therapies in conventional health care.

6 GLENOHUMERAL MUSCLE ARCHITECTURE DIFFERS WITH AGE

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Purpose of Study: The purpose was to provide a detailed architectural comparison of middle-aged and elderly rotator cuff and deltoid muscles.

Methods Used: Forty-one cadaveric shoulders were used and were split into middle-aged (12) and elderly (29) specimens. Mass (M) and muscle length (Lm) were measured for each muscle. Length of predetermined fiber bundles was measured, and sarcomere length (L0) was determined using laser diffraction. L0 was used to normalize muscle fiber length (Lm) to 2.7 μm. Physiological cross-sectional area (PCSA), a measure of...
a muscle’s capacity for force generation, was calculated according as follows: PCSA = (T × cos ϑ)/(ρ × Lf) where ϑ is pennation angle and ρ is muscle density, 1,055 g/cm³. 

Summary of Results: The PCSA of middle-aged deltoids (24 ± 2.1 cm²) was greater than that of younger deltoids (16.0 ± 1.8 cm²) (P < 0.0001). The PCSA of middle-aged infraspinatus (19.85 ± 2.11 cm²) was greater than that of young subscapularis (14.7 ± 1.0 cm²) (P < 0.005), and middle-aged infraspinatus (10.4 ± 0.7 cm²) tended to be larger than young subscapularis (10.4 ± 0.7 cm²) (P = 0.316). There was no main effect of age on fiber lengths (Fig. 1), and fiber length variability due to age differences was small (2-10%).

Conclusions: The rotator cuff muscles have relatively short fiber lengths and large PCSAs. As muscles age, their force-generating capacity is reduced, while their excursion range remains constant.

7 LACK OF ENDOGENOUS β-ENDORPHIN AND ENKEPHALINS REDUCES DRUG SEEKING BEHAVIOR IN COCAINE ADDICTED MICE

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Purpose of Study: Cocaine addiction continues to be a paramount problem in the United States, without any effective pharmacological treatments. Pre-clinical studies have clearly associated the endogenous opioid system as one of the principle modulators of the mesolimbic dopaminergic reward circuitry and one of the possible substrates of cocaine reward and addiction. Thus, it is well established that cocaine administration causes the release of endogenous β-endorphin in the nucleus accumbens, where it induces conditioned place preference (CPP), a behavioral paradigm used to study reward. Additionally, evidence exists to implicate enkephalins which may alter the function of the mesolimbic dopaminergic circuitry and the rewarding effects of cocaine. In this study we investigated whether β-endorphin and enkephalin have any role in memory retrieval or consolidation in regards to drug seeking behavior in mice treated chronically with cocaine. In order to study the role that these endogenous opioids have in memory, we used a CPP paradigm.

Methods Used: In the CPP paradigm, mice were tested for baseline preference on day 1. On day 2-9, mice received an alternate day of saline/cocaine (30 mg/kg) conditioning training and then tested for post conditioning preference on day 10. Mice were then tested again following a saline challenge on day 17 followed by a cocaine challenge on day 18. In addition we used HPLC to assess the dopamine levels and immunohistochemistry for histological changes within the brain.

Summary of Results: Our results show that wild type mice expressed significant CPP on day 10, which was also observed on day 17. Conversely, mice lacking β-endorphin and enkephalin failed to show CPP on day 17, indicating that memory retrieval of the conditioned response was altered in mutant mice. However, a cocaine challenge reinstated the CPP response in mutant mice.

Conclusions: The present results suggest that endogenous β-endorphin and/or enkephalin may be important in the memory retrieval of the cocaine induced-conditioned response but not in the consolidation of memory. Consequently, β-endorphin and enkephalin may be the target proteins in designing pharmacological agents to treat cocaine addiction.

8 ROLE OF LIVER X RECEPTOR IN CALCIFICATION OF MURINE AORTIC CELLS

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Purpose of Study: Vascular calcification is prevalent in atherosclerotic lesions and correlates with an increased risk of cardiovascular events, including myocardial infarction and death. Recent studies have shown that activation of the nuclear receptor Liver X Receptor (LXR) promotes cholesterol efflux from macrophages and decreases inflammatory gene expression. Accordingly, LXR activation reduces atherosclerotic lesion size in an animal model, and has thus received great interest for its therapeutic potential in atherosclerosis. Yet its effect on vascular calcification is largely unknown. Since LXR activation is widely considered to be anti-atherogenic, we investigated its effects on in vitro vascular cell calcification.

Methods Used: Primary murine aortic cells were induced to calcify with forskolin, as we have shown previously in vitro, and were cotreated with T0901317, a known synthetic LXR ligand. Osteogenic markers, including alkaline phosphatase activity, intracellular phosphate levels, and matrix calcification, were assessed after 4, 7, and 10 days, respectively.

Summary of Results: Results showed that LXR activation with T0901317 augmented forskolin induction of matrix calcification. Treatment with T0901317 alone, however, did not induce calcification. Cotreatment with T0901317 also augmented forskolin-induced alkaline phosphatase activity. Gene expression analysis by real-time quantitative polymerase chain reaction revealed that LXR activation induced the expression of the phosphate cotransporter Pit-1 and inhibited forskolin induction of ectonucleotide pyrophosphatase/phosphodiesterase 1. Cotreatment with levamisol, an inhibitor of alkaline phosphatase, and phosphonoformic acid, an inhibitor of Pit-1, attenuated the effects of T0901317 on matrix calcification.

Conclusions: Overall, these data suggest that LXR activation promotes matrix calcification of vascular cells and may pose potential adverse effects in the vasculature.

9 SHH IS NECESSARY FOR LIMB REGENERATION

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Purpose of Study: The Shh-Fgf feedback loop is important for patterned limb outgrowth. Shh emanating from the ZPA in the posterior limb bud up-regulates Fgfs in the apical ectodermal ridge (AER). Fgfs secreted from the posterior AER maintain the expression of Shh in the posterior distal aspect of the limb bud. However, it is not clear how this feedback loop works during limb regeneration. Amputation of a chick limb bud (at stage 23) with implantation of an FGF bead into the posterior stump will
trigger SHH reactivation and limb regeneration. Interestingly, if amputation is performed a few hours later (stage 25), FGF application is unable to up-regulate SHH and regeneration. We wondered whether SHH promotes or indicates regenerative capacity.

**Methods Used:** To determine the role of Shh in patterned regeneration, we amputated developing chick wings (stage 23) and implanted FGF soaked beads. We then applied a chemical known to inhibit Shh signaling, Cyclopamine. Chicks were harvested either 6 hours after treatment to verify inhibition of downstream SHH targets (Ptc - by whole mount *in situ* hybridization) or after 6 days for skeletal analysis using alcian green.

**Summary of Results:** Application of Cyclopamine inhibited Shh-mediated up-regulation of the Ptc receptor at 6 hrs; evidence of FGF-induced SHH up-regulation was confirmed by whole mount *in situ* hybridization. Furthermore, skeletal analysis revealed only a single shortened zeugopod bone formed in the absence of SHH signaling. In contrast, both zeugopod bones regrew in response to FGF and functional SHH.

**Conclusions:** Collectively, these data suggest that Shh is necessary for the promotion of limb regeneration. The use of Shh in conjunction with Fgfs may provide a mechanism to promote regenerative limb or after healing following amputation.

### 10 MOUSE MODEL OF TESTOSTERONE-INDUCED MUSCLE FIBER HYPERTROPHY


**Purpose of Study:** Previously, we have shown that testosterone (T) supplementation increases muscle size in healthy young and old men, hypogonadal men, and men with illness. We have also demonstrated that T-induced increase in muscle size is linked to fiber hypertrophy and significant increase in myonuclear and satellite cell numbers. The mechanisms by which T increases muscle cell number and promotes muscle growth are not well known. An understanding of the mechanistic pathways that mediate T-induced muscle fiber hypertrophy may unveil novel targets for the development of anabolic therapies. As a prerequisite for studies using mutant mice, we wish to establish a mouse model for studying the molecular mechanisms by which T promotes muscle growth. T induces skeletal muscle hypertrophy by promoting satellite cell activation and proliferation through activation of Notch (N)-signaling pathway.

**Methods Used:** Adult mice (C57BL/6) received gonadotropin-releasing hormone antagonist to suppress endogenous T production and were implanted subdermally under anesthesia with empty (sham control) or 2 cm PDS T- filled tubule to ensure a supraphysiologic dose of T. Mice were sacrificed 1, 2, 4, 6, and 8 wk after treatment.

**Summary of Results:** T treatment for 8 wk resulted in a significant (P < 0.001) increase in fiber area of gastrocnemius muscles (C: 1675 ± 47 vs. T: 2414 ± 105 μm²). T-induced fiber-hypertrophy was preceded by up-regulation of the Notch ligand Delta-1 and activation of N-signaling, as evidenced by an increase in cleaved intracellular activated forms of N-1, N-2, and N-4 in gastrocnemius muscle lysates by western blotting. Consistent with this, we also observed an increase in the number of PCNA-positive nuclei in muscles of T-treated mice, indicating that activation of N-signaling enhanced cell proliferation. T supplementation also resulted in suppression of JNK activation but concurrently promoted p38 MAPK activation with 2 wk of treatment, as evidence by immunometric assay for phospho-JNK and phospho-p38 MAPK, respectively.

**Conclusions:** T induces muscle fiber hypertrophy in mice by cell proliferation induced by Notch-signaling. The inactivation of JNK together with the activation of p38 MAPK may be critical for T-induced muscle fiber hypertrophy.

### 11 MECHANISM OF PTEN INTERACTIONS IN COLORECTAL CANCERS AND HAMARTOMATOUS POLyps

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**Purpose of Study:** The phosphatase and tumor suppressor gene PTEN is mutated in endometrial and colon cancers with microsatellite instability, and is thought to play a role in their pathogenesis. PTEN encodes two coding hexadene microsatellites that often change in length within these cancers. Germline PTEN mutations occur in certain hamartomatous polyposis syndromes, some with high colorectal cancer risk in which the polyps may lose PTEN expression indicating somatic inactivation of the wild-type allele. However, the mechanism for silencing the second PTEN allele is unclear. We examine whether familial hamartomatous polyps harbor defects in DNA mismatch repair, with instability in coding microsatellites in PTEN.

**Methods Used:** 99 microsatellite unstable (MSI) sporadic colon cancers and 10 MSI hamartomatous polyps were analyzed for mutations in two hexadene tracts (exons 7 and 8) of PTEN. PTEN expression was determined by immunohistochemistry using an antibody targeting an epitope beyond the predicted truncated protein.

**Summary of Results:** 5/10 patients with familial hamartomatous polyposis syndrome showed germline PTEN mutations and complete loss of PTEN expression. 11/99 MSI cancers (11%) demonstrated frameshifts in exon 7 and 8 of PTEN while 0/10 MSI hamartomatous polyps showed no frameshifts. Of tumors with mutant PTEN, all demonstrated down regulation or complete loss of PTEN in the epithelium.

**Conclusions:** Microsatellite unstable colon cancers exhibit PTEN exon 7 and 8 frameshift mutations and subsequent loss of PTEN expression. Hamartomatous polyposis syndrome patients exhibit microsatellite instability. Loss of PTEN expression was noted in patients with PTEN germline mutations, but was not associated with microsatellite instability in PTEN. Loss of PTEN expression may be due to other epigenetic phenomenon.

### 12 EARLY POSTNATAL BODY FAT DEPOSITION IN INTRAUTERINE GROWTH RESTRICTED (IUGR) TERM INFANTS

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**Purpose of Study:** To compare early postnatal fat deposition by air displacement plethysmography (ADP) in IUGR and AGA infants from birth to 6 weeks of age.

**Methods Used:** Term IUGR (Birth Wt <10th%ile) and appropriately for gestational age (AGA) infants were studied. Maternal consent was obtained during the 2nd trimester to assess fetal growth by 2-D ultrasound at 20 and ~35 wks gestation. Maternal and infant medical histories and serum glucose (mg/dL) were obtained. Percentage infant body fat (%BF) by ADP (PEA POD, Life Measurement, Inc.) and dual energy x-ray absorptiometry (DXA, QDR4500, Hologic), and anthropometrics (body Wt, length, and head and regional circumferences) were measured in at 48 hrs and 6 wks of age. Derived anthropometric measurements included weight/length, body mass index (BMI, W/ length²), ponderal index (PI, w/length³), and regional to head circumference (HC) ratios. ANOVA and correlations and were performed with p < 0.05.

**Summary of Results:** A total 33 infants (IUGR = 13, 3M; AGA = 20, 7M) were studied. Asymmetrical growth (Birth wt only <10th%ile) was present in 9/13 (70%) of IUGR. Feeding source was distributed equally (60%
breast-fed, 20% breast+ formula, 20% formula only from birth to 6 wks. Gestational age and length and head circumferences at birth and 6 wks were similar. Wt, BMI, and circumference ratios (abdominal, mid-arm and -thigh) at birth and 6 wks and %BF at birth were significantly lower in IUGR (p < 0.001). Wt %ile and %BF gains were greater and wt/length %ile gain lower in IUGR infants from birth to 6 wks (p < 0.05; Table). Greater postnatal %BF gain was positively related to greater fetal abdominal circumference %ile loss from 20 wk to 36 wk 2-D ultrasound (R = 0.45, p = 0.01). Postnatal growth changes were greatest in asymmetrical IUGR infants while 75% (3/4) of symmetrical IUGR infants remained <10%ile for growth measures at 6 wks.

Conclusions: These findings demonstrate that body fat is the major component of ‘catch-up’ growth observed in asymmetrical IUGR infants during the first six weeks of life.

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13 AN ABUNDANT EVOLUTIONARILY CONSERVED CSB-PIGGYBAC FUSION PROTEIN EXPRESSED IN COCKAYNE SYNDROME

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Purpose of Study: Cockayne syndrome (CS) is a devastating progeria most often caused by mutations in the CSB gene encoding a SWI/SNF family chromatin remodeling protein. Although all CSB mutations that cause CS are recessive, the complete absence of CSB protein does not cause CS. In addition, most CSB mutations are located beyond exon 5 and are thought to generate only C-terminally truncated protein fragments. We undertook a study of the CSB gene in order to understand the critical role of the N-terminal region of CSB and how complete absence of the protein does not cause disease.

Methods Used: We used a variety of genomic, molecular and biochemical techniques, including a survey of the human genome sequence, quantitative PCR, Western blotting, and comparative genomic analysis of several primate genomes.

Summary of Results: We show that a domesticated PiggyBac-like transposon PGBD3, residing within intron 5 of the CSB gene, functions as an alternative 3’ terminal exon. The alternatively spliced mRNA encodes a novel chimeric protein in which CSB exons 1-5 are joined in frame to the PiggyBac transposase. The resulting CSB-transposase fusion protein is as abundant as CSB protein itself in a variety of human cell lines, and continues to be expressed by primary CS cells in which functional CSB is lost due to mutations beyond exon 5. The CSB-transposase fusion protein has been highly conserved for at least 43 Myr since the divergence of humans and marmoset, and appears to be subject to positive selection. The human genome contains over 600 nonautonomous PGBD3-related MER85 elements that were dispersed when the PGBD3 transposase was last active at least 37 Mya. Many of these MER85 elements are associated with genes which are involved in neuronal development, and are known to be regulated by CSB.

Conclusions: The CSB-PGBD3 fusion protein is a highly abundant - but never before observed - alternative splice product of the CSB locus. We speculate that this fusion protein has been conserved for host antitransposon defense, or to modulate gene regulation by MER85 elements, but may cause CS in the absence of functional CSB protein.
Methods Used: The cancer cells, with either the Ral pathway activated with the activating mutants or the Ral pathway down-regulated using RNAi technology, were injected into the left cardiac ventricle or the spleen of athymic nude mice. Metastatic tumor burden was analyzed by bioluminescence imaging, X-ray, and histology.

Summary of Results: Results show that when prostate cancer cells were inoculated via intracardiac injection, activating the RalGEF pathway, but not the Raf/MEK/ERK pathway, promoted metastasis to bone, while down-regulating Ral GTPases inhibited metastasis to bone. It’s also important to notice that differences between cancer cell lines do exist. Knocking down either Ral A GTPase or Ral B GTPase alone inhibited bone metastasis in DU145/G37 cells. However, in PC3 cells, the same inhibitory effect was only observed in Ral A knockdown, but not in Ral B knockdown cells. Interestingly, results from HT29 colon cancer cell line show no obvious effects on metastasis when Ral A is knocked down in the intra-cardiac injection model.

Conclusions: We have shown that RalGEF pathway plays an important role in Ras-mediated metastasis in certain types of human cancer, while other cancers may utilize different downstream pathways of Ras to mediate metastasis. A broader survey of cancer types and metastatic properties will be needed to dissect the role of the Ral pathway in cancer metastasis.

16
THE ROLE OF TGF-ALPHA AND VITAMIN D IN KERATINOCYTE HOST DEFENSE
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Purpose of Study: The expression of antimicrobial peptides by epithelial cells represents a first line of defense against microbial pathogens. TGF-alpha, an important growth factor involved in wound healing, can increase the expression of human catherelicin antimicrobial peptide (LL-37). In addition, LL-37 can also be induced by activation of the Vitamin D receptor (VDR) pathway. The purpose of our study was to examine if TGF-alpha can augment the VDR-mediated induction of LL-37 in human epithelial keratinocytes.

Methods Used: To study the expression of LL-37 in human skin, we used air-lifted organotypic keratinocyte cultures that form layers resembling normal human skin and the HaCAT keratinocyte cell line derived from immortalized adult epithelium. We first examined if these keratinocytes were capable of responding to active vitamin D (1,25D3), by measuring downstream Vitamin-D pathway gene activity (cathelicidin and CYP24) by quantitative PCR (qPCR). After confirming these cell were responsive to 1,25D3, we measured the expression of CYP27, the enzyme that converts inactive vitamin D to the active form, in the presence of TGF-alpha by qPCR. Finally, we challenged both models with inactive Vitamin D (25D3) and TGF-alpha to determine if the increased expression of cathelicidin seen in the presence of TGF-alpha was due to the upregulation of the Vitamin D pathway.

Summary of Results: TGF-alpha stimulation, in the absence of VDR activation, resulted in increased mRNA expression of CYP27, which converts inactive 25D3 (25-hydroxyvitamin D3) to active 1,25D3, and also increased expression of VDR. CYP24 and LL37 mRNA were up-regulated in the presence of both 25D3 and TGF-alpha to a greater extent than that of 25D3 alone. In summary, these results provide evidence that TGF-alpha increases expression of CYP27, which can convert inactive 25D3 to active 1,25D3, resulting in VDR-mediated up-regulation of CYP24 and LL-37 and enhanced host defense mechanisms at epithelial surfaces.

Conclusions: TGF-alpha, increases the expression of CYP27 and VDR of the Vitamin D pathway, allowing greater conversion of active Vitamin D, which results in increased expression of LL-37, a potent antimicrobial peptide. This may be an important mechanism in keratinocyte host defense against pathogens.

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ROLE OF EPITHELIAL MEMBRANE PROTEIN (EMP2) IN CHLAMYDIA INFECTIVITY IN VIVO
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Purpose of Study: Chlamydia trachomatis produces significant human disease of the eye and genitourinary tract (GT). Epithelial membrane protein-2 (EMP2) is a candidate host molecule that mediates Chlamydia infection. Our study has two goals: 1) to investigate the effect of EMP2 blockade on GT chlamydial infectivity in vivo; 2) to optimize a murine model of chlamydial conjunctivitis.

Methods Used: In a GT model, BALB/C mice received either negative control or anti-EMP2 diabody (10ug diabody/mouse) preceding infection with C.muridarum (MoPn). Bacterial load and interferon-gamma in GT tissue were respectively quantified by immunofluorescence and ELISA. Short-term and long-term ocular infections involved inoculating CH/Hen mice with UV-inactivated MoPn or live MoPn (5 x 105 IFU/mouse). Bacterial load in ocular tissue and interferon-gamma secretion were quantified.

Summary of Results: Pretreatment of BALB/C mice with anti-EMP2 diabody significantly reduced bacterial burden and interferon-gamma secretion in the GT model, with greater reduction of ascending infection. In the eye model, transient conjunctivitis was observed only with live organism and there was an associated immunity which appeared protective against repeated ocular infection. Infection-associated immunity is demonstrated by induction of IFN-gamma producing splenic T cells by live MoPn but not by UV-inactivated Chlamydia.

Conclusions: Blockade of EMP2 significantly decreases ascending GT chlamydial infection, supporting a potentially important role for EMP2 as a therapeutic target. The ocular Chlamydia infection model for chlamydial conjunctivitis produces a transient infection followed by an immune response which is protective against repeated infection. Future studies that define specific interactions between Chlamydia and EMP2 may reveal potential targets for disease prevention.

18
EFFECT OF CARELINK, AN INTERNET-BASED INSULIN PUMP MONITORING SYSTEM, ON GLYCEMIC CONTROL IN RURAL AND URBAN CHILDREN WITH TYPE 1 DIABETES MELLITUS
E. Corriveau1, P. Durso1, E. Kaufman3, B. Skipper2, L. Laskaratos3, and K. Heintzman1. 1University of New Mexico School of Medicine, Albuquerque, NM; 2University of New Mexico School of Medicine, Jules Stein Eye Institute, Los Angeles, CA and 3Presbyterian Medical Group, Albuquerque, NM.

Purpose of Study: To determine whether use of the internet-based insulin pump monitoring system, Carelink, improved glycemic control in rural and urban children treated with insulin pump therapy.

Methods Used: We reviewed records of 94 children treated with insulin pump therapy between the years 2004-2007. Records were divided into 3 groups: No Access consisted of patients without Carelink access due to software incompatibilities (33 patients). Non-Users consisted of patients who had access to Carelink but did not use it (20 patients). Carelink Users consisted of patients who used Carelink to upload and review their pump and glucometer data (41 patients). Through record review, we also assessed glycemic control, diabetes self-care measures, frequency of clinic visits and patient geographic location associated with Carelink use.
Summary of Results: Carelink Users showed significant improvement in HbA1c levels after initiation of Carelink use (8.0 ± 0.1 (SE) vs 7.7 ± 0.1 (SE), p = 0.002). No Access patients followed in a conventional manner, showed no change in HbA1c (8.0 ± 0.2 (SE) vs. 8.1 ± 0.2 (SE), p = 0.17) during the study period. Carelink Non-Users had a higher HbA1c level than Carelink Users and No Access patients at the start of the study and did not change over the study period (8.9 ± 0.2 (SE) vs. 9.0 ± 0.3 (SE), p = 0.82). Rural Carelink Users (patients who live >1 hour drive from clinic) showed significant improvement in HbA1c levels following Carelink use (7.9 ± 0.2 (SE) vs. 7.4 ± 0.2 (SE), p = 0.001) compared to urban Carelink Users (patients who live within 1 hour drive from clinic) (8.1 ± 0.2 (SE) vs. 7.9 ± 0.1 (SE), p = 0.15), yet had significantly fewer clinic visits per year as compared to urban patients (2.8 ± 0.2 (SE) vs. 3.5 ± 0.1 (SE), p = 0.001).

Conclusions: Carelink use was significantly associated with improved glycemic control in children with Type 1 diabetes on insulin pump therapy. Carelink may be an especially useful tool to help improve glycemic control in patients who do not live in close proximity to their diabetes care clinic.

19
COMPARISON OF SINGLE VERSUS MULTIPLE ECHOCENIC FOCI IN THE FETAL HEART REGARDING THE RISK OF ANEUPLOIDY

Purpose of Study: The purpose of this study was to determine if multiple echogenic foci within the fetal heart were associated with an increased risk of fetal chromosomal aneuploidy as compared to a single focus in our patient population.

Methods Used: During a span of 30 months, all women referred to our institution for obstetrical ultrasound were evaluated prospectively for the presence of echogenic cardiac foci in the fetal heart. Each patient was also evaluated for the presence of other risk factors for aneuploidy including other ultrasound findings, biochemical screening and maternal age. A population of patients with two or more fetal echogenic cardiac foci and a comparable population of patients with single fetal echogenic cardiac foci were then identified and their neonatal outcomes were followed.

Summary of Results: 53 patients with multiple (more than one) fetal echogenic foci were identified. During the time period that these patients were identified, 55 patients with single fetal echogenic foci were also identified for comparison and a control group with normal fetal cardiac findings on ultrasound was also identified to use as a control. Of the 53 patients with multiple fetal echogenic foci there were 5 cases of Trisomy 21, 3 of these cases without other ultrasound abnormalities and in women under 35 years of age. Of the 55 patients with the finding of single fetal echogenic foci, there was one case of Trisomy 21, the patient in this case was 40 years old but without other abnormalities on ultrasound. In the control group there were no cases of Trisomy 21, 3 of these cases without other ultrasound abnormalities and in women under 35 years of age. Of the 55 patients with the finding of single fetal echogenic foci, there was one case of Trisomy 21, the patient in this case was 40 years old but without other abnormalities on ultrasound. In the control group there were no cases of Trisomy 21.

Conclusions: Though the significance of echogenic cardiac foci in fetal ultrasound remains controversial, this data suggests that the identification of more than one echogenic cardiac focus may be of greater significance in terms of association with aneuploidy when compared to having a single echogenic cardiac focus which in turn carries greater risk than having no echogenic cardiac focus. This was found to be the case even in the absence of other risk factors such as other sonographic anomalies and advanced maternal age.

20
COMPARISON OF NITRITE METABOLISM IN FETAL AND ADULT BLOOD


Purpose of Study: SWL has become a mainstay in the treatment of kidney stones. It has been proposed that patients undergoing SWL have a higher risk of developing diabetes. We determined the prevalence of diabetes in patients who underwent SWL 20 years ago. The study rate was compared to background provincial rates.

Methods Used: 357 patients who had undergone SWL between 1985 and 1987 were identified from our database at Vancouver General Hospital. A telephone survey was conducted and patients were questioned on whether they had developed diabetes since SWL. Information was gathered regarding BMI, hypertension, smoking, recurrent stone disease, and family history of diabetes. It was difficult to identify patients diagnosed with kidney stones who did not have SWL during the study period and who have never had subsequent SWL; therefore, the prevalence of diabetes in our study group was compared to the provincial prevalence as reported by the BC Ministry of Health Services in 2002.

Summary of Results: Of the 357 contact letters mailed, 247 were ineligible due to: deceased (18), incorrect address (104), unable to reach
(82), refused/unable to consent (11), other (12). 130 patients completed the telephone questionnaire (36.4% response rate). 4 were excluded for diagnosis of diabetes prior to SWL. The median age was 67.4 year, and the median BMI was 26.7 kg/m². Background BMI and smoking rates for this age group in BC are comparable with the study group. The overall prevalence of diabetes in the study group was 25.4%. Males had a prevalence of diabetes of 28.7% (25/87) and females 17.9% (7/39). The provincial prevalence of diabetes for this age group is 12-18% for men and 9-15% for women.

**Conclusions:** There is an elevated prevalence of diabetes in male patients who had undergone SWL 20 years previously. The shortcomings of this study are that proper controls were difficult to obtain, and it would be premature to conclude that SWL alone causes this increased prevalence. Part may be attributable to suggestions that people with stone disease have a higher risk of diabetes due to a common precipitating metabolic derangement. The observation that patients have a higher prevalence of diabetes 20 years following SWL remains to be completely explained, but this study supports that an association exists.

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**22 HISTOLOGICAL ANALYSIS OF POST MORTEM ALZHEIMER'S DISEASE AND CEREBRAL AMYLOID ANGIOPATHIC BRAIN AT POINT OF SMALL HYPINTENSITIES IN SUSCEPTIBILITY WEIGHTED MR IMAGES**

M. Schrag¹, G. McAuley ¹, B. Holshouser ¹, H. Vinters ², and W. Kirsch ¹. ¹Loma Linda University, Loma Linda, CA and ²University of California Los Angeles, Los Angeles, CA.

**Purpose of Study:** The etiology of sporadic Alzheimer’s dementia (AD) is a subject which has been subjected to a great deal of research but despite the volume we now know about the disease, its origins remain enigmatic. The hope for a cure or effective treatment must lie in early detection and diagnosis, as damage to mature neural pathways is all-too-often irreversible. A few key pieces of evidence have emerged that are consistent with vascular degeneration as a major finding in AD. Extracellular and intracellular iron deposits are present in the AD brain. Whether this is due to endogenous dyshomeostasis or exogenous deposition from the blood, or both, is not yet clearly established. The blood brain barrier is also frequently compromised in AD, leading to the extravasation of plasma components. The complete significance of this finding is not yet known. Finally, cerebral amyloid angiopathy (CAA) which results from the deposition of amyloid-beta peptide (Ab) in the walls of cerebral vessels is found in as many as 95% of confirmed AD brains. Ab is toxic to vascular smooth muscle cells and has been shown to cause a thinning of the vessel wall. Hemorrhaging from the microvasculature is a frequent finding in CAA. In light of these observations, we seek to implicate vascular degeneration as a factor in the etiology of AD.

**Methods Used:** Ongoing in vivo studies of mildly cognitive-impaired human subjects with an MR imaging technique that is sensitive to iron, namely susceptibility weighted imaging (SWI), have detected small hypointensities (SH) which may prove to correlate with the risk of conversion to AD. The current study seeks to clarify the identity and significance of SHs in confirmed CAA and AD post mortem brain by histological and immunological techniques.

**Summary of Results:** Hypointense residues on SWI correlate with microvascular hemorrhages and iron deposition accompanied by significant apoptotic neuronal loss and HO-1 dysregulation in the perifocal zone. 10 of these patients have had thalamic strokes and 6 of these patients have had MELAS syndrome. The correlation between iron deposition and SHs is consistent with the presence of SHs in CAA. The presence of SHs in CAA and AD post mortem brain suggests a role of vascular degeneration in the etiology of AD.

**Conclusions:** There is an elevated prevalence of diabetes in male patients who had undergone SWL 20 years previously. The shortcomings of this study are that proper controls were difficult to obtain, and it would be premature to conclude that SWL alone causes this increased prevalence. Part may be attributable to suggestions that people with stone disease have a higher risk of diabetes due to a common precipitating metabolic derangement. The observation that patients have a higher prevalence of diabetes 20 years following SWL remains to be completely explained, but this study supports that an association exists.

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**23 TRANSITION TO ORAL VASODILATOR THERAPY FOR SEVERE PULMONARY ARTERIAL HYPERTENSION (PAH) - A SINGLE CENTER SERIES**

M.S. Sidhu, R. Saggar, J.A. Belperio, R. Saggar, J.P. Lynch III, S. Weigt, D.A. Zisman, and D.J. Ross. David Geffen School of Medicine, Los Angeles, CA.

**Purpose of Study:** Severe PAH portends a grave prognosis if left untreated. Parenteral prostacyclin (PGL2) has been long considered most appropriate for severe, functional class (FC) IIIIB/IV PAH. However, intravenous PGL2 is associated with potential catheter related infections. Experience with transition from intravenous to oral therapy is limited. Herein, we report our single center clinical experience for patients who requested transition to oral therapies after parenteral PGL2 complications.

**Methods Used:** A retrospective review of seven patients with PAH [idiopathic (4), anorexigen (2), ASD (1)] who were determined during Phase I of our protocol, to be “clinically stable.” In Phase II, PGL2 was decreased by 0.5-1mg/kg/min weekly during close monitoring of physical examination, transthoracic echocardiography, six-minute walking distance (6MWD), FC (I-IV) and B-type natriuretic peptide (BNP) until discontinuation. Concurrently, oral vasodilator therapies were introduced with the combination of bosentan and sildenafil. This combination was selected to target both the endothelin-1 and phosphodiesterase type-5 pathways in PAH. In Phase III, after discontinuation of PGL2, surveillance was maintained to detect and intervene for potential clinical worsening.

**Summary of Results:** For the seven patient group during phase I, the optimal achieved FCs were: Classes I (2), II (4), and III (1). At the end of phase III, the FCs were: I (3) and II (4). One patient later experienced right ventricular failure after five months, resulting in requirement for therapy with subcutaneous treprostinil and return to “active listing” for lung transplant. The mean duration (mos) [±SD] for phase I was 15.9 [±4.8], phase II 7 [±3.3], and phase III 10.9 [±7.4]. Data for 6MWD and BNP is summarized in the TABLE.

**Conclusions:** Selected patients with severe PAH at initial presentation, may be transitioned from parenteral PGL2 to oral therapies during strict clinical monitoring. However, longer term results and a larger series will be necessary to ascertain the efficacy of this strategy.

**Summary of Results:**

<table>
<thead>
<tr>
<th></th>
<th>Phase I</th>
<th>Phase II</th>
<th>Phase III</th>
</tr>
</thead>
<tbody>
<tr>
<td>6MWD (m)</td>
<td>304.3 [±90]</td>
<td>355.4 [±10.4]</td>
<td>416.3 [±36.1]</td>
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<tr>
<td>BNP</td>
<td>230.5 [±182.3]</td>
<td>86 [±462.5]</td>
<td>85.4 [±510]</td>
</tr>
</tbody>
</table>

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**24 ANTECEDENT SIMULATION TRAINING ACCELERATES LEARNING OF SHOULDER ARTHROSCOPY**


**Purpose of Study:** To determine whether antecedent simulation training accelerates the ability of a novice medical student to learn how to perform a diagnostic arthroscopy on a cadaveric shoulder.

**Methods Used:** Twenty-four medical students with no arthroscopy experience were recruited and divided randomly into two groups. The first group (experimental group) trained on the Procedicus VA shoulder arthroscopy simulator for a total of six hours divided into six sessions, and then performed three abbreviated diagnostic arthroscopies on a cadaveric shoulder, divided into three separate sessions. The second group (control...
group) performed the same arthroscopies as the first group, but did not receive any simulation training. The cadaveric arthroscopies were videotaped and graded in a blind manner. Performance was measured by several objective parameters including efficiency of probe movement (number of probe misdirections en route to an anatomical structure), total time, number of scope collisions, proper structure identification, and number of scope pullout incidents.

Summary of Results: Students who trained on the simulator were able to locate anatomical structures with significantly fewer probe misdirections (better efficiency) than those students without any simulation training. Simulation-trained subjects were also less likely to leave out structures during their initial trial on the cadaver. Conversely, total time, number of scope collisions, and number of scope pullout incidents were no different between the two groups.

Conclusions: As simulation training continues to develop in all medical fields, it is important to critically evaluate the effectiveness of such training. This is the first prospective, randomized study exploring whether simulation training can enhance the ability of a novice to learn shoulder arthroscopy. Based upon our results, it appears that the Procedicus VA simulator can teach students how to move the probe more efficiently and identify structures more accurately. However, simulation training does not appear to enhance other performance measures. This is likely due to the inherent limitations of simulation in mimicking the reality of actual arthroscopy. Further studies are needed to continue to evaluate the utility of simulation training in development of arthroscopic skills.

Behavior and Development

Concurrent Session

8:30 AM

Friday, February 1, 2008

25

THE USE OF MASSAGE AMONG CHILDREN WITH CEREBRAL PALSY

G. Glew1,3, M. Fan2, H. Hagland1, K. Bjornson4, S. Beider4, and J. McLaughlin1,3, 1University of Washington, Seattle, WA; 2University of Washington, Seattle, WA; 3Children’s Hospital and Regional Medical Center, Seattle, WA; 4University of Washington, Seattle, WA and 5Integrative Touch Therapy, Beverly Hills, CA.

Purpose of Study: To determine the prevalence of the use of massage among children with cerebral palsy, reasons for which massage is being used, and limits of recruitment for a future randomized, controlled trial.

Methods Used: 100 families of children with cerebral palsy were surveyed in neurodevelopmental and neurology subspecialty clinics at Seattle’s Children’s Hospital.

Summary of Results: 80% of children surveyed had received massage. The majority of families use massage for musculoskeletal relief, to improve quality of life, and to help their children sleep. Lower maternal income was associated with relatives, as opposed to professional massage therapists, providing massage. The use of massage by the mother herself was significantly associated with the current use of massage by the child. Similarly, a more severe GMFCS level is associated with significantly more current use of massage.

Conclusions: The majority of families with children with cerebral palsy in Seattle have tried massage for musculoskeletal and other concerns. Additional research is needed to determine whether massage should routinely be used in children with cerebral palsy.

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FIRST CASE REPORT OF MONOZYGOTIC TWINS WITH SMITH-MAGENIS SYNDROME

J. Lemay and M. Hicks. University of Calgary, Calgary, AB, Canada.

Purpose of Study: To report the first description in the literature of monozygotic twins with Smith-Magenis Syndrome (SMS).

Methods Used: Behavioral / Developmental and clinical assessment in monozygotic 3.5-year-old twins with a history of global developmental delay, behavioral issues including self-harm, and severe receptive and expressive language delays.

Summary of Results: Chromosome testing confirmed for both children a 17p11.2 interstitial microdeletion commonly seen in SMS. Despite their zygosity the twins had some differences in presentation including cardiac and renal anomalies (only Twin A affected), language development delays (Twin B was more verbal) and maladaptive behaviors (Twin B self-injurious behavior more severe than Twin A). In addition both twins displayed abnormal communication, impairments in social interaction and stereotyped behaviors consistent with autism spectrum disorder. Both twins were functioning at development age equivalence of approximately 12 months with an overall adaptive behaviour score well under the 1st percentile consistent with cognitive delays and significant adaptive functioning deficits. Fine Motor skills were significantly delayed in both children. No sleep issues were reported. Neither child had ocular or spine anomalies. However they had midface hypoplasia with broad square-shaped face commonly seen in SMS. Finally growth parameters were normal. This is in contrast to short stature and obesity commonly seen in SMS.

Conclusions: Examining the differences in behavioural/developmental and clinical phenotype in theses monozygotic twins with may lead to a better understanding of the etiology of the clinical variability seen in SMS, as well as the natural history of this syndrome.

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ABERRANT BEHAVIOR IN SMITH LEMLI OPTIZ SYNDROME: BIO-BEHAVIORAL INFLUENCES

R.F. Eagle, K.A. Freeman, R.D. Steiner, L. Merkens, and J. Penfield. Oregon Health & Science University, Portland, OR.

Purpose of Study: A bio-behavioral classification model of aberrant behavior displayed by people with developmental disorders suggests 3 possible subtypes of aberrant behavior: learned (operant), biological, or both. Such a model may have particular relevance when studying individuals with genetic or metabolic disorders, as it may shed light on the biochemical etiology of aberrant behavior. Further, using this model may help lead to appropriate treatment selection and decrease incidence of nonresponders to both behavioral intervention and medication. Smith Lemli Optiz Syndrome (SLOS) is a multiple malformation, mental retardation syndrome caused by a defect in cholesterol metabolism associated with dehydrocholesterol (DHC) accumulation, a defect that occurs on a continuum. Aberrant behavior is reported as common in SLOS. We apply the bio-behavioral classification model to aberrant behavior in SLOS, testing the association between biochemical indicator of disease severity (defined by DHC/sterol ratio) and behavior subtype (i.e., biological or learned). We hypothesized that the higher biochemical severity scores, the greater the association with behavior being classified as biological.

Methods Used: Parents of 8 children with SLOS (ages 2-13) completed the Motivation Assessment Scale (MAS), a 16-item measure used to subtype aberrant behavior as biological or learned. Parents completed the MAS considering a specific behavior they identified as problematic. Biochemical severity was measured when participants were on a no-cholesterol diet (baseline DHC/total sterols).

Summary of Results: Aberrant behavior included self-injury (2 children), aggression (2 children), and destructive behavior (4 children). MAS results suggested that aberrant behavior was learned for 6 children and biological for 2. One-tailed point bi-serial correlation showed a
significant association between biochemical severity and MAS subtype \((r = .703, p = .026)\), specifically that higher biochemical severity was associated with the biological subtype.

**Conclusions:** Results confirmed the a priori hypothesis and indicate that severity of metabolic defect in SLOS relates to whether aberrant behavior is learned or biological. Findings highlight importance of attending to both biological and behavioral influences on aberrant behavior, and may inform future intervention research.

**28 CHILDREN AND PARENTS DIFFERENTIAL PERCEPTION ON SELF REPORT OF FUNCTIONAL STATUS AND WELL-BEING OF CHILDREN ATTENDING SPECIALIZED SUMMER CAMPS**

B. Creel\(^1\), W.N. Evans\(^1,2\), G.A. Mayman\(^1,2\), J.C. Collazos\(^1,2\), and H. Restrepo\(^1,2\). \(^1\)Children’s Heart Center, Las Vegas, NV and \(^2\)University of Nevada, School of Medicine, Las Vegas, NV.

**Purpose of Study:** To assess parental and children perceptions of functional status and well-being of children attending specialized summer camps.

**Methods Used:** This report includes data from 36 parents of children with congenital heart disease who attended a 4-day camp. We measure their perceptions with Child Health Questionnaire (CHQ) pre and post camps. CHQ covers 12 physical/psychosocial domains. Each domain was transformed into a score from 0 to 100 (worst to best). We used for comparison tables for U.S. school children and non-parametric tests for statistical analysis.

**Summary of Results:** Each group had 50% of males, with children’s mean age of 12.1 years (range 8-16 years), and the parents’ civil status was: married: 48%, single or divorced: 45%, unknown or non-reported: 7%. Table shows those domains with significant low scores in pre-camp data. Only physical functioning domain showed normal scores after camps.

**Conclusions:** Parents and campers reported different perceptions on the same physical/psychosocial domains. Parents perceived their children with low self-esteem, limited physical functioning, and low general health. They also reported high disruption in family activities and high emotional impact on parents. Children perceived themselves with low general health and lack of cohesion in their families.

**Table:**

<table>
<thead>
<tr>
<th>Domain</th>
<th>Pre-Camp</th>
<th>Post-Camp</th>
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**30 LACK OF ENDOGENOUS \(\beta\)-ENDORPHIN AND ENKEPHALINS REDUCES DRUG SEEKING BEHAVIOR IN COCAINE ADDICTED MICE**

D. Mahato\(^1\) and K. Lutfy\(^2\). \(^1\)Western University of Health Sciences, Pomona, CA and \(^2\)Western University of Health Sciences, Pomona, CA.

**Purpose of Study:** Cocaine addiction continues to be a paramount problem in the United States, without any effective pharmacological treatments. Pre-clinical studies have clearly associated the endogenous opioid system as one of the principle modulators of the mesolimbic dopaminergic reward circuitry and one of the possible substrates of cocaine reward and addiction. Thus, it is well established that cocaine administration causes the release of endogenous \(\beta\)-endorphin in the nucleus accumbens, where it induces conditioned place preference (CPP), a behavioral paradigm used to study reward. Additionally, evidence exists to implicate enkephalins which may alter the function of the mesolimbic dopaminergic circuitry and the rewarding effects of cocaine. In this study we investigated whether \(\beta\)-endorphin and enkephalin have any role in memory retrieval or consolidation in regards to drug seeking behavior in mice treated chronically with cocaine. In order to study the role that these endogenous opioids have in memory, we used a CPP paradigm.

**Methods Used:** In the CPP paradigm, mice were tested for baseline preference on day 1. On day 2-9, mice received an alternate day of saline/ cocaine (30 mg/kg) conditioning training and then tested for post conditioning preference on day 10. Mice were then tested again following a saline challenge on day 17 followed by a cocaine challenge on day 18. In addition we used HPLC to assess the dopamine levels and immunohistochemistry for histological changes within the brain.
Summary of Results: Our results show that wild type mice expressed significant CPP on day 10, which was also observed on day 17. Conversely, mice lacking β-endorphin and enkephalin failed to show CPP on day 17, indicating that memory retrieval of the conditioned response was altered in mutant mice. However, a cocaine challenge reinstated the CPP response in mutant mice.

Conclusions: The present results suggest that endogenous β-endorphin and/or enkephalin may be important in the memory retrieval of the cocaine induced-conditioned response but not in the consolidation of memory. Consequently, β-endorphin and enkephalin may be the target proteins in designing pharmacological agents to treat cocaine addiction.

31 EFFECT OF AN OSTEOPATHIC MANIPULATIVE PROCEDURE ON PSYCHOEMOTIONAL PARAMETERS

A. Bagla¹, D. Ananyev¹, C. Rodriguez², R. Mercado², M. Seffinger¹, and E.J. Wagner¹, Western University of Health Sciences, Pomona, CA and ²University of La Verne, La Verne, CA.

Purpose of Study: The CV4 (Cerebral Ventricle 4) is an osteopathic cranial manipulation with reported effects including relaxation and general parasympathetic activation. Previous studies using the Drug Reaction Scale (DRS) and blood serum analysis demonstrated that cannabimimetic activation occurs during manipulation of joints. Behavioral surveys present a potential method of assessing neurochemical activity within the CNS during various drug-induced and endogenous states. The purpose of this study was to characterize changes in behavioral states due to 7 minutes of manipulation.

Methods Used: Method: Our study used the Visual Analog Survey (VAS) and Likert surveys to assess behavioral and mood changes before and after CV4 or sham. Subjects completed a survey prior to and after intervention. The administration of the surveys was randomized according to a 2 x 2 paradigm. Possibilities included receiving a redundant survey or any combination of the two surveys. Healthy volunteers were recruited from university students and faculty and were randomly assigned to either a manipulation or sham (light touch) group.

Summary of Results: Pre and post CV4 surveys previously used in a medical course teaching the technique revealed significant changes (p < .05) in nearly all mood states. In the subsequent randomized controlled study approved by the IRB, various t-test, ANOVA, and post-hoc statistics computed in SPSS compared the experimental group surveys to the control group surveys. We found significant changes in the following descriptors with their respective p-values: Energetic (.007), Listless (.013), Maddled (.017), Blue (.048), Discouraged (.048), Gloomy (.025), Weary (.021), Bewildered (.048), Agreeable (.030), and Anxious (.047).

Conclusions: These data indicate a possible activation of endocannabinoid receptors. This study demonstrated that CV4 elicits a change in a subset of behavioral states found in the VAS and Likert surveys, relative to sham. There were no perceivable adverse effects due to CV4, suggesting that neither light touch nor CV4 cause psychological or physiologic harm. One subject reported transient dizziness and head pain following sham, which she later ascribed to her breathing rate.

33 EFFECTS OF DEPLOYMENT ON MILITARY CHILDREN

E.M. Flake, B. Davis, and P.L. Johnson. Madigan Army Medical Center, Tacoma, WA.

Purpose of Study: To describe the psychosocial profile of school age children during parental military deployment utilizing standardized psychosocial health and stress measures, and to seek predictors of children at “high risk” psychosocial morbidity during deployment.

Methods Used: A voluntary convenience sample consisting of Army spouses with a deployed service member and a child age 5–12. Caregivers consented to complete demographic questions, the Pediatric Symptom Checklist (PSC), the Parenting Stress Index - Short Form (PSI-SF) and the Perceived Stress Scale (PSS-4). Children were labeled “high risk” according to normed data (PSC > 28, PSI-SF > 85, PSS > 9). The data was stratified by demographic profile and analyzed using SPSS.

Summary of Results: 87% (101/116) of participants met inclusion criteria and completed questionnaires. Spouses (86% female) of service members (26% officers and 74% enlisted), reported that 47% had moved ≥ 3 times in the past 5 years. Child characteristics included a mean age of 8.6 years, 52% male, and 64% non-Hispanic white. 32% of respondents reported above cutoff PSC scores for their child. 44% of participants reported “high risk” stress on the PSI-SF and 19% reported “high risk” perceived stress on the PSS. Demographic predictors of “high risk” families included: young enlisted families (p < 0.05), perception of poor military or community support (<0.01), low parental education (p < 0.07), and marriage < 5 years (p < 0.07). The length of service member separation, time with deployed unit, child gender, child age, and race or ethnic background did not demonstrate statistical significance. Regression results of the PSC score on demographic factors and parent stress were performed. Caregivers reporting positive military support...
were 68% less likely to have a child with a positive PSC (p < 0.01) and caregivers employed outside the home were 62% less likely to report a positive PSC (p < 0.04).

Conclusions: This is the first study to report the prevalence of child symptoms during parental military deployment. Families experiencing deployment report high stress levels and identify children at “high risk” for psychosocial morbidity twice as often as national norms. Specific demographic predictors of psychosocial morbidities will assist providers in screening for “high risk” children during deployment.

34 AUTISM WITH REGRESSION, FRAGILE X PREMUTATION AND EEG ABNORMALITIES WITHOUT CLINICAL SEIZURES

R.S. Akins, and R. Hagerman. M.I.N.D. Institute, U.C. Davis Department of Pediatrics and the American Academy of Neurology recommend a comprehensive evaluation of children with the diagnosis of autism, including high resolution chromosome studies and DNA analysis for Fragile X, should be performed in the presence of mental retardation, if mental retardation cannot be excluded, if there is a family history of Fragile X or undiagnosed mental retardation, or if dysmorphic features are present. Indications for an adequate sleep-deprived electroencephalogram include clinical seizures or suspicion of subclinical seizures, and a history of regression. There is evincing evidence regarding the association of both fragile X premutation and autism with EEG abnormalities without clinical seizures.

Methods Used: We present the case of a four year old boy with language regression, fragile X premutation with 79 CGG repeats and EEG abnormalities without clinical seizures on EEG.

Summary of Results: Developmental testing in our clinic demonstrated an Early Learning Composite at the first percentile on the Mullen Scales of Early Learning and significant delay on the Adaptive Behavior Composite of the Vineland. Results of the Autism Diagnostic Observation Scale were consistent with a diagnosis of autism.

Conclusions: This case provides an opportunity to discuss three evolving and controversial areas of autism in an evidenced based manner. Carriers of the Fragile X premutation were previously thought to be cognitively and behaviorally unaffected. However, recent research has demonstrated an association of premutation carriers and autism. Emerging research regarding epileptiform EEG abnormalities in the absence of clinical seizures in children with autism and the evidence for treatment of EEG abnormalities will be reviewed. Recommendations for Fragile X testing of family members and current understanding of conditions affecting premutation carriers will also be reviewed.

35 DYSMORPHOLOGY AND AUTISM

K. Angkustsiri1,2, B. Moghaddami1,2, T. Wardinsky1, J. Gardner2, N. Kalamkarian2, P. Krakowiak1, I. Hertz-Picciotto3,4, and R.L. Hansen2,1. UC Davis Medical Center, Sacramento, CA; 1UC Davis, Sacramento, CA; 2Alta California Regional Center, Sacramento, CA and 3UC Davis, Davis, CA.

Purpose of Study: There is clinical heterogeneity among the autistic spectrum disorders (ASD). The presence of dysmorphic features, which may represent insults in early embryogenesis, is one possible tool for defining an etiologically relevant subset in ASD. This study investigates dysmorphology status among ASD, developmentally delayed (DD), and typically developing (TD) children.

Methods Used: Children between the ages of 2–5 years were recruited through a larger population study, the CHARGE (Childhood Autism Risks from Genetics and the Environment) study. Group status (ASD, DD, TD) was confirmed with the Autism Diagnostic Interview-Revised (ADI-R), Autism Diagnostic Observation Schedule (ADOS), Mullen Scales of Early Learning (MSEL), and Vineland Adaptive Behavior Scales (VABS). Children with known genetic syndromes were excluded from analysis, yielding 251 subjects (128 ASD, 48 DD, 77 TD). In addition to a medical exam, 3 clinicians trained in genetics or pediatrics and blinded to group status evaluated photographs of each child’s face, profile, and hands. Children were classified as dysmorphic if 3 or more minor physical anomalies (MPAs) were present and nondysmorphic if fewer than 3 MPAs were present. Each photograph was reviewed by at least 2 clinicians, and disagreements were resolved by consensus of all 3 raters. Frequencies of dysmorphology status were compared using chi2 analyses.

Summary of Results: Significantly more children with ASD were classified as dysmorphic compared to typically developing children (18% vs. 8%, p < 0.04). Developmentally delayed children were also more likely to be considered dysmorphic compared to typically developing children (27% vs. 8%, p < 0.01). Children with developmental delay did not have significantly higher rates of dysmorphology than children with ASD.

Conclusions: Preliminary data suggest that children with ASD have higher rates of dysmorphology than typically developing children and have rates similar to that of children with idiopathic developmental delay. Further study is needed to determine if there is a characteristic pattern of MPAs in dysmorphic children with ASD and if this group differs clinically from nondysmorphic children with ASD.

Cardiovascular I
Concurrent Session
8:30 AM
Friday, February 1, 2008

36 ISOLATED RIGHT VENTRICULAR ENDOCARDIAL FIBROELASTOSIS ASSOCIATED WITH MATERNAL ANTI-SSA AND ANTI-SSB ANTIBODIES IN THE ABSENCE OF ATRIOVENTRICULAR BLOCK

P. Ngeth1,2, B.K. Iriye3, W.N. Evans1,2, K.T. Kip1,2, H. Restrepo1,2, and R.J. Acherman1,2,1. Children’s Heart Center, Las Vegas, NV and 2University of Nevada, School of Medicine, Las Vegas, NV.

Purpose of Study: Maternal lupus erythematosus can be associated with fetal atrioventricular block, sinus bradycardia, myocarditis, endocarditis, dilated cardiomyopathy, and endocardial fibroelastosis. To the best of our knowledge, we report the first prenatal diagnosis of isolated right ventricular endocardial fibroelastosis without atrioventricular block in the presence of maternal anti-SSA and anti-SSB antibodies.

Methods Used: Case report.

Summary of Results: A 29-year-old, gravida 3, para 0 was referred for a fetal echocardiogram due to history of maternal lupus erythematosus and positive anti-SSA and anti-SSB antibodies. An obstetric ultrasound at 18 weeks gestation was normal. A fetal echocardiogram at 25 weeks gestational age showed a fetal heart rate of 136, a regular rhythm with 1:1 atrioventricular conduction, and a normal mechanical PR interval of 128 msec. There was a small pericardial effusion. The left ventricle showed no endocardial echo brightness and normal function. However, the right ventricle demonstrated decreased systolic function, endocardial
echo brightness, and moderate tricuspid regurgitation. We administered maternal intravenous immunoglobulin at 1gm/kg every four weeks coupled with oral dexamethasone at 0.25mg/kg/dose every 6 hours. A subsequent fetal echocardiogram showed moderate cardiomegaly with a cardiothoracic ratio of 40%, right atrium and right ventricle enlargement, and a normal mechanical PR interval. A postnatal echocardiogram demonstrated: right ventricular enlargement, decreased systolic function, endocardial echo brightness; pulmonary hypertension; normal left ventricle; and no pericardial effusion. A postnatal ECG showed diffuse ST-T wave changes but no conduction abnormalities.

Conclusions: Fetuses of mothers positive for anti-SSA and anti-SSB antibodies need frequent echocardiographic evaluations. Isolated right ventricular endocardial fibroelastosis may be a manifestation of fetal cardiac pathology secondary to maternal lupus.

37
IDENTIFICATION OF BIOMARKERS OF HEART FAILURE IN SINGLE VENTRICLE PHYSIOLOGY

Purpose of Study: Single ventricle (SV) physiology is associated with congenital heart disease characterized by one functional ventricle. Early identification of heart failure is critical to the timing of surgical palliation. However, current methods for grading heart failure in this patient population are limited. Serum biomarkers of heart failure for SV patients would provide objective, accessible, and inexpensive indicators of disease progression and response to therapies. In this pilot study, we sought to define normal serum values for the candidate biomarkers B-type natriuretic peptide (BNP), high-sensitivity C reactive protein (CRP), and cardiac troponin I (cTnI) in children with SV physiology compared to controls.

Methods Used: We are enrolling children 1 month - 3 years old with SV (all tricuspid atresia) or PDA presenting to the UCSF Pediatric Heart Center for cardiac catheterization or surgery. Enrolled patients are assigned Ross and International Society for Heart and Lung Transplantation (ISHLT) heart failure scores, evaluated by echocardiography, and to follow, and results can be compared to BSA indexed normal values. Currently, we are evaluating patients with other pre and postoperative congenital heart disease and pulmonary hypertension.

Summary of Results: For continuous measures, all data expressed as mean ± sem (Table). Two-sample t tests used to test for significance.

Conclusions: SV patients tend to be younger at enrollment than PDA patients (p = 0.0017), reflecting the early timing of invasive procedures for SV patients. In children without clinical evidence of heart failure enrolled to date, mean values for all three biomarkers appear marginally elevated in children with single ventricle physiology relative to controls, although with our current patient numbers these trends do not reach statistical significance. Significant associations may emerge as additional data accrue.

39
LONG-TERM FOLLOW-UP OF VENTRICULAR FUNCTION IN PATIENTS WITH D-TRANSPOSITION OF THE GREAT ARTERIES FOLLOWING ATRIAL SWITCH OPERATIONS: AN MRI AND ECHOCARDIOGRAPHY STUDY
M. Shiota, J. Abouhousn, D. Lohan, and J. Child. UCLA, Los Angeles, CA.
Purpose of Study: The prevailing theory on D-TGA patients post atrial switch operation is that right ventricular function decreases over time, leading to the functional deterioration seen in these patients. The results of a long-term follow-up on D-TGA patients were reviewed.

Methods Used: Data was collected on 16 patients with D-TGA post atrial switch operation. There were 11 men and 5 women, and 13 had the Mustard operation while 3 had the Senning. MRI and transthoracic echocardiogram were obtained on all patients. Ventricular function and exercise tolerance were assessed. MRI variables were compared with age matched controls controls.

Summary of Results: MRI and echocardiographic studies were undertaken in D-TGA patients a mean of 28.5 years following atrial switch operation. Mean age was 30+/−8 years, 5 women, 11 men. Sixteen age matched controls also underwent MRI. Left ventricular diastolic index was lower in D-TGA patients than controls (2.16 l/min/m2 vs. 2.89 l/min/m2, p = 0.049). Surprisingly, systemic right ventricular ejection fraction, as quantified by MRI using a modified Simpson’s method, did not differ significantly from healthy controls (52+/−8% versus 52+/−9%, p = 0.39). There were no differences found in right ventricular cardiac index nor stroke volume in D-TGA versus controls.

Conclusions: Sub-pulmonic left ventricular stroke volume and cardiac index in adult D-TGA patients followed long-term status post atrial switch operation are reduced. An inadequate sub-pulmonic left ventricle, rather than the systemic right ventricle, may be the cause of the functional deterioration seen in D-TGA patients after atrial switch operation.

40 VENTRICULAR FIBRILLATION WAVEFORM PREDICTS SHOCK OUTCOME

J.D. Waters1, D.B. Jorgenson2, L.D. Sherman3, W.E. Cronc, and T.D. Rea1, 1University of Washington, Seattle, WA; 2Philips Medical Systems, Seattle, WA and 3St. Francis Hospital, Federal Way, WA.

Purpose of Study: Optimal treatment for out-of-hospital ventricular fibrillation (VF) cardiac arrest is uncertain. Initial shock may provide the best chance of survival for some, while for others better survival might be achieved if shock were delayed until therapies such as CPR could be provided. We evaluated whether a measure of VF waveform could discriminate survival, and compared the potential impact of strategies using VF waveform versus emergency medical services (EMS) response intervals to guide initial shock. We hypothesized that, compared to EMS response interval, a measure of VF waveform prior to initial shock better discriminates survival.

Methods Used: Design, setting, and population: Cohort study of persons = 18 years suffering VF arrest treated with ForeRunner AED by an EMS system using a shock-first protocol (n = 129). Exposure: Quantitative VF measure that incorporates amplitude and frequency upon initial AED application. Two commercially available thresholds for the VF waveform (VF1 and VF2) were compared to EMS response intervals of 6 and 5 minutes respectively. Outcome: Neurologically intact hospital survival. Analyses: We used chi-squared statistic to compare the proportion with neurologically intact survival better than EMS interval and has potential to guide care and improve outcome.

Conclusions: A measure of VF waveform discriminated neurologically intact survival better than EMS interval and has potential to guide care and improve outcome.

41 THE SAFETY OF DISPATCHER-ASSISTED TELEPHONE CPR INSTRUCTIONS FOR NON-CARDIAC ARREST PATIENTS

J. Rogers1, M. Eisenberg1,2, and L. White3, 1University of Washington, Seattle, WA and 2King County Public Health, Seattle, WA.

Purpose of Study: Dispatcher-assisted telephone CPR significantly increases bystander CPR and the chance of survival from cardiac arrest (CA). A number of conditions can mimic the presentation of cardiac arrest leading to false positive identification by dispatchers and unwarranted initiation of telephone CPR. The purpose of this study is to 1) Determine the incidence of dispatcher-assisted, bystander chest compressions performed on non-CA patients and 2) Determine the morbidity associated with inappropriate chest compressions.

Methods Used: This investigation is a retrospective cohort study of patients who received dispatcher-assisted, bystander CPR instructions in King County, Washington, from June 1, 2004 through January 31, 2007. Dispatcher recordings and EMS incident report forms were analyzed from each incident to ascertain the extent of bystander CPR performed and the underlying etiology of each patient’s medical emergency. We also attempted to telephone interview the non-CA patients who received chest compressions to determine any associated morbidity.

Summary of Results: During the study period, dispatchers initiated CPR instructions for 1702 presumed cardiac arrests. Nine hundred eight were true CA or dead on arrival, 720 were non-CA patients, and 74 of the incidents had incomplete or missing EMS incident reports. Of the non-CA patients, 299 received dispatcher-instructed bystander chest compressions. The major underlying etiologies of the non-CA patients who received chest compressions were: 77 overdoses (26%), 59 seizures (20%), 40 CVA/TIA (13%), 36 syncope (12%) and 32 diabetic emergencies (11%). Seventy-four of the 299 non-CA chest compression patients were contacted for interview. 57 reported no injury (77%), 12 reported pain/soreness (16%), and 2 reported fractured rib(s) (3%).

Conclusions: 911 dispatchers frequently initiate CPR instructions over the telephone for patients whose condition mimics cardiac arrest. These false positive conditions involve overdoses, seizures, hypoglycemia and other emergencies mistaken for cardiac arrest. It is reassuring that the majority of patients contacted reported no injury and there was no major morbidity or mortality associated with inappropriate chest compressions.

42 CLINICAL REASONING IN CARDIAC EXAMINATION


Purpose of Study: Deficiencies in cardiac examination (CE) are widely published, existing at all levels of medical training, including faculty. When performing CE, deficiencies can occur either at the stage of detection (identifying findings) or at the stage of interpretation (giving a diagnosis).

Methods Used: To measure clinical reasoning in medical students, the first year class (n = 86) were tested on two virtual patient examinations (VPEs), interactive multimedia vignettes featuring patients with auscultatory and visible manifestations of cardiovascular pathology. Four
cardiac auscultation findings were possible for each patient, with a single correct diagnosis. NEGATIVELY CONSISTENT: a student identifies incorrect findings, compounds the error by interpreting them inconsistently, arriving at an implausible diagnosis. NEUTRARILY CONSISTENT: the findings and diagnosis have an ambiguous relationship; the correct diagnosis may have been identified, but it is not clear that the findings were interpreted correctly. POSITIVELY CONSISTENT: a student identifies findings, and interprets them consistently to arrive at a plausible diagnosis.

**Summary of Results:** Answers for patient 1 are plotted below. Diagnoses had the greatest dispersion among the negatively consistent group: no one identified a normal, physiologic S3. For the neutrally consistent group, the diagnoses were less dispersed, with 14% giving the correct diagnosis. For the positively consistent group the range of diagnoses were tightly clustered: 70% made the correct diagnosis.

**Conclusions:** Students whose identified findings were positively consistent with their given diagnosis were in the minority, but were overwhelmingly correct in both findings and diagnosis.

**43 REMOTE TESTING OF CARDIAC EXAMINATION COMPETENCY**


1St Mary Medical Center, Long Beach, CA; 2Blaufuss Multimeda, Rolling Hills Estates, CA and 3Harbor-UCLA Medical Center, Torrance, CA.

**Purpose of Study:** Audiotape testing for identification of heart sounds and murmurs has indicated severe deficiencies among contemporary medical trainees. Since bedside cardiac examination (CE) expertise requires integration of auscultation with other modalities, we have developed Virtual Patient Examinations (VPEs), interactive multimedia vignettes featuring patients with auscultatory and visible manifestations of cardiovascular pathology for training and testing CE competency. Published results from administration of a standardized 50-question multimedia CE Test using VPEs demonstrated no improvement in scores after the second year of medical school, even through residency, up to full-time non-cardiology faculty. Cardiologists and cardiology fellows were the only group with significantly better scores (p < .001). Lack of postgraduate improvement could be attributed to inadequate patient exposure, less qualified mentoring, or both.

**Methods Used:** To obtain a broader geographic survey and overcome faculty reluctance to be tested, the 50-question interactive multimedia CE Test was installed at 17 major U.S. teaching centers. Answers were automatically collected, scored, and sent over the internet to a secure database.

**Summary of Results:** 421 participants, including 71 full-time faculty, were tested with mean scores (out of a possible 100) listed in descending order in the table below.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean</th>
<th>Std. Dev.</th>
<th>Games-Howell Significance (p &lt; 0.028)</th>
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</thead>
<tbody>
<tr>
<td>Volunteer Cardiology Faculty</td>
<td>12</td>
<td>82.6</td>
<td>10.2</td>
<td>*</td>
</tr>
<tr>
<td>Full-Time Cardiology Faculty</td>
<td>52</td>
<td>81.8</td>
<td>12.7</td>
<td>*</td>
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<tr>
<td>Cardiology Fellows</td>
<td>127</td>
<td>77.6</td>
<td>12.7</td>
<td>*</td>
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<tr>
<td>Medical Students</td>
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<td>17.8</td>
<td></td>
</tr>
<tr>
<td>Residents: Internal Medicine</td>
<td>110</td>
<td>61.4</td>
<td>17.2</td>
<td></td>
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<tr>
<td>Residents: Family Medicine</td>
<td>40</td>
<td>58.9</td>
<td>16.1</td>
<td></td>
</tr>
<tr>
<td>Full-Time Non-Cardiology Faculty</td>
<td>19</td>
<td>57.6</td>
<td>28.4</td>
<td></td>
</tr>
</tbody>
</table>

44 **AN EVALUATION OF USING VIRTUAL PATIENTS FOR TEACHING AND TESTING CARDIAC EXAMINATION SKILLS IN FIRST YEAR MEDICAL STUDENTS**


1St Mary Medical Center, Long Beach, CA and 2Stanford University School of Medicine, Stanford, CA.

**Purpose of Study:** We implemented a Cardiac Exam (CE) teaching module for first-year medical students (MS1s), and tested their CE skills using virtual patients (VPs)-audiovisual recordings of actual patients.

**Methods Used:** The entire MS1 class (n = 86) participated in structured teaching in CE using standardized patients (2 hours) to practice visual and stethoscope placement skills, and VPs (6 hours) to illustrate the relationship between electromechanical activity in the heart with blood flow and subsequent auscultatory findings. We measured the accuracy of clinical assessment using two VPs: VP1 had a normal first (S1) and second (S2) heart sound and a physiologic third heart sound (S3); VP2 had bicuspid aortic stenosis (AS), with S1, S2, and ejection sound (ES), and midystolic murmur (MSM).

**Summary of Results:** In VP1, students correctly identified S1 (71%, n = 61); S2 (70%, n = 60); S3 (53%, n = 46), any extra sound (73%, n = 63). Students had more difficulty correctly identifying a benign condition (26%, n = 22), or physiologic S3 (12%, n = 10). In VP2, students correctly identified S1 (69%, n = 59); S2 (83%, n = 71), split S1 (14%, n = 12) and ES (19%, n = 16). Eighty six percent of students (n = 74) heard systolic murmur, 8% (n = 7) diastolic, 41% MSM, and 16% (n = 14) thought the murmur was holosystolic. Students correctly diagnosed AS (62%, n = 53), identifying correctly bicuspid AS (7%, n = 6.0).

**Conclusions:** Our findings demonstrate the feasibility of teaching and assessing CE skills with MS1 using VPs, and identify areas of CE skills that are more challenging for these learners. To improve CE skills, teaching and evaluating CE can begin in first year of medical school, emphasizing findings rather than diagnosis. Further CE training, with emphasis on accurate identification of abnormal findings, will aid clerkships and residency training.
45
THE ELECTROCARDIOGRAPHIC BRUGADA PATTERN IS RARELY ASSOCIATED WITH THE BRUGADA SYNDROME BUT IS COMMON WITH ACUTE CORONARY SYNDROME

N. Farraj1, K. Desser1, N. Laufer1,2, K. Benson1, and M. Burns1.
1Banner Good Samaritan Medical Center/Carl T. Hayden VA Medical Center, Phoenix, AZ and 2Heart and Vascular Center of Arizona, Phoenix, AZ.

Purpose of Study: Previously designated incomplete or complete right bundle branch block with early or high J point/ST segment take-off, this electrocardiographic (ECG) configuration is now interpreted as “Brugada pattern” by many cardiologists. The purpose of this study was to determine the incidence and clinical significance of this ECG finding.

Methods Used: Over a 2 year period, 67, 233 consecutive ECGs were prospectively studied for the presence of the Brugada pattern at an ECG work station of a large teaching hospital. Clinical correlation was then determined by patient data extraction.

Summary of Results: One hundred fifty-eight patients (0.24%) manifested the Brugada pattern with the following Arrhythmia Working Group distribution: type 1 (prominent coved ST segment elevation) = 3, type 2 (saddleback configuration above baseline) = 98, type 3 (saddleback, coved or both < 1mm above baseline) = 57. Only 4/158 (2.5%) had pre-syncope, syncope and/or polymorphic ventricular tachycardia (3 with type 1 and 1 with type 2). Of the other 154 subjects, 64 (42%) had cardiovascular diagnoses with 50 acute coronary syndromes and 14 cases of suspect or diagnosed pericarditis. Forty-one of the fifty (82%) with acute coronary syndrome were troponin positive. Of these forty-one patients, all but three cases were non-ST elevation myocardial infarctions. The Brugada saddleback pattern in 2/3 adjacent leads (V1, V2, V3) did not resolve after therapy and no subject received thrombolytics. Ninety subjects had a variety of non-cardiovascular medical and surgical diagnoses.

Conclusions: Only a small minority of patients with the Brugada ECG pattern have findings which satisfy Brugada Syndrome criteria and this finding has been encountered in subjects with ischemic heart disease. Whether the latter association is incidental remains to be determined.

46
TROPOIN FOR THE DIAGNOSIS OF ACUTE CORONARY SYNDROME IN PATIENTS WITH CHRONIC KIDNEY DISEASE

C. Macias1 and R. Komaragiri2. 1University of New Mexico HSC, Albuquerque, NM and 2University of New Mexico HSC, Albuquerque, NM.

Purpose of Study: By the year 2010 it is estimated that patients on dialysis will number 13.3 million where the prevalence of cardiovascular disease is 40 to 50% with an annual mortality of 9%. In light of this, the accurate and timely diagnosis of an acute coronary syndrome (ACS) is a priority. Biomarkers such as Troponin I (cTnI) are pivotal in diagnosing an Acute Myocardial Infarction (AMI), we studied its utility in patients with Chronic Kidney Disease (CKD).

Methods Used: A three year retrospective case control study was performed using a database of patients with CKD admitted to the hospital for presumed ACS. Patients were excluded if there was a history of recent AMI (<30 days) or an admitting diagnosis such as pulmonary embolus, sepsis, systemic inflammatory response, pericarditis, congestive heart failure exacerbation or rhabdomyolysis. cTnI was evaluated for its diagnostic utility in acute myocardial infarction (AMI). Serial (cTnI) measurements with a cutoff value of >0.8 ng/mL were considered positive. Severity of CKD was classified in Stage 3 (GFR 30–59 mL/min), Stage 4 (GFR 15–29 mL/min) and Stage 5 (GFR < 15 mL/min).

Summary of Results: A total of 308 patients were included in the study with 37 (12%) of them diagnosed with an AMI. Patients studied included 160 (51.94%) males and 148 (48.06%) females with a mean age of 64 years. Prevalence of diabetes was 42% of total patients and 82% in the MI group. The degree of CKD was as follows: Stage 3 (210 (68.18%), Stage 4 (71 = 23.05%) and Stage 5 (27 = 8.77%). Of the 37 patients diagnosed with an MI, 35 (94.54%) had a positive (>0.8 ng/mL) troponin level at presentation with 37 (100%) being positive at 6 and 12 hours after presentation. In the control group (271 patients) where MI was not the final diagnosis, troponin measurements were negative in 254 (93.72%), 263 (97%) and 266 (98%) at 0, 6 and 12 hr respectively. Sensitivity, specificity, PPV and NPV by CKD stage was 100%, 99%, 95% and 100% for stage 3 (p < 0.01), 100%, 96%, 84%, 100% for stage 4 (p < 0.01) and 100%, 80%, 63% and 100% for stage 5 (p < 0.05) respectively.

Conclusions: cTnI is highly sensitive for the diagnosis of AMI in patients CKD although its specificity does decrease with worsening renal function its sensitivity does not.

47
LEFT VENTRICULAR VOLUME IS A STRONG PREDICTOR OF ENDOCARDIAL VOLTAGE IN SUB-ACUTE AND CHRONIC SETTINGS OF MYOCARDIAL INFARCTION


Purpose of Study: The extent of myocardial damage after infarction (MI) has been assessed in previous studies by measurements of endocardial voltages. It is also known that left ventricular (LV) volume directly correlates with QRS amplitude and R wave voltage, and that voltages decrease markedly when LV diameters are acutely increased. However, the direct relationship between endocardial unipolar voltages (UpV) and volumes in sub-acute and chronic settings of MI has not been fully elucidated.

Methods Used: An anterior MI was induced by a 90 minute occlusion of the left anterior descending artery in 16 pigs. LV UpV mapping was performed at baseline (T0), 1 week post-MI (T1), and at 6 weeks post-MI (T6), and 324 +/-72 points were collected throughout the ventricle walls per case. Echocardiography was used to assess volumes (end-diastolic (EDV) and end-systolic (ESV)), and wall thickness (WT).

Summary of Results: MI induced a significant decrease in LV UpV at T1 (p < 0.01) and at T6 (p < 0.01) compared to T0. The UpV were lower in antero-septal (AS) wall when compared to the postero-lateral (PL) wall at T1 (p < 0.01) and at T6 (p < 0.01). The EDV and ESV increased at T1 (both p < 0.01), and T6 (both p < 0.01). The AS-WT increased from T0 to T1 (p < 0.01), and decreased from T0 to T6 (p < 0.01), while the PL-WT remained stable from T0 to T1 and increased at T6 (p < 0.01). At T1, EDV and ESV were inversely correlated, respectively, with LV UpV (r = -0.85; r = -0.84), AS-UpV (r = -0.8; r = -0.83), and PL-UpV (r = -0.88; r = -0.87). Even stronger correlation was found at T6, respectively (r = -0.9; r = -0.92), (r = -0.89; r = -0.92), and (r = -0.89; r = -0.89). However, a multiple linear analysis revealed that EDV, ESV, and AS-WT predicted LV UpV and AS-UpV at T1 and T6 (both p < 0.05) whereas only EDV and ESV were the only predictors of PL-UpV at T1 and T6 (both p < 0.05).

Conclusions: These results demonstrate that LV volume is a strong predictor of LV UpV after MI. If the results of this study are extrapolated to patients, then the inverse correlation of LV volume and E-UpV may reduce the diagnostic sensitivity of LV mapping criteria of myocardial damage if volumetric changes are not taken into account.
48  INDUCING HEAT STRESS WITH THERMALLY RESTRICTIVE CLOTHING IN MAXIMAL BRUCE PROTOCOL ELECTROCARDIOGRAPHY: A HOT BRUCE TEST
L.W. Raymond1,2 and T.A. Barringer1,2. 1Univ of North Carolina, Chapel Hill, Charlotte, NC and 2Carolinas HealthCare System, Charlotte, NC.
Purpose of Study: Stress testing should be considered in the medical evaluation of hazardous materials (Hazmat) workers when heat stress is expected, as is usually the case. However, our earlier testing of 71 subjects who wore gym clothes for maximal Bruce Protocol treadmill electrocardiography found that body temperature rose by only 0.08 +/- 0.06SD degrees F per minute of MBPTE, with little sweating or thermal discomfort. We wondered if using thermally-restrictive clothing might enable us to add more substantial thermal stress during MBPTE.
Methods Used: Twenty non-smoking, healthy subjects (85% male, age 36 +/- 11, BMI 27 +/- 4) underwent MBPTE, first in gym clothes (Test 1) and later wearing a cotton flannel sweat suit under a vinyl "Sauna Suit" and a diver’s neoprene wet suit hood (Test 2). Heat stress was self-assessed by Young’s Index (4 = neutral, 8 = very hot) and by oral "pill", Test 2 only.
Summary of Results: Substantial thermal stress was created in Test 2, with a rise in body temperature similar to that of Hazmat drills (1.8 - 2.2 F) and more than twice that of Test 1 (Table). The rise in tympanic temperature exceeded that of the "pill" (2.4 vs. 1.1 degrees F, p = 0.002) though the two were closely correlated (r = 0.65, p < 0.01). Test 2 elicited heavy sweating, whereas Test 1 yielded little or no visible perspiration.
Conclusions: Readily available, inexpensive apparel induced substantial heat stress without the need of a temperature-controlled chamber. Since heat stress may play a role in the risk of sudden death in workers such as firefighters, we believe it should be incorporated in medical evaluations performed on them and on Hazmat workers.

<table>
<thead>
<tr>
<th>Test #</th>
<th>MBPTE Time, min.</th>
<th>Young Index</th>
<th>Tympanic Temperature Rise, oF</th>
<th>Rise per minute of MBPTE, oF</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14.1 +/- 3.1</td>
<td>6.4 +/- 0.6</td>
<td>1.1 +/- 0.8</td>
<td>0.08 +/- 0.05</td>
</tr>
<tr>
<td>2</td>
<td>15.6 +/- 2.8</td>
<td>7.1 +/- 0.5</td>
<td>2.4 +/- 1.1</td>
<td>0.17 +/- 0.06</td>
</tr>
<tr>
<td>p</td>
<td>0.65</td>
<td>0.001</td>
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</table>

Endocrinology I
Concurrent Session
8:30 AM
Friday, February 1, 2008

49  PHARMACOKINETICS AND PHARMACODYNAMICS OF ORAL TESTOSTERONE ENANTHATE PLUS DUTASTERIDE FOR FOUR WEEKS IN NORMAL MEN: IMPLICATIONS FOR MALE HORMONAL CONTRACEPTION
Purpose of Study: Testosterone (T) exerts its contraceptive effect by suppressing the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the pituitary. Low levels of LH and FSH deprive the testis of stimulatory signals required for spermato- genesis, leading to markedly decreased sperm concentrations. Current male hormonal contraceptive regimens, while effective in ~90% of men, rely upon transdermal or subcutaneous injection of T for delivery, limiting their acceptability to some men. We have previously shown that a single dose of orally administered T enanthate (TE) plus the 5a-reductase inhibitor dutasteride (D) normalizes serum T in medically castrate men. We hypothesized that prolonged administration of oral TE+D might suppress gonadotropins and therefore be useful for male hormonal contraception. Objective: To ascertain the degree of gonadotropin suppression mediated by 4 weeks of daily, oral TE+D.
Methods Used: Twenty normal young men were randomly assigned to 4 weeks of either 400 mg oral TE twice daily or 800 mg oral TE once daily, in a double-blinded, controlled fashion at a single site. All men received 0.5 mg of D daily. Blood for measurement of serum LH, FSH, T, dihydrotestosterone (DHT) and estradiol was obtained prior to treatment, weekly during treatment, and 1, 2, 4, 8, 12, 13, 14, 16, 20 and 24 hours after the dose on the last treatment day. Hormones were measured by immunoassay except for DHT which was measured by LC-MS.
Summary of Results: FSH was significantly suppressed throughout treatment with 800 mg TE once daily and after 4 weeks of treatment with 400 mg TE twice daily. LH was significantly suppressed after 2 weeks of treatment with 800 mg TE, but not with 400 mg TE. Serum DHT was suppressed and serum estradiol increased during treatment in both groups. HDL cholesterol was suppressed during treatment, but liver function tests, hematocrit, creatinine, mood and sexual function were unaffected.
Conclusions: The oral administration of 800 mg TE + 0.5 mg D for 28 days significantly suppresses gonadotropins in men without untoward side effects and might have utility as part of a male hormonal contraceptive regimen or in the treatment of male hypogonadism.

50  MOUSE MODEL OF TESTOSTERONE-INDUCED MUSCLE FIBER HYPERTROPHY
Purpose of Study: Previously, we have shown that testosterone (T) supplementation increases muscle size in healthy young and old men, hypogonadal men, and men with illness. We have also demonstrated that T-induced increase in muscle size is linked to fiber hypertrophy and significant increase in myonuclear and satellite cell numbers. The mechanisms by which T increases satellite cell number and promotes muscle growth are not well known. An understanding of the mechanistic pathways that mediate T-induced muscle fiber hypertrophy may unveil novel targets for the development of anabolic therapies. As a prerequisite for studies using mutant mice, we wish to establish a mouse model for studying the molecular mechanisms by which T promotes muscle growth. T induces skeletal muscle hypertrophy by promoting satellite cell activation and proliferation through activation of Notch (N)-signaling pathway.
Methods Used: Adult mice (C57BL/6) received gonadotropin-releasing hormone antagonist to suppress endogenous T production and were implanted subdermally under anesthesia with empty (sham control) or 2 cm PDS T- filled tubule to ensure a supraphysiologic dose of T. Mice were sacrificed 1, 2, 4, 6, and 8 wk after treatment.
Summary of Results: T treatment for 8 wk resulted in a significant (P < 0.001) increase in fiber area of gastrocnemius muscles (C: 1675 +/- 47 vs. T: 2414 +/- 105 mm2). T-induced fiber-hypertrophy was preceded by up-regulation of the Notch ligand Delta-1 and activation of N-signaling, as evidenced by an increase in cleaved intracellular activated forms of N-1, N-2, and N-4 in gastrocnemius muscle lysates by western blotting. Consistent with this, we also observed an increase in the number of PCNA-positive nuclei in muscles of T-treated mice, indicating that activation of N-signaling enhanced cell proliferation. T supplementation also resulted
in suppression of JNK activation but concurrently promoted p38 MAPK activation. T induces muscle fiber hypertrophy in mice by cell proliferation induced by Notch-signaling. The inactivation of JNK together with the activation of p38 MAPK may be critical for T-induced muscle fiber hypertrophy.

**51 COMBINED TRANSDERMAL NESTORONE AND TESTOSTERONE GELS SUPPRESS GONADOTROPINS IN HEALTHY MEN**

V. Mahabadi1, C. Wang1, J. Amory2, S. Page2, P.D. Christensen1, R. Sitruk-Ware3, N. Kumar1, Y.Y. Tsong1, D. Blithe4, W.J. Brenner2, and R.S. Swerdloff1. 1Harbor-UCLA Medical Center, Torrance, CA; 2University of Washington, Seattle, WA; 3Population Council, New York, NY and *NICHD, NIH, Bethesda, MD.

**Purpose of Study:** Nestorone (Nes) is a progestin without estrogenic or androgenic activity. To investigate whether Nes has any effect on suppression of gonadotropins and whether it has any additive effect when administered with testosterone (T), we recruited healthy men to a proof of concept study.

**Methods Used:** The 140 participants applied these agents daily as a transdermal gel for 20 days. The transdermal route was chosen because of ease of use and delivery of steroids at steady levels. The subjects were randomized into 5 groups of 20 to receive: 10 g T gel; 2 mg Nes gel, 4 mg Nes gel; 10 g T gel + 2 mg Nes gel; and 10 g T gel + 4 mg Nes gel. Prior to initiating treatment and on day 21, 5 blood samples were drawn to measure serum LH and FSH levels. The suppression of gonadotropins to very low levels occurred only in small proportion of subjects, 2 additional groups of 20 men were enrolled to receive 10 g T gel + 6 mg Nes gel and 10 g T gel + 8 mg Nes gel.

**Summary of Results:** Data are presented from 119 subjects who were compliant with the application of the gels based on preset criteria. Nes alone suppressed gonadotropin levels significantly but not to very low levels. As shown in the table below T gel 10г plus Nes gel 6 or 8 mg suppressed both serum LH and FSH to very low levels. Prior studies suggested that suppression of both gonadotropins to such low levels is compatible with inhibition of spermatogenesis if the gels are applied for longer periods. No significant adverse events were reported.

**Conclusions:** We conclude that this proof of concept study showed that Nes gel had some gonadotropin suppressing activity. Nes when applied at 8mg plus T gel 10 g per day suppressed gonadotropins in the majority of subjects warranting a study to assess the suppression of spermatogenesis with this combination of Nes and T gel.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>T 10g</th>
<th>Nes 2 mg</th>
<th>Nes 4 mg</th>
<th>T 10g + Nes 2 mg</th>
<th>T 10g + Nes 4 mg</th>
<th>T 10g + Nes 6 mg</th>
<th>T 10g + Nes 8 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>19</td>
<td>16</td>
<td>16</td>
<td>19</td>
<td>15</td>
<td>18</td>
<td>16</td>
</tr>
<tr>
<td>Both LH &amp; FSH &lt; 0.5 mU/L</td>
<td>21.1</td>
<td>0</td>
<td>36.8</td>
<td>40.0</td>
<td>61.1</td>
<td>68.8</td>
<td></td>
</tr>
<tr>
<td>Both LH &amp; FSH &lt; 1.0 mU/L</td>
<td>31.6</td>
<td>6.3</td>
<td>12.5</td>
<td>57.9</td>
<td>53.3</td>
<td>72.2</td>
<td>81.3</td>
</tr>
</tbody>
</table>

**52 TRANSPLANTED XY SPERMATOGONIAL STEM CELLS PRODUCE SPERM IN TESTES OF XXY MICE**


**Purpose of Study:** Klinefelter syndrome (XXY males) is the most common sex chromosome aneuploidy, occurring in about 1 per 500 men. To study the underlying molecular mechanisms caused by the extra X chromosome, we have developed an experimental mouse model for men with Klinefelter’s syndrome. We have demonstrated that adult XXY mice have absence of germ cells, decreased serum testosterone levels, and elevated gonadotropin levels. Testicular failure begins early as a result of massive germ cell loss that precedes the initiation of meiosis. Loss of germ cells is mediated through apoptosis. It is unclear however if the germ cell defects are a primary problem or secondary to abnormal Sertoli cell function.

**Methods Used:** To test XXY Sertoli cell function, we harvested testicular cells from 10-day-old GFP transgenic mice (XY) and injected them into the seminiferous tubules of young adult (6-week-old) XXY mice. Twelve weeks after transplantation, the recipient testes were removed and decapsulated to detect GFP positive cells under fluorescence microscopy. Immunohistochemistry was also performed to detect cells with GFP expression.

**Summary of Results:** The results showed that the segments of green fluorescence (GFP positive cells) seminiferous tubules were observed in the recipient XXY testes. Immunohistochemistry on serial testicular sections demonstrated that donor-derived GFP positive spermatogonia, spermatocytes, round spermatids and spermatzoa were present in some of the seminiferous tubules of XXY mice. Thus, we demonstrate that transplanted XY spermatogonial stem cells were able to survive for at least 12 weeks in XXY testes. Donor XY spermatogonial stem cells can colonize the seminiferous tubules, go through meiosis and spermogenesis, and ultimately form sperm in testes of young adult XXY mice.

**Conclusions:** We conclude that 1) the XXY testicular environment is able to support XY spermatogonial stem cells to finish spermatogenesis; 2) XY donor derived patchy spermatogenesis in XXY testes may reflect the functional variability of the XXY Sertoli cells in supporting the development of XY spermatogonial stem cells; 3) whether these XY spermatozoas are able to fertilize oocytes and generate pups has to be addressed.

**53 COMPARABLE HEIGHT GAIN WITH 3-MONTH AND 1-MONTH DEPOT LEUPROLIDE THERAPY IN CENTRAL PRECOCIOUS PUBERTY**

H.K. Chiu1, D.F. Gunther1, and G.B. Kletter2. 1Children’s Hospital & Regional Medical Center, Seattle, WA and 2Swedish Physician Division, Swedish Hospital, Seattle, WA.

**Purpose of Study:** Central precocious puberty is associated with accelerated skeletal maturation and compromise of adult stature. Depot 1-month formulations of synthetic analog agonist of gonadotropin-releasing hormone are effective treatments that retard precocious puberty and thus augment adult height. Recently, 3-month depot formulations have been introduced and used for this indication. Previous comparisons between the one and three month preparations have looked at LH suppression as the dependent variable, with no clear rationale as to its clinical interpretation.

**Methods Used:** We compared the expected height gain between the use of depot 1-month leuprolide (19 children: 14 girls and 5 boys) with depot 3-month leuprolide (31 children: 26 girls and 5 boys) in a retrospective, observational study. The principal outcome assessments were bone age change relative to chronologic age, absolute height changes, and progressive deviation of predicted eventual height from established normative data.

**Summary of Results:** The 3-month depot leuprolide preparation was as effective as the 1-month depot leuprolide in slowing the rate of bone-age maturation, resulting in similar adult stature between the cohorts.
Conclusions: The 3-month depot leuprolide regimen offers comparable clinical efficacy and safety as compared to the 1-month regimen with the advantage of reducing the annual injections from 12 to 4.

Depot Leuprolide

<table>
<thead>
<tr>
<th>Race</th>
<th>Fasting insulin (μU/mL)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hispanics</td>
<td>22.5 ± 14.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Caucasians</td>
<td>17.9 ± 11.3</td>
<td>N.S.</td>
</tr>
<tr>
<td>African-Americans</td>
<td>18.7 ± 13.8</td>
<td>N.S.</td>
</tr>
<tr>
<td>Other races</td>
<td>21.5 ± 8.7</td>
<td>0.001</td>
</tr>
</tbody>
</table>

54 ENDOCRINE ABNORMALITIES IN OBESE CHILDREN

W.N. Evans 1,2, G.A. Mayman 1,2, R.J. Acherman 1,2, K.T. Kip 1,2, and H. Restrepo 1,2. 1 Children’s Heart Center, Las Vegas, NV and 2 University of Nevada, School of Medicine, Las Vegas, NV.

Purpose of Study: To explore the presence of subclinical thyroid abnormalities and hyperinsulinemia in overweight children referred to a life-style modification program.

Methods Used: We analyzed data from 730 children with BMIs = 95th percentile enrolled in our 12-week lifestyle intervention program from March 2003 to April 2007. We excluded diabetic and clinically hypothyroid children from analysis. All participants had initial fasting levels of insulin, free thyroxine (T4), T3 uptake, and TSH. We used t-test for statistical analysis.

Summary of Results: The mean age was 11.3 ± 2.5 years (range 6 to 18) with a gender distribution of 45% females and 55% males. Racial distribution was Hispanics 57%, Caucasians 26%, African-Americans 18%, and other races 7%. Mean fasting insulin was 20.5 ± 13.5 μU/mL, significantly higher (p < 0.001) than the reference laboratory’s upper normal value (17 μU/mL). Analysis of fasting insulin by race showed Hispanics and other races with significantly higher levels than Caucasians or African-Americans (table summarizes results). We found a subgroup of 125 (17%) children with subclinical hypothyroidism (TSH between 3 and 5 μU/mL and normal T4 and T3- according to the American Association of Clinical Endocrinologists); these patients had also significant elevated values for fasting insulin (23.9 ± 15.9 μU/mL, p < 0.001).

Conclusions: This group of obese children had elevated levels of fasting insulin, especially Hispanic children. An important percentage of children also had subclinical hypothyroidism. The evaluation of obese children should include fasting insulin and thyroid hormones.

55 ROLE OF p38 MITOGEN ACTIVATED PROTEIN KINASE/MITOGEN ACTIVATED PROTEIN KINASE ACTIVATED PROTEIN KINASE 2/HEAT SHOCK PROTEIN 27 PATHWAY IN MALE GERM CELL APOPTOSIS AFTER MILD TESTICULAR HYPERTERMIA IN THE RAT

Y. Ju 1, A. Hikim 1, C. Wang 1, Y. Lu 1, V. Atienza 1, J. Castellanos 1, C. Johnson 1, and R. Swerdloff 1. 1 Harbor-UCLA Medical Center, Torrance, CA and 2 The First Affiliated Hospital of Nanjing Medical University, Nanjing, China.

Purpose of Study: Previously, we have shown that p38MAPK signaling is the key upstream pathway in male germ cell apoptosis across species induced by testicular hypertermia. Expression of Hsp27 mRNA has also been found dramatically increased in heat treated testis, but its role in heat triggered male germ cell death is unclear. The goal of this study was to define the role of p38MAPK/MK2/Hsp27 pathway in male germ cell apoptosis in rats after mild testicular hypertermia.

Methods Used: Groups of 3-5 adult Sprague-Dawley male rats were pre-treated 1 h before local testicular heating with intratesticular of 50 ul vehicle (DMSO) or 50 ug of SB203580, a p38 MAPK inhibitor. They were then exposed to 22°C (control) or 43°C (Heat) local testicular heating for 15 minutes and killed 6 h later. Frozen tissue was used for Western Blot experiment and Bouin’s solution fixed tissue for TUNEL and immunohistochemistry staining.

Summary of Results: Compared with controls (15.4 ± 2.7), mild testicular hypertermia resulted in a marked activation (45.1 ± 18.2) of germ cell apoptosis. SB 203580 administered IT at this dose level significantly (P < 0.05) attenuated heat-induced germ cell apoptosis by 50.6%. Expression of phosphorylated Hap27 (pHap27) and phosphorylated MK2 (pMK2) were increased, as evidenced by Western Blot, in heat group, but not in SB203580 alone group. Pre-treatment with SB203580 decreased pHap27 and pMK2 expression after heat. pMK2 and pHsp27 staining was mainly found in germ cells, especially in the apoptotic germ cells.

Conclusions: Our results indicate that p38MAPK/MK2/Hsp27 pathway may play a role in male germ cell apoptosis triggered by heat stress in rats.

56 EVALUATION OF GENE PROMOTERS FOR EFFICIENT PRODUCTION OF HUMAN FACTOR VIII IN A MURINE MODEL OF HEMOPHILIA A

A. Nguyen, A. Dow, R. Busuttil, and G.S. Lipshutz. University of California, Los Angeles, Los Angeles, CA.

Purpose of Study: Hemophilia A is a bleeding disorder caused by mutations in factor VIII (FVIII). Severely affected patients present with bleeding in joints and muscles and can die from exsanguination. Gene replacement therapy with AAV represents a promising treatment in that small levels of gene expression can convert a severe to a mild phenotype. The purpose of the present study was to 1) evaluate promoters for efficient expression of human (h) FVIII and 2) construct AAV plasmids to correct a murine model of the disease.

Methods Used: Human cytomegalovirus (hCMV), chicken beta-actin/CMV enhancer (CBA), elongation factor 1 alpha (EF1alpha) and
57 EVALUATION OF CHITOSAN AS A GENE TRANSFER AGENT TO FETAL MurINE ORGANS AND HEK293T CELLS IN VITRO, AND FETAL ORGANS FOLLOWING IN UTERO GENE DELIVERY

L. Hoang1,2, P.T. Yang1,2, L. Huang3, W.W. Jia3,4, and E.D. Skarsgard1,2  
1University of British Columbia, Vancouver, BC, Canada; 2Child & Family Research Institute, Vancouver, BC, Canada; 3University of British Columbia, Vancouver, BC, Canada and 4University of British Columbia, Vancouver, BC, Canada.

Purpose of Study: In utero gene therapy offers novel treatment for prenatally diagnosed monogenic disorders. Approaches using viral vectors have raised significant safety concerns. The polysaccharide chitosan offers the substantial benefit of non-viral, site-restricted transgene delivery using a non-viral vector strategy. Chitosan-pGFP-pPGK vectors have raised significant safety concerns. The polysaccharide chitosan however, shows promise as a non-viral gene transfer agent, as fetal tissues are highly permeable to cell-penetrating peptides.

Methods Used: Fetal trachea, segmental lung and intestine were harvested from CD-1 pregnant mice on gestational day (GD) 17 and transfected in vitro with chitosan-pGFP-pdC31. Fluorescence microscopy (FM) was used to monitor GFP expression. HEK293T cells were transfected in medium containing 50%, 25%, 12.5% or 0% amniotic fluid (AF) and GFP positive cells counted by flow cytometry. Finally, chitosan-pGFP-pdC31 was injected into the amniotic sacs of pregnant mice on GD 16. Mice gave birth and 1 day post-delivery, tissues were procured for analysis using PCR, RT-PCR and FM.

Summary of Results: Fetal organs transfected in vitro exhibited greater fluorescence than controls. Efficiency of transfection of HEK293T cells was inversely proportional to the concentration of AF in culture medium. In utero transfection by amniotic injection resulted in GFP gene delivery and expression in mice pup tissues postnatally. In utero transfection by amniotic injection resulted in GFP gene delivery and expression in mice pup tissues postnatally.

Conclusions: This study demonstrated that chitosan-pGFP-pdC31 transfects fetal organs and a human epithelial cell line in vitro, as well as fetal tissues in vivo, providing proof-of-principle for in utero gene therapy using a non-viral vector strategy. Chitosan’s favorable biosafety profile and potential for increased stability in AF via biochemical modification, justify further investigation of its use in fetal gene therapy.

58 AN ABUNDANT EVOLUTIONARILY CONSERVED CSB-PIGGYBAC FUSION PROTEIN EXPRESSED IN COCKAYNE SYNDROME

J. Newman1, A. Bailey1, T. Pavelitz1, H. Fan2, and A. Weiner1  
1University of Washington, Seattle, WA and 2Fox Chase Cancer Center, Philadelphia, PA.

Purpose of Study: Cockayne syndrome (CS) is a devastating progeria most often caused by mutations in the CS gene encoding a SWI/SNF family chromatin remodeling protein. Although all CSB mutations that cause CS are recessive, the complete absence of CSB protein does not cause CS. In addition, most CSB mutations are located beyond exons 5 and 6 and are thought to generate only C-terminally truncated protein fragments. We undertook a study of the CSB gene in order to understand the critical role of the N-terminal region of CSB and how complete absence of the protein does not cause disease.

Methods Used: We used a variety of genomic, molecular and biochemical techniques, including a survey of the human genome sequence, quantitative PCR, Western blotting, and comparative genomic analysis of several primate genomes.

Summary of Results: We show that a domesticated PiggyBac-like transposon PGBD3, residing within intron 5 of the CSB gene, functions as an alternative 3’ terminal exon. The alternatively spliced mRNA encodes a novel chimeric protein in which CSB exons 1–5 are joined in frame to the Piggybac transposase. The resulting CSB-transposase fusion protein is as abundant as CSB protein itself in a variety of human cell lines, and continues to be expressed by primary CS cells in which functional CSB is lost due to mutations beyond exon 5. The CSB-transposase fusion protein has been highly conserved for at least 43 Myr since the divergence of humans and marmoset, and appears to be subject to positive selection. The human genome contains over 600 non-autonomous PGBD3-related MER85 elements that were dispersed when the PGBD3 transposase was last active at least 37 Mya. Many of these MER85 elements are associated with genes which are involved in neuron development, and are known to be regulated by CSB.

Conclusions: The CSB-PGBD3 fusion protein is a highly abundant - but never before observed - alternative splice product of the CSB locus. We speculate that this fusion protein has been conserved for host antitransposon defense, or to modulate gene regulation by MER85 elements, but may cause CS in the absence of functional CSB protein.
ISOLATION AND CHARACTERIZATION OF INTERLEUKIN-24 SPlice VARIANTS

D. Whang, E. Schmidt, V. Filipov, and P. Duerksen-Hughes. Loma Linda University, Loma Linda, CA.

Purpose of Study: Discovery of a novel cytokine called interleukin-24 (mda-7/IL-24) has shown promise in the field of cancer therapy. Overexpression of the gene was found to inhibit cell growth in tumor cell lines, while remaining innocuous for non-cancerous cells. Its potential as a cancer therapy comes from its capabilities of inducing apoptosis, inhibiting angiogenesis, enhancing radiation lethality, and playing a key role in growth prenatally and the GH-IGF axis postnatally. We have demonstrated that the histone code along the hepatic IGF-1 gene is altered in day 0 (d0) and d21 IUGR rats. IGF-1 plays a key role in growth prenatally and the GH-IGF axis postnatally. GH promotes hepatic IGF-1 gene expression via STAT5b activation. The primary and secondary STAT5b binding sites lie within intron 2 (I2SBS) and within 5’ flanking region (5’SBS) respectively. Therefore we hypothesize that the postnatal histone code associated with the two STAT5b binding sites will be altered in correlation with the IUGR induced prenatal changes.

Methods Used: Bilateral uterine artery ligation was performed on e19 pregnant Sprague-Dawley rats. ChiP/Real-time PCR was performed using anti-acetyl histone H3 (Ac) K9, K14, dimethyl (me2) -K4, me3-K4, K9 and K36.

Summary of Results: We initially defined the normal histone code around STAT5b binding sites of rat hepatic IGF-1 gene. The normal histone code pattern around the two STAT5b binding sites had no gender difference. In d0 controls me3K36 and me2K4 were increased at I2SBS relative to 5’SBS. In d21 controls, me3K36 was still increased while me2K4, me3K4, me3K9 and AcK9 were decreased at I2SBS relative to 5’SBS. We then characterized the influence of IUGR on the histone code around STAT5b sites. IUGR altered the histone code at the two STAT5b sites in a gender specific manner. At I2SBS, me3K36 was decreased at d0 and remained low at d21 in IUGR females relative to controls, while me3K4 and AcK14 increased. At 5’SBS, AcK14 was increased in d21 IUGR females while me3K4 was decreased in d21 IUGR males relative to controls.

Conclusions: The normal histone code is unique between the primary and the secondary STAT5b binding site on rat hepatic IGF-1 gene. Uteroplacental insufficiency and subsequent IUGR alter the histone code at the two sites prenatally and postnatally in a gender specific manner. We speculate that the sequence of events is that 1) uteroplacental insufficiency induces the previously described changes in the histone code of the transcribed regions; and 2) this affects the normal ontological changes of the STAT5b sites, which subsequently leads to the decreased mRNA levels of the IUGR rat liver.

THIN FILAMENT LENGTH REGULATION IN PATIENTS WITH NEMALINE MYOPATHY

J.E. Popper. University of Arizona, Tucson, AZ.

Purpose of Study: Nemaline myopathy (NM) is a slowly progressive or non-progressive disorder characterized by muscle weakness and the presence of rod bodies in affected muscle fibers. NM is caused by mutation in genes coding for skeletal muscle thin filament proteins such as troponin, tropomyosin, actin, and (in 50% of all cases) nebulin. Nebulin is a giant protein that functions as a thin filament template,
regulating thin filament length and thus muscle contraction. Therefore, we hypothesized that NM patients with a nebulin mutation show loss of thin filament length regulation and depressed contractility. To further investigate this we studied protein composition, thin filament length, and Ca2+-activated force generation of striated muscle from nemaline myopathy patients with a nebulin mutation.

Methods Used: Skeletal myofibrillar protein expression was determined by western blot. Myofibrils were structurally characterized using electron microscopy (EM), immunoelectron microscopy (IEM), and immunofluorescence confocal microscopy (IF). Muscle force from maximally activated skinned fibers was measured over a range of sarcomere lengths and normalized to the cross sectional area of the fiber.

Summary of Results: Protein expression results from three different antibodies directed at either the N- or C-terminus epitopes indicated a marked reduction of nebulin in NM muscle fibers. EM revealed that the overall sarcomeric structure was similar in both control and NM muscle fibers, yet the NM fibers lacked H-zones, indicating that thin filaments are not of defined length in NM. IEM of control fibers labeled with phallolin showed an even distribution from the Z-disk out to 1.2 μm, indicating all thin filaments are of equal length. In contrast, NM fibers contained thin filaments that were shorter and more variable, ranging in length from 0.2 μm to 1.2 μm. IF of the filament capping protein tropomodulin-1 also indicated an overall decrease in thin filament length and increased variability. Maximal force generated in muscle fibers obtained from NM patients was consistent with thin filament data and tropomodulin-1 also indicated an overall decrease in thin filament length.

Conclusions: These findings demonstrate the importance of thin filament length and its role in decreased force production in patients with NM.

64
A NOVEL MICRODELETION AT 4Q28.3 CAUSING MICROTIA AND CRANIOFACIAL ANOMALIES

Purpose of Study: Microtia is a congenital abnormality with an incidence of 1.5/10,000 live births. Both autosomal dominant and autosomal recessive modes of inheritance have been described. Microtia can be isolated or occur with other associated anomalies in syndromes such as oculo-auriculo-vertebral syndrome and Treacher-Collins syndrome. However, for both isolated and syndromic microtia, few causative genes have been described. We report a neonate with right unilateral microtia and non-development of the right external auditory meatus with a normal left ear, growth retardation and facial features that included hypertelorism, small palpebral fissures, a broad nasal bridge and micrognathia. Other anomalies were wide-spaced nipples, aberrant palmar creases and overlapping toes. An echocardiogram showed a small VSD, but renal and cranial ultrasound scans were normal. There was no significant family history. We sought to evaluate the underlying genetic cause in this patient.

Methods Used: Blood samples were obtained for high resolution karyotyping in the patient and his parents. Further cytogenetic investigation was performed by array comparative genomic hybridization (array CGH) using a targeted BAC array (CA1000) at Bicolormixat.

Summary of Results: High resolution karyotyping revealed a de novo pericentric chromosome inversion of chromosome 4 in the proband, karyotype 46, XY.inv(4) (p14q28.2). Karyotypes of the parents were normal. Further testing by array CGH revealed a deletion of a single BCA clone at 4q28.3, the distal inversion breakpoint. Deletion of this clone is not recognized to be a normal copy number variant.

Conclusions: A microdeletion at 4q28.3 is the most likely cause of microtia and other associated congenital anomalies in this child. Parental array CGH studies are pending to confirm absence of the deletion in parents. The maximum size of the deleted region can be estimated to be 3–4 Mb. Further mapping of the 4q28.3 deletion with a whole genome array will enable us to determine which genes are included in the deletion and thus whether they can be considered to be candidates for microtia in humans.

65
FUNCTIONAL IN VITRO CHARACTERIZATION OF MEK MUTATIONS IN CFC SYNDROME: IMPLICATION FOR THERAPEUTIC OPTIONS
K.A. Rauen1,2, A.L. Estep2, and O. Tetsu2. 1UCSF, San Francisco, CA, and 2UCSF, San Francisco, CA.

Purpose of Study: Cardio-facio-cutaneous (CFC) syndrome is a rare multiple congenital anomaly disorder in which individuals have characteristic dysmorphic craniofacial features, cardiac defects, ectodermal anomalies, developmental delay and hypotonia. CFC is caused by
alteration of activity through the mitogen-activated protein kinase (MAPK) pathway due to heterogeneous de novo mutations in protein kinases B-Raf, MEK1 and MEK2.

Methods Used: Approximately 25% of known mutation-positive CFC individuals have a MEK1 or MEK2 mutation. The majority are missense substitutions located in exons 2 and 3 with many of the mutations located 5 of the kinase domain. The most common mutation is MEK1 Y130C comprising about one third of all the MEK mutations. Original functional studies of a limited number of MEK1/2 CFC mutants performed in our lab have demonstrated increased activity in vitro over wild-type MEK in stimulating ERK phosphorylation, but CFC mutants were not as active as an artificially generated constitutively active MEK mutant.

Summary of Results: In this present study, we have examined the biochemical properties of all CFC MEK mutants identified to date and demonstrated that all are hypermorphic, but to varying degrees. In addition, we determined that mutants are sensitive to the MEK inhibitor U0126.

Conclusions: Understanding the function of these novel mutations is important for understanding MEK’s role in the MAPK signaling pathway in CFC and cancer. Because CFC has an evolving phenotype, systemic treatment by inhibiting the Ras/MAPK pathway merits investigation.
current perspectives on how to transform the challenges into opportunities for providing more effective culturally competent care.

**Methods Used:** In July, 2007 an intervention was designed to address the growing trend of inactivity in the Latino diabetic patient population of the SeaMar Community Health Clinic in Marysville, WA. Various community organizations were contacted in order to facilitate the intervention. When difficulties were encountered, a literature review was executed to research how the challenges are currently being addressed.

**Summary of Results:** During the intervention, difficulties were encountered in the attempt to identify a geographic Latino community and support for the planned intervention. The project highlighted the importance of redefining the meaning of “community” to include non-geographic components, the importance of considering cultural characteristics in planning interventions, and taking advantage of culturally distinct social networks (such as church and family) and existing Latino-focused service programs.

**Conclusions:** Using results of a the community intervention and a literature review, the presentation will describe the difficulties encountered in Marysville, WA, characteristics to consider when working with a Latino population that is both socially isolative and geographically dispersed, and how these considerations could be utilized in the design and implementation of effective community interventions. The results of the literature review reflects a growing understanding of the importance of redefining community intervention techniques and philosophies in order to provide culturally competent care which can ultimately help eliminate current health disparities in the urban, underserved Latino population.

**69**

**DOES MEDICARE PART D OUTREACH TO ASIAN AMERICANS WORK?**

P. Lu, and F. Chen, 1University of Washington School of Medicine, Seattle, WA and 2University of Washington School of Medicine, Seattle, WA.

**Purpose of Study:** Medicare Part D is the prescription drug program for Medicare beneficiaries. Though there have been outreach efforts to minorities, few studies have documented the effectiveness of outreach to Asian Americans. Asian Americans face challenges when accessing health care because many Asian American elderly are foreign-born and have limited English proficiency. Given these barriers, we wanted to assess Asian American elderly’s knowledge about Part D and whether they enrolled in a Part D prescription drug plan.

We hypothesized that not speaking English was a barrier to participating in Part D.

**Methods Used:** We created a survey instrument containing questions about ethnicity, age, language, Part D knowledge, and enrollment. The survey was translated into Chinese, Korean, and Vietnamese. The survey population consisted of a convenience sample of Asian American, Medicare beneficiaries residing in Seattle. We analyzed how demographics and language relate to knowledge and enrollment in Part D.

**Summary of Results:** We surveyed 132 Medicare beneficiaries with a mean age of 77, 53% were female and 44% spoke English. Most respondents (62%) knew “a little” about Part D; 15% reported knowing “a lot”, and 24% reported knowing nothing. English speaking was not associated with knowledge of Part D (p=0.32).

The majority of respondents (62%) had signed up for Part D. English speaking was not associated with enrolling in Part D (p=0.16). Those who knew more about Part D were more likely to have enrolled in a plan; 88% who knew a lot, 70% who knew a little, and 19% who knew nothing had enrolled or been enrolled (p=0.01).

**Conclusions:** In this study, we found that English language ability was not a barrier to enrollment, but the majority of respondents had little or no knowledge of Part D. The lack of knowledge among most respondents raises questions of whether the enrollees actually understand Part D or are passively enrolled by others with little explanation to them. Results indicate that those who knew more about Part D were more inclined to enroll. It is likely that informed beneficiaries will be able to weigh the risks and benefits of the plans more effectively than less informed ones. Without adequate knowledge, many of this vulnerable group might make poor decisions regarding plans.

**70**

**PATIENT-CENTERED SATISFACTION MEASURES IN A LAY PATIENT NAVIGATOR PROGRAM FOR UNDERSERVED POPULATIONS IN ONCOLOGY**

N. Hanna, 1 M. Steinberg, 2 H. Knapp, 1 D. Khan, 2 D. Huang, 1 O. Streeter, 1 and A. Freeman, 3 UCLA, Los Angeles, CA; 2Centinela Freeman Regional Medical Center, Inglewood, CA; 3RAND Corporation, Santa Monica, CA and 4University of Southern California—Norris Cancer Center, Los Angeles, CA.

**Purpose of Study:** Cancer care in underserved populations entails many barriers. Navigators serve to reduce those barriers. However, there is little information about patient acceptance, satisfaction, and benefit of navigation.

**Methods Used:** We previously published regarding our program to address disparity in cancer care by implementation of a lay patient navigator program for the underserved. A patient-centered satisfaction self-evaluation multi-scale survey (P-CS) and interview instruments were used to evaluate patients who accepted or refused navigation services, in four areas: demographics, reason(s) for refusing navigation, treatment satisfaction, and FACIT (Functional Assessment of Chronic Illness Therapy) analyzing quality of life/well being in social/family, physical, emotional and functional domains.

**Summary of Results:** 178 patients offered navigation were surveyed in the post treatment allotted period (1–6 months). 64 (36.6%) accepted and 114 (64.0%) declined navigation. Analysis of demographics revealed no significant differences (p ≥ .05) between the two groups. The top 3 reasons for declining navigation were: I am an independent person; I have a supportive family; I have supportive friend(s). P-CS satisfaction noted the was no significant different between the non-navigated patients and the navigated patients (p ≥ .05). This held true for ANOVA and categorical variables (X2) analysis. Overall P-CS satisfaction among navigated patients was 91.61% and 87.1% of patients choose to continue navigation post treatment. FACIT revealed no statistical difference in well being between the two groups.

**Conclusions:** The top three reasons given by patients who declined navigation, suggest that these patients are disadvantaged by pre-existing personal and external support systems supported by the statistically significant finding: “During my cancer treatment, I felt supported”. The equivalence of navigated to non-navigated patients in areas of P-CS satisfaction and FACIT, plus high patient satisfaction scores among navigated patients suggest a heretofore unreported augmentation in care enabled by navigation.

**71**

**NURSE PRACTITIONER SERVICES AT THE FOUNDRY: THREE-YEAR STUDY OF IMPACT ON HEALTH CARE COSTS**

L.W. Raymond, 1, D. Chenoweth, 2 N.L. Martin, 3 and J. Pankowski, 3

1Univ of North Carolina, Chapel Hill, Chapel Hill, NC; 2Chenoweth & Associates, New Bern, NC and 3Carolinas HealthCare System, Charlotte, NC.
**Purpose of Study:** Initial analysis of the impact on health care costs (HCC) of on-site Nurse Practitioner (NP) services showed favorable results, so both the client company and health care service organization wished to see if these savings would be sustained.

**Methods Used:** We measured the effects of the NP program on HCC in two ways. Method 1 compared actual HCC for 2005–2007 vs. projected HCC, the latter based on medical payments in 2002–2004, prior to the NP intervention. Method 2 was a micro-analytic comparison of the HCC of nine Major Diagnostic Categories (MDCs) responsible for 88.5% of all conditions treated by the NP from 7/05 to 12/06. As baseline HCC for these MDCs, the actual values from 2003 were used, adjusting upward by 26.7% as a three-year, composite medical inflation rate. Neither method claimed a credit for improved productivity due to possible reductions in medical absenteeism or other indirect effects. Workers Compensation HCC were also excluded from our analysis.

**Summary of Results:** The large differences in HCC savings and Return on Investment (Table) are partly due to the HCC of the 11.5% of MDCs not captured by Method 2 which are included by Method 1. In addition, Method 1 reflects HCC savings which may be due to the addition of a 24/7 Nurse Help Line and a Wellness Program. Studies by others indicated that on-site programs such as ours reduce medical absences and do not induce utilization.

**Conclusions:** This three-year analysis confirms our preliminary findings that an on-site NP has a favorable benefit-to-cost function. Longer-term analyses are needed to confirm these findings and assess possible impact of NP programs on HCC due to remediable risk factors for chronic diseases, on worksite illness and injury, and on productivity.

<table>
<thead>
<tr>
<th>Method</th>
<th>HCC Savings, $</th>
<th>Cost of NP Program</th>
<th>Return on Investment</th>
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<tbody>
<tr>
<td>1</td>
<td>1,089,466</td>
<td>124,750</td>
<td>8.7 to 1</td>
</tr>
<tr>
<td>2</td>
<td>244,455</td>
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**Purpose of Study:** To evaluate the comparability of several commonly used measures of acculturation in predicting contraceptive use by Latinas in an agricultural community.

**Methods Used:** A sub-sample of all (n = 326) premenopausal women not currently pregnant or attempting pregnancy was derived from a larger population-based study of farmworker families in Mendota, California. Data collected included reproductive history and current contraceptive practices, a formal acculturation scale (the ARSMA II-SV), place of birth, age at immigration, and length of residence in the United States. Logistic regression analyses were used to examine the effect of each measure on the odds of contraceptive use.

**Summary of Results:** All women completed the survey in Spanish. Mean participant age was 34.9 years (range 18–53) and 96.0% were born outside the US, primarily in Mexico and El Salvador. The average age at immigration was 22.1 years, and 70.0% of women scored as moderately to highly acculturated. Mexican birth (versus US birth) was positively correlated with contraceptive use (OR 3.83, 95% CI 1.10, 13.29), while Salvadoran birth was associated with a nonsignificant trend toward increased contraceptive use (OR 2.42, 95% CI 0.65, 8.98). Conversely, a high score on the Mexican-orientation subscale (MOS) of the ARSMA II-SV, place of birth, age at immigration, and length of residence in the United States. Logistic regression analyses were used to examine the effect of each measure on the odds of contraceptive use.

**Conclusions:** Among rural Latinas, the observed effect of acculturation on contraceptive use is highly dependent on the measure (or proxy) used to determine acculturation. Studies of reproductive health and acculturation should be cautious in extrapolating conclusions from specific measure-associated effects to a more general acculturative effect as each measure likely captures a different dimension of this complex phenomenon. In addition, the observation of independent and distinct effects of the MOS, ARSMA II, and linear ARSMA II scores suggests a powerful new method for improving understanding of the acculturative process and its effect on health-related behaviors.

**Purpose of Study:** To describe how medical students and residents disclose medical errors. Disclosing harmful errors to patients is recommended, but such disclosure appears to be uncommon. Little is known about how trainees approach these difficult conversations. Understanding how trainees would disclose hypothetical errors could help educators design programs to address this gap.

**Methods Used:** A survey of 488 students (MSII, MSIV) and 270 residents in internal medicine at two academic medical centers (U. of Washington, Washington U.). Surveys contained one of two scenarios depicting a serious error that varied by how apparent the error would be to the patient. The more apparent scenario involved a ten-fold overdose of insulin due to physician handwriting error. The less apparent scenario involved a cardiac arrest after a physician overlooked a high potassium level caused by a new medicine. Ten questions measured attitudes towards the errors and what respondents would disclose using scripted responses.

**Summary of Results:** Of 999 trainees surveyed, 76% (758) responded. Most (85%) agreed their scenario represented a serious error. A majority (78%) believed the doctor was very or extremely responsible, although nearly all (98%) would apologize, but trainees were split between a general expression of regret (52%) and an explicit apology (46%). Regarding discussing preventing error recurrences, 37% chose a general pledge to prevent recurrences, and 57% would discuss detailed plans.

**Conclusions:** Trainees vary widely in how they would disclose errors to patients. The approach to disclosure may be affected by how apparent the error is to the patient. As disclosure standards emerge, they may provide a framework for educational programs.
cancer than other ethnic groups. Despite this evidence, cancer screening utilization remains low. This intervention sought to promote cancer education and to increase awareness about community resources in Latinas living in Walla Walla, Washington.

**Methods Used:** I created an interactive educational session with five components: a breast self-exam (BSE) demonstration, a hands-on example of an abnormal lump using a breast model, a tutorial about mammograms and clinical breast exams, a pap smear demonstration using a pelvic model, and a HPV vaccine tutorial. This session attempted to address previously documented barriers to screening in Latinas including time, costs, transportation, fear of pain, fear of diagnosis/treatment, cultural beliefs about modesty, and cultural beliefs about preventative services. Pamphlets detailing local resources and BSE shower cards were also distributed.

**Summary of Results:** Two sessions were held for a total of approximately 70 Latinas at the Walla Walla Farm Labor Camp, a housing project for families involved in seasonal and migrant labor. Another session was held for 12 women at a YWCA support group. Attendees were responsive to conversations about health improvement and education about free services.

**Conclusions:** This project exposed Latinas living in Walla Walla to the breast and cervical health resources in their community and resulted in an increase in their knowledge of preventive screening and vaccines for these conditions.

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**75 THE EFFECT OF ETHNICITY ON LONG BONE FRACTURE ANALGESIA IN NATIVE AMERICAN PATIENTS AT REGIONAL CHILDREN’s HOSPITAL**

A.M. Greening, K. Gnauck, and B. Skipper. University of New Mexico School of Medicine, Albuquerque, NM.

**Purpose of Study:** The study objective was to determine whether pediatric Native American patients with isolated long bone fractures are as likely to receive adequate analgesia as non-Native Americans with similar fractures at a regional academic hospital in the Southwest.

**Methods Used:** A retrospective cohort study was conducted by chart review with 61 Native Americans and 121 non-Native American patients age 2 months to 15 years who were discharged from the pediatric emergency department (PED) or the pediatric urgent care (PUC) with a discharge diagnosis of long-bone fractures between June 2005 and May 2007. Insurance status, either Indian Health Service or exempt Medicaid, was used as a proxy for Native American ethnicity. Potential confounding variables: age, language, gender, need for fracture reduction, previous analgesia, fracture location, and site of treatment were abstracted. Differences in age, language, gender, pain score, and duration of analgesia at discharge were analyzed. Dose adequacy (mg/kg) and the likelihoods of receiving any analgesia or narcotic analgesia, both in the PED or PUC and at discharge, were also calculated. Univariate analysis was performed to assess potential confounding variables on the likelihood of receiving analgesia; multivariate analysis was performed to control for those variables shown to have an effect.

**Summary of Results:** Demographic data and pain scores did not differ significantly between the two groups. 61% of Native Americans received analgesia, as compared to 65% non-Native Americans (p = 0.53). Native Americans were as likely to receive narcotic analgesia (p = 0.24) and to receive an adequate dose of analgesia than non-Native Americans (p=0.24). Age, language, and gender were found to correlate with the likelihood of receiving analgesia. Pain score did not correlate with likelihood of receiving analgesia (p = 0.09).

**Conclusions:** This study is unique in assessing analgesia and pain scores in Native Americans in the emergency/acute care setting. We found no difference in the analgesia received by Native American pediatric patients compared with non Native American patients in this multi-ethnic southwest regional academic hospital. We conclude that Native American ethnicity did not affect quality of analgesia care in this setting.

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**76 FAMILY STRUCTURE AND THE TREATMENT OF CHILDHOOD ASTHMA**

A.Y. Chen1, and J. Escarce2. 1Childrens Hospital Los Angeles, Los Angeles, CA and 2UCLA, Los Angeles, CA.

**Purpose of Study:** To examine how family structure influences the treatment and control a child’s asthma using a nationally representative sample of US children.

**Methods Used:** Our data sources were the 1996–2003 Medical Expenditure Panel Survey (MEPS) and the 2003 National Survey of Children’s Health (NSCH). The study samples consisted of children 2–17 years of age with asthma who lived in single-mother or two-parent families. We assessed the effect of number of parents and number of other children in the household on office visits for asthma and use of asthma medications using negative binomial regression, and we assessed the effect of family structure on the severity of asthma symptoms using binary and ordinal logistic regression. Our regression models adjusted for sociodemographic characteristics, parental experience in child-rearing and in caring for an asthmatic child and, when appropriate, measures of children’s health status.

**Summary of Results:** Asthmatic children in single-mother families had fewer office visits for asthma and filled fewer prescriptions for controller medications than children with two parents. In addition, children living in families with three or more other children had fewer office visits and filled fewer prescriptions for reliever and controller medications than children living with no other children. Children from single-mother families had more health difficulties from asthma than children with two parents, and children living with two or more other children were more likely to have an asthma attack in the past 12 months than children living with no other children.

**Conclusions:** For children with asthma, living with a single mother and the presence of additional children in the household are associated with less treatment for asthma and worse asthma outcomes.

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**77 PNEUMONIA: PATIENT QUALITY OF CARE AT UCLA MEDICAL CENTER**

J. Wang, and J. Napolitano. David Geffen School of Medicine at UCLA, Los Angeles, CA.

**Purpose of Study:** The Infectious Diseases Society of America (IDSA) and the American Thoracic Society (ATS) jointly develop guidelines for the treatment of community-acquired pneumonia (CAP). These guidelines detail several core measures that assess the quality of care of CAP. The objective of this study was to determine the proportion of patients admitted to UCLA Medical Center for treatment of CAP who had a radiograph diagnostic of pneumonia during their stay. A lack of diagnostic radiographs may indicate that patients are being treated inappropriately with an overuse of antibiotics resulting in a poorer quality of care. This study also seeks to find a correlation between radiographic interpretation and subsequent treatment course.

**Methods Used:** The last 100 charts from 2006 with the discharging diagnosis of CAP from UCLA Medical Center were reviewed retrospectively. Each chart’s admitting radiograph was categorized as either “probable diagnosis of pneumonia,” “possible diagnosis of pneumonia,” or “no diagnosis of pneumonia” according to the impressions in the official radiology report. Days of antibiotics, adverse effects due to antibiotics, and length of stay in hospital were also recorded.
**Western Regional Meeting Abstracts**

**Concurrent Session**
8:30 AM Friday, February 1, 2008

**Hematology and Oncology I**

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**78 ROLE OF RAL GTPASES IN RAS-MEDIATED CANCER METASTASIS**

J. L. Zhou1,2, M. Obers3, and K. Kelly2, 1UCLA, Los Angeles, CA and 2National Cancer Institute, Bethesda, MD.

**Purpose of Study:** Thirty percent of all human cancers contain activating Ras mutations, and that percentage rises to approximately 50% in metastatic cancers. Furthermore, Ras is mutated at a later stage in the progression of certain types of cancer, such as colorectal carcinoma, indicating its role in tumor progression and metastasis in these tumors. Studies in a variety of human cell lines have indicated that the Ral GTPase pathway is the most transforming pathway among the Ras-mediated metastasis in animal models. We are currently investigating the role of Ral GTPases in cancer metastasis using human prostate (LnCap C4-2, PC3, DU145) and colon cancer cell lines (HT29, SW620) in a xenograft model.

**Methods Used:** The cancer cells, with either the Ral pathway activated or knocked down in the intra-cardiac injection model, were injected into the left cardiac ventricle or the spleen of athymic nude mice. Metastatic tumor burden was analyzed by bioluminescence imaging. X-ray, and histology.

**Summary of Results:** Results show that when prostate cancer cells were inoculated via intra-cardiac injection, activating the RalGEP pathway, but not the Raf/MAPK pathway, promoted metastasis to bone, while down-regulating Ral GTPases inhibited metastasis to bone. It’s also important to notice that differences between cancer cell lines do exist. Knocking down either Ral A GTPase or Ral B GTPase alone inhibited bone metastasis in DU145/G37 cells. However, in PC3 cells, the same inhibitory effect was only observed in Ral A knockdown, but not in Ral B knockdown cells. Interestingly, results from HT29 colon cancer cell line show no obvious effects on metastasis when Ral A is knocked down in the intra-cardiac injection model.

**Conclusions:** We have shown that RalGEF pathway plays an important role in Ras-mediated metastasis in certain types of human cancer, while other cancers may utilize different downstream pathways of Ras to mediate metastasis. A broader survey of cancer types and metastatic properties will be needed to dissect the role of the Ral pathway in cancer metastasis.

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**79 EXPRESSION AND FUNCTION OF ERYTHROPOIETIN AND VASCULAR ENDOTHELIAL GROWTH FACTOR INCREASED IN OXYGEN INDUCED RETINOPATHY**

S. Patel1,2, H. Chen3,4, N. London3, Z. Tong2,3, Z. Yang2,3, D. Li2, and K. Zhang3,4, 1University of Utah, Salt Lake City, UT; 2University of Utah, Salt Lake City, UT and 3University of Utah, Salt Lake City, UT.

**Purpose of Study:** Retinopathy of prematurity (ROP) is a prevalent morbidity among preterm infants and is characterized by retinal neovascularization. Recent research in diabetic retinopathy, thought to have similar pathogenesis to ROP, has shown that erythropoietin (Epo) and vascular endothelial growth factor (VEGF) are involved in neovascular angiogenesis. It is not known whether and how these growth factors are involved in ROP. We hypothesized that Epo and VEGF mRNA expression is up-regulated in oxygen induced retinopathy (OIR) and these proteins are involved in endothelial cell proliferation, migration and permeability.

**Methods Used:** After exposure to hyperoxic conditioning, murine vitreous samples were obtained at postnatal day 17, retina isolated and RNA extracted for Epo and VEGF mRNA determination. This procedure was repeated in control mice. Migration and proliferation assays were performed according to manufacturer’s instruction (Promocell, Germany; Cambrex, Walkersville) on in vivo endothelial cells using increasing concentrations of Epo and VEGF. Cell growth and migration was measured manually and using Cell Counting Kit 8 (Dojindo Molecular Technologies, Gaithersburg). Finally, Epo was injected into the vitreous of murine retina, followed by intravenous injection of Evan’s blue dye 6 hours later. Retinal vascular permeability was assessed by measuring Evan’s blue leakage into vitreous cavity.

**Summary of Results:** Both Epo and VEGF mRNA levels were significantly greater in hyperoxia exposed mice (p<0.001,n=4). However, Epo levels were significantly higher than VEGF levels (15-fold increase vs. 2.5 fold increase, p<0.05). In addition, both Epo and VEGF stimulated endothelial cell migration and proliferation in a dose dependent manner (p<0.05,n=4). Finally, intravitreal Epo injection increased retinal vascular permeability (p<0.05,n=4). Howev-, both proteins stimulate endothelial cell proliferation and migration. Like VEGF, Epo increases endothelial cell permeability. We speculate that Epo and VEGF play an important role in OIR.

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**80 SMALL MOLECULE THERAPY: OVERCOMING IMATINIB RESISTANCE IN GASTROINTESTINAL STROMAL TUMORS**

D. Kurtzman, and D. Mahadevan, University of Arizona, Tucson, AZ.

**Purpose of Study:** Gastrointestinal stromal tumors (GIST) are rare, metastatic tumors of the gastrointestinal tract that have responded somewhat successfully to small molecule therapy, particularly with the KIT selective tyrosine kinase inhibitor imatinib mesylate. However, patients are relapsing and developing imatinib resistance with poor prognoses. As the number of imatinib-failed patients increases, alternate effective therapies are required. Recent work suggests a kinase switch governs the progression to imatinib insensitivity. Out of such data, a novel, multiple tyrosine kinase inhibitor (TKI), MP470, has emerged and its efficacy has been established in vitro. Here we describe the combination of imatinib with MP470 as an effective modality to elicit complete GIST abrogation in vitro.

**Methods Used:** The cell line GIST 882 was seeded in 96 well plates at a density of 5000 cells/well. IC50 values for imatinib and MP470 were...
established separately through monotherapy. Combination assays were performed at one molecule’s IC50 concentration while varying the second molecule’s concentration, and vice versa. Cell viability was determined four days after initial drug administration by MTS assay. Experimental work was conducted in sextuplets twice.

Summary of Results: The IC50 values of imatinib and MP470 were 0.1µM and 10µM respectively. Combining the molecules reduced their IC50 values significantly suggesting these molecules act synergistically: imatinib was reduced to less than 0.001µM and MP470 was reduced to 2µM. A combination of 8µM imatinib with 2µM MP470 or a combination of 0.1µM imatinib with 10µM MP470 were sufficient to elicit a complete response, or 0% GIST viability. It is important to note that each drug was administered within its clinically-relevant dose.

Conclusions: Such data demonstrates the effectiveness of combining the two small molecules. Herein lies the potential to reach a complete response, whereas monotherapy of either small molecule is inadequate. Furthermore, achieving a complete response may prevent disease relapse and substantially prolong patient survival.

81 IDENTIFICATION OF THE PROTEIN BINDING PARTNERS OF THE H37 TUMOR SUPPRESSOR LOCATED AT THE MOST FREQUENTLY DELETED CHROMOSOMAL REGION IN LUNG CANCER

I. Giuroiu1, C. Washington3, B. Doctor2, I. Delgado3, A. Koegel1, J. Oh1, and D. Slamon1. David Geffen School of Medicine at UCLA, Los Angeles, CA; 2University of California, San Diego, School of Medicine, San Diego, CA; and 3University of California, Los Angeles, Los Angeles, CA.

Purpose of Study: Lung cancer is the leading cause of cancer death in the U.S. Thorough knowledge of the molecular pathogenesis of this malignancy is pivotal for developing novel diagnostics/therapeutics. Chromosomal deletion at 3p21.3 is the earliest and most frequent genetic alteration observed in lung cancer, occurring in ~80% of all lung cancer types and even in the normal epithelia of smokers’ lungs. Therefore, the putative tumor suppressor gene(s) (TSGs) contained in this region are particularly promising for lung cancer clinical management. One of the 19 genes residing at 3p21.3, H37 has manifested prominent TSG characteristics: 1) Decreased expression of H37 mRNA/protein in ~75% of the primary lung tumors compared with adjacent normal lung epithelia; 2) In vitro and in vivo growth inhibition of lung cancer cells induced by H37; and 3) A TS mechanism that involves G1/S arrest and apoptosis. Its exact cellular functions, however, remain yet to be uncovered for the long-term development of novel H37 gene-based cancer therapies.

Methods Used: Yeast two-hybrid screening was performed to find H37’s protein binding partners.

Summary of Results: Presently, the interaction of 9 proteins with H37 has been verified in yeast cells, and their identity was revealed by sequencing. Thus far, Western blotting confirmed the expression of Insulin Receptor Substrate of 53 kD and Transducer of Regulated CREB 1 in various lung cancer cell lines.

Conclusions: Future validation of H37’s true biologic protein partners is expected to help map out the H37-mediated tumor suppressor pathway and streamline the most effective clinical strategies based on H37’s tumor suppressor activity.

82 CANCER STEM CELL MARKER EXPRESSION IN HUMAN MELANOMA CELL LINES

M. Chiu, R. Koya, B. Comin-Aoduixi, S. Mok, J. Jallil, H. Sazevar, and A. Ribas. UCLA, Los Angeles, CA.

Purpose of Study: Many cancers are derived from a single cell that has self-renewal capability and has undergone frequent genetic mutations - a cancer stem cell. These stem cells generate malignant daughter cells that proliferate uncontrollably, ultimately invading and destroying normal tissues. Cancer stem cells and their surface marker profiles have been described in tumors of the breast, brain, and prostate, as well as in hematologic malignancies. Alternatively, emerging data suggest that some cancers are sustained not by rare cancer stem cells, but rather by a more homogeneous cell population that consists of the majority of cancer cells.

The purpose of this study was to analyze melanoma cell lines for expression of cancer stem cell markers previously identified in other malignancies.

Methods Used: Twenty-two melanoma cell lines derived from biopsy samples taken from patients with metastatic melanoma were cultured in standard growth medium and analyzed for expression of ten cancer stem cell markers by flow cytometry. In addition, melanoma cell lines were cultured in mouse embryonic fibroblast-conditioned embryonic stem cell (MEF-cESC) medium to enrich for potential cancer stem cells. Cell lines that displayed morphological changes in MEF-cESC medium were analyzed for cancer stem cell marker expression.

Summary of Results: Our data indicate high expression of several cancer stem cell markers, including CD44, CD71, CD105 and CD166, in the majority of melanoma cell lines cultured in standard growth medium. Furthermore, six out of 22 cell lines treated with MEF-cESC medium generated non-adherent, spheroid cells consistent with cancer stem cell morphology. Analysis of these cells showed no difference in cancer stem cell marker expression when compared with adherent melanoma cells in standard growth medium.

Conclusions: Human melanoma cell lines demonstrated high expression levels of several cancer stem cell markers, including CD44, CD71 (TFR), CD105 (ENG) and CD166 (ALCAM). Positively-staining cells encompassed a large proportion of the cell population (~30%), rather than a small subset of cells. These data support the hypothesis that melanoma cell lines are maintained by a relatively homogeneous population of cells rather than a small subset of cancer stem cells.

83 EVALUATING ANTI-HLA FLUORESCENCE IN QUANTIFYING HUMAN TUMOR BURDEN IN A NUDE MOUSE MODEL

J.M. Moretz, S. Baldwin, Y. Amaar, and M. Reeves. Loma Linda University, Loma Linda, CA.

Purpose of Study: Preclinical nude mouse models are often used to assess the efficacy of new therapies on human cancer in an attempt to decrease cancer mortality in humans. We evaluated laser infrared (IR) as a potential imaging modality for the detection of liver metastasis in nude mice.

Methods Used: A “hemispleen” model was utilized for injections as described by Hansen et al. Male nude mice were obtained at 9 weeks old from Taconic (Hudson, NY). The mice were anesthetized using 1.75% isoflurane and the surgical area was prepped using povidone iodine. Incisions were made in the left flank and the spleen was mobilized. The inferior hemispleen was tucked into the peritoneal cavity. The spleen was then sutured using 5-0 Prolene. The nude mice received 1.5 L of anti-HLA fluorescently labeled antibody (Sigma Corporation) per load subcutaneous injections of Buprenex and were monitored until ambulatory.

The human pancreatic cancer cell line PL45 was cultured in DMEM containing 10% calf bovine serum, all obtained from the ATCC (Manassas, VA). 50 µL of anti-HLA fluorescently labeled antibody (Sigma Corporation) was injected retro-orbitally into the cavernous venous plexus.
The nude mice were imaged with anti-HLA Antibody using a LI-COR Odyssey Model 9120 Laser Infrared Scanner (Lincoln, NE).

**Summary of Results**: Direct comparison between the image obtained by the Odyssey and actual tumor burden of the mouse was done. At 8 hours after antibody injection, the liver tumor burden is clearly visualized with minimal background. There is excellent correlation between actual tumor burden and tumor detected by imaging. A small area of local recurrence on the left side was not detected on imaging. This is most likely due to the fibrotic and avascular nature of the local recurrence.

**Conclusions**: Laser infrared imaging is an easy, comparatively inexpensive method for detection of tumor in vivo. Use of this technique can allow monitoring of viable tumor burden without the need for sacrificing the animal so long as an antibody specific for the tumor relative to the animal it is in is available.

### 84
**INDUCTION OF CXCR4 EXPRESSION BY NF-KappaB IN PROSTATE CANCER CELLS IS INHIBITED BY POMEGRANATE UROLITHIN A**

D. Pandya, A. Wong, K. Hong, and D. Heber. David Geffen School of Medicine at UCLA, Los Angeles, CA.

**Purpose of Study**: Prostate cancer is the second leading cause of cancer death among American males today. Mortality associated with prostate cancer stems from metastatic disease and has been shown to be dependent on activation of CXCR4/CXCL12 chemokine axis. Induction of CXCR4 is found in aggressive tumor phenotype, which is also frequently associated with intracellular NF-kappaB activation. Whether CXCR4 expression is directly regulated by the NF-kappaB pathway is not clear. Whether induction of CXCR4 is found in aggressive tumor phenotype, which is also dependent on activation of CXCR4/CXCL12 chemokine axis. Induction of CXCR4 is found in aggressive tumor phenotype, which is also frequently associated with intracellular NF-kappaB activation.

**Methods Used**: Prostate cancer cell lines LNCaP, LNCaP-AR, DU-145, PC-3 and 22Rv1 were treated with TNF-alpha to induce the NF-kappaB pathway. Differential changes in protein levels of IKK-alpha were examined as inverse surrogate marker for NF-kappaB activation. Changes were examined by western blot, quantitative real time RT-PCR and transfected luciferase construct.

**Summary of Results**: TNF-alpha treatment led to rapid activation of NF-kappaB pathway in LNCaP, DU-145 and PC-3 cell lines as indicated by IkB-alpha protein degradation and increased NF-kappaB-induced luciferase activity. Corresponding time-dependent NF-kappaB activation was observed and confirmed by IkB-alpha protein degradation and increased NF-kappaB-induced luciferase activity. Corresponding time-dependent NF-kappaB activation led to induction of CXCR4 expression, and that UroA may exert its anti-tumor effects through inhibition of this process.

**Methods Used**: Prostate cancer cell lines LNCaP, LNCaP-AR, DU-145, PC-3 and 22Rv1 were treated with TNF-alpha to induce the NF-kappaB pathway. Differential changes in protein levels of IKK-alpha were examined as inverse surrogate marker for NF-kappaB activation. Changes were examined by western blot, quantitative real time RT-PCR and transfected luciferase construct.

**Summary of Results**: TNF-alpha treatment led to rapid activation of NF-kappaB pathway in LNCaP, DU-145 and PC-3 cell lines as indicated by IkB-alpha protein degradation and increased NF-kappaB-induced luciferase activity. Corresponding time-dependent NF-kappaB activation may induce CXCR4 expression (both gene and protein) in LNCaP, DU-145 and PC-3 cells. Pre-treatment with UroA prior to TNF-alpha inhibited NF-kappaB activation which may abrogate CXCR4 induction.

**Conclusions**: Our results suggest that NF-kappaB activation by TNF-alpha may be associated with downstream CXCR4 expression. Urolithin A in pomegranate may exert its anti-tumor and anti-inflammatory effect through inhibition of this pathway, thus demonstrating promise as a prostate cancer protective agent.

### 85
**EVALUATION OF CLINICAL PREDICTORS AND OUTCOMES IN ABANDONED VERSUS COMPLETED RADICAL HYSTERECTOMY FOR CERVIX CANCER**

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**Purpose of Study**: Approximately 8–10% percent of patients with early stage cervix cancer are found intraoperatively to have disseminated disease, and the planned hysterectomy is abandoned. The aim of this study is to evaluate preoperative presentation and outcomes in these patients.

**Methods Used**: A review of records of early stage cervical cancer patients evaluated at the University of Washington between 1993 and 2003 identified 268 women who presented for primary surgical management of invasive cervical cancer. The abandoned group consisted of nineteen patients (7%) whose planned radical hysterectomy was aborted for treatment with primary radiotherapy. Comparison was made to a group of 44 patients who received adjuvant radiation for high risk features following completed hysterectomy.

**Summary of Results**: At time of surgery, reasons for abandonment were grossly positive lymph nodes (84%) or pelvic spread of tumor (16%). Preoperatively, 16% of the abandoned group were diagnosed following an abnormal Pap while 68% presented primarily with vaginal bleeding. Only half of patients who underwent CT had findings suspicious for pelvic lymphadenopathy. In comparison to the completed group, there was no difference in age, stage or histology distribution. Major complications were less frequent in the abandoned group (16 v. 32%). Six patients recurred in the abandoned group with a median time to recurrence of 25 months, compared to eight patients following completed hysterectomy (32 v. 18%). At the time of study conclusion, progression free survival was 47 versus 50% and overall survival was 80 versus 73%.

**Conclusions**: Women who are at risk for abandoned radical hysterectomy for early invasive cervical cancer have few preoperative clinical predictors. Abandoning radical surgery for primary chemoradiation does not appear to worsen outcome, and may be associated with decreased morbidity.

### 86
**DEFINING THE INCIDENCE AND CHARACTERISTICS OF PICC-INDUCED UPPER EXTREMITY DEEP VEIN THROMBOSIS IN 6,513 UCLA PATIENTS**

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**Purpose of Study**: In this large retrospective study, we define the characteristics of patients with PICC-related upper-extremity deep vein thrombosis (UEDVT).

**Methods Used**: Between January 2003 and December 2006, 4,223 patients underwent placement of 6,513 PICCs at UCLA. Patient charts of those with ultrasound diagnosis of UEDVT soon after PICC placement were analyzed.

**Summary of Results**: 189 patients were determined to have PICC-induced UEDVT. Among those, the most common primary indications for PICC placements were: IV access (35.9%), antibiotics (28.2%), chemotherapy infusion (17.4%), and total parenteral nutrition (9.3%). Predisposing risk factors were: coagulopathy (13.2%), specific organ-related disease (13.2%), malignancy (10.7%), cardiac disease (10.1%), and vasculopathy (9.5%). 42.3% had one PICC placement over the four-year period, 27.5% had two placements, 15.3% had three placements, and 14.8% had more than three. Recurrent DVT occurred as a post-UEDVT complication in 8%, pulmonary embolism in 6.3%, infection in 3.2%, mortality in 1% and other minor complications in 19%.

**Conclusions**: In the largest PICC-induced UEDVT study to our knowledge, we report an overall incidence of 3.16%. We found a striking four-fold increase in risk for patients who received multiple PICCs (10.4%) versus those with only one PICC placement (2.5%). We detected a slightly higher rate of PICC-induced UEDVT in lines placed by MDs (4.2%) versus nurses (3.0%), probably due to the more complicated cases performed in the radiology suite. We report the overall risk of a complication from PICC placement as 1.1%, with recurrent DVT in...
0.23%, pulmonary embolism in 0.18%, infection in 0.09%, and mortality in 0.03%.

87 LONGITUDINAL VOLUME ANALYSIS FROM COMPUTED TOMOGRAPHY: REPRODUCIBILITY USING THE ADRENAL GLANDS AS SURROGATE TUMORS
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Purpose of Study: To determine the precision (reproducibility) of volume assessment in routine clinical computed tomography (CT) using the adrenal glands as surrogate tumors.

Methods Used: Seven patients at our institution were identified retrospectively as having received many abdominal CT scans (average 13.1, range 4 to 20). Typical CT section thickness was 5 mm, with some scans having up to 10 mm section thickness. The adrenal glands were used as surrogate tumors, assuming no actual volume change. The volumes of both the left and right adrenal glands were assessed by hand segmentation for each patient and each scan. The reproducibility, expressed as the coefficient of variation (COV = standard deviation / mean), was used to characterize measurement imprecision.

Summary of Results: The average volumes were 5.93 and 4.55 cm³ for the left and right adrenals, respectively, with COVs of 18.7% and 21.2%, respectively. With this degree of imprecision and using one patient's data (20 scans) as an example, it was calculated that a 13% change in volume could be determined with statistical significance at p=0.05, and a 17.8% change in volume could be determined at a significance of p=0.01.

Conclusions: The imprecision of volume determination was strongly dependent on total volume. Given the small dimensions of the adrenal glands, the ~20% COV is likely to be a high estimate compared to larger tumors. Modern CT scanners working with thinner sections (i.e. <1 mm) would likely produce better measurement precision.

88 A CASE-CONTROL PROTEOMIC BREAST CANCER STUDY IN BLACK WOMEN
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Purpose of Study: Black women are more likely to be diagnosed with breast cancer at a younger age and with more aggressive form of the disease than White women. This study’s purpose is to describe the baseline characteristics of Black women who participated in an earlier proteomic biomarker study and to evaluate the association between incidence of breast cancer and Body Mass Index (BMI) controlling for characteristic risk factors including age, parity, age at first delivery, age at menarche, menopausal status, exercise, diet, family history, breastfeeding, smoking and alcohol use.

Methods Used: A case-control study consisting of 50 Black participants; 21 breast cancer cases and 29 controls was performed. Each participant provided a blood sample for proteomic analysis and completed a health questionnaire. A logistic regression model was developed that initially contained BMI and age. Additional variables listed above were retained if they changed the odds ratio (OR) for BMI by more than 10%.

Summary of Results: The age-adjusted OR for BMI was 0.83 (95% CI: 0.73–0.95). Controlling for menopausal status did not change the OR for BMI or associated confidence interval. Alcohol intake was associated with an increased OR of 1.55 (0.50–4.14). Dairy use and early menarche were also associated with increased ORs; ORs for moderate and frequent dairy use were 1.51 (0.26–8.86) and 3.10 (0.32–30.71) respectively. Age at menarche between ages 9–10 was associated with an OR of 4.02 (0.21–75.91). A vegetarian lifestyle, regular exercise and parity were associated with decreased ORs of 0.29 (0.04–2.26), 0.46 (0.11–1.97) and 0.66 (0.09–5.00) respectively.

Conclusions: BMI appears to be slightly protective against the development of breast cancer in this Black population regardless of age or menopausal status. Alcohol use, nulliparity, early menarche and eating a diet high in fatty foods such as diary products may increase an individual’s odds of developing breast cancer. Late menarche, regular exercise, having children and eating a vegetarian diet may represent factors that protect against developing breast cancer. Further research and increased sample size are needed in order to establish these variables as effect modifiers or confounders in breast cancer research. Funded by The Susan G. Komen Breast Cancer Foundation.

89 PRECORE MUTANT HEPATITIS B REACTIVATION FOLLOWING R-CHOP CHEMOTHERAPY: A CASE REPORT
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Purpose of Study: To identify chemotherapy as a risk for fulminant hepatitis in hepatitis B (HBV) carriers and to examine the evidence for prophylactic antivirals in HBV infected patients receiving chemotherapy.

Methods Used: Case report and literature review.

Summary of Results: A 75 year old HBV positive Vietnamese woman was diagnosed with Stage IVB Diffuse Large B Cell Lymphoma. Prior to chemotherapy, the patient was HBsAg and HBeAb positive and HBsAb negative. PCR for HBV was negative. For antiviral prophylaxis, lamivudine was started prior to receiving six cycles of R-CHOP. Restaging PET/CT showed no evidence of disease. One month following chemotherapy lamivudine was discontinued. One month later, the patient developed epigastric pain. Two months later, the patient presented with abdominal pain, nausea, anorexia and jaundice. Total bilirubin was 4.3, AST 3267, ALT 1501 and alkaline phosphatase 215. Her symptoms worsened and three days later total bilirubin was 12.1, AST 5181, ALT 2460 and alkaline phosphatase 247. She was admitted for reactivation viral hepatitis. Lamivudine was resumed and supportive treatment initiated. PCR for HBV was greater than 38,000,000 IU/mL of HBV genotype B containing the precore mutation G1896A. The hepatitis worsened despite lamivudine and the patient developed severe coagulopathy with fulminant hepatic failure. The patient became obtunded and died. Autopsy was declined.

Conclusions: This case represents a fatal example of reactivation of HBV after chemotherapy with R-CHOP. The optimal length of antiviral...
prophylaxis after chemotherapy is unknown. Current practice is for prophylaxis in patients with HBV infection or risk factors prior to and throughout chemotherapy. The literature demonstrates HBV reactivation in 20–50% of HBsAg positive patients following chemotherapy. Rituximab, steroids, positive HBsAg or HBeAg, high viral titer and male gender are risk factors. Prophylaxis reduces the incidence of reactivation by five to ten-fold if continued after chemotherapy. There is no consensus on the duration of therapy. Further studies are required to optimize the length of antiviral prophylaxis. The role of precore mutation in HBV reactivation also merits future consideration.

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PHYSICIAN COMMUNICATION SKILLS IN DISCUSSING TRANSITIONS TO END-OF-LIFE CARE: BIOMEDICAL VS. MEANING-MAKING PARADIGMS

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Purpose of Study: Physician communication skills are fundamental to providing high quality palliative care. This qualitative study identified patterns of communication in physician discussions with patients about transitions from palliative anticancer therapy to end-of-life care.

Methods Used: Audiotaped interviews between physicians and standardized patients were transcribed and coded based on the initial theory-driven code list that was grounded in a conceptual framework. Researchers collaborated to perform a componential analysis that identified patterns, variations, and conceptual networks used by physicians in these interviews.

Summary of Results: Analysis identified two paradigms that shape the patterns of communication. Physicians working from the “death as a scientific problem” paradigm lectured the patient, emphasizing biomedical logic in explanations of disease progression and presenting options for the patient (usually more chemotherapy versus no chemotherapy). These physicians were less likely to respond to patient emotions by empathizing and exploring. Physicians working from the “paradigm making meaning” paradigm engaged in a dialogue with the patient and presented the transition as a shift in focus. They took a holistic view of the patient’s needs while introducing palliative care and hospice and made an effort to explore the patient’s concerns and feelings about death and dying. These physicians were more likely to respond to patient emotions by empathizing and exploring.

Conclusions: Most physicians operate from some combination of the two paradigms, but often draw from one more than the other. The paradigm lectured the patient, emphasizing biomedical logic in explanations of disease progression and presented the transition as a shift in focus. They took a holistic view of the patient’s needs while introducing palliative care and hospice and made an effort to explore the patient’s concerns and feelings about death and dying. These physicians were more likely to respond to patient emotions by empathizing and exploring.

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EXENATIDE SENSITIZES INSULIN-MEDIATED GLUCOSE UPTAKE AT SKELETAL MUSCLE AND LIVER


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Purpose of Study: Exenatide (EX) is a long-acting mimetic of GLP-1 (glucagon-like peptide 1) which in addition to its currently recognized actions (↑ insulin, ↓glucagon, ↓IGF II ratio), may mediate a glucose-lowering effect independent of increased β-cell response. The aim of our study was to elucidate whether EX stimulates insulin-independent glucose uptake directly or if it potentiates insulin-mediated glucose uptake.

Methods Used: First, we performed a modified hyperinsulinemic euglycemic clamp in dogs: EX (20 μg/kg) or saline (SAL) were administered at t = 0; glucose was infused intraportally to co-stimulate the putative portal sensor together with EX. Peripheral insulin was raised to 250 pm, totally suppressing endogenous glucose production (EGP). Total intraportal infusion (PoGinf) was therefore equal to the sum of peripheral glucose disposal (Rd) and first-pass hepatic glucose uptake (HGU). It is known that an increase in glycemia induces a higher glucose disposal at the same insulinemia through glucose transporters recruited by insulin. We hypothesized that if EX enhanced glucose uptake by sensitizing insulin action its effect would be amplified at higher glycemia. A hyperinsulinemic hyperglycemic clamp was then performed with intraportal glucose infusion to raise peripheral glycemia to 150 mg/dL. A third experiment was performed to confirm that high insulin was necessary for EX-enhanced glucose uptake.

Summary of Results: At euglycemia, EX raised the PoGinf by 18% (13.2 ± 1.9 vs. 15.6 ± 2.1, p < 0.01; all units in mg/kg/min), due to an 11% increase in Rd (13.1 ± 2.4 vs. 14.5 ± 2.4, p < 0.05) and a 50% increase in HGU (1.1 ± 0.1 vs. 1.7 ± 0.5, p = 0.12). At hyperglycemia, EX enhanced PoGinf by 31% (16.3 ± 1.9 vs. 21.3 ± 2.7, p < 0.001), while Rd increased by 20% (16.1 ± 1.9 vs. 19.3 ± 2.5, p < 0.05) and HGU doubled (1.2 ± 0.3 vs. 2.4 ± 0.6, p = 0.06). Without hyperinsulinemia, despite elevated glucose, EX did not cause greater PoGinf (2.2 ± 0.2 SAL vs. 1.9 ± 0.3 EX). Rd increased slightly and similarly and EGP was similarly suppressed with or without EX.

Conclusions: We conclude that there exists a systemic glucose-lowering effect of EX as a result of the sensitization of insulin-mediated glucose uptake at skeletal muscle and liver. Therefore, along with its other known actions, EX represents an acute insulin sensitizer, possibly rescuing the impaired insulin sensitivity in type 2 diabetes.

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PARTIAL KNOCK-DOWN OF P85α IN VIVO WITH ANTI-SENSE OLIGONUCLEOTIDE IMPROVES INSULIN SENSITIVITY IN OB/OB AND CONTROL MICE

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Purpose of Study: Insulin-stimulation of the insulin receptor substrate 1 (IRS-1)–phosphoinositide-3 kinase (PI3K) pathway in skeletal muscle results in many metabolic changes including glucose uptake. Results from mouse and human studies show that increased levels of p85α, the regulatory subunit of PI3K, are associated with decreased insulin sensitivity (IS), whereas a reduction in p85α expression is associated with greater IS. The object of this study was to: 1. Attempt in vivo knock-down of p85α with anti-sense oligonucleotides (ASO) in normal (C57/B16) mice and, 2. Assess whether p85α knock-down is associated with improvement in IS in insulin-resistant ob/ob mice.
Methods Used: Study 1: Three different ASOs (#2–4, received from ISIS Pharmaceuticals) were tested in C57/B16 mice. Mice were injected with either one of the ASOs or saline twice weekly at a dose of 100 µg/g/dose for 5 doses. Insulin tolerance test (ITT) was used to assess IS before and after ASO treatment. Western blot analysis was used to assess p85α knockdown (KD) in muscle and liver. Results from the ITT and Western blots identified ASO#4 as the most effective at targeted KD of p85α. Study 2: To identify whether ASO can rescue insulin sensitivity, we injected ASO#4 and a control ASO into ob/ob mice (10 mice/group, 5 doses, 35 µg/g/dose). ITT was used to assess IS before and after injections.

Summary of Results: Study 1: There were no significant differences in IS between the C57/B16 groups at baseline. After ASO injections, mice injected with ASO#4 had improved IS by ITT compared with PBS-injected mice. They also had decreased expression of p85α in skeletal muscle. Study 2: No significant differences were found in IS at baseline between the ob/ob ASO and control groups. At the end of the injection period, the ASO group was more insulin sensitive vs. controls, with significantly lower blood glucose levels at 30 and 60 minutes of ITT (p < 0.05).

Conclusions: Skeletal muscle knock-down can be achieved with p85α ASO injection. Furthermore, this knock-down is associated with improvement in insulin sensitivity in both normal and ob/ob mice. Thus, p85α could be an important therapeutic target in attempt to ameliorate insulin resistance.

93 VISFATIN: A GENETIC DETERMINANT OF INSULIN SENSITIVITY
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Purpose of Study: Adipose tissue is no longer considered a simple depot for triglyceride storage. On the contrary, adipose tissue secretes a variety of adipokines into the circulation that can influence insulin sensitivity in distant sites as well as in adipose tissue itself. Adipokines have increasingly been implicated as mediators of obesity-related insulin resistance and the increased risk of type 2 diabetes associated with obesity. Visfatin, also known as pre-B cell colony-enhancing factor (PBEF1), is highly enriched in visceral fat of mice and humans; circulating visfatin levels are increased in obesity and correlate with measures of visceral adiposity. However, its role in human obesity and insulin resistance remains unknown. In this study, we sought to determine whether visfatin is a genetic determinant of metabolic traits.

Methods Used: We genotyped 5 single nucleotide polymorphisms (SNPs) in PBEF1 in 975 Mexican Americans from 190 families. These SNPs were selected using HapMap data to tag the majority of the variation (SNPs) in PBEF1. Genotyping was performed using the Sequenom MassARRAY (Sequenom, San Diego, CA) at the University of Arizona. In addition, we genotyped 10 single nucleotide polymorphisms (SNPs) in the proinsulin gene (INS) in 450 Mexican Americans from 200 nuclear families. These SNPs were selected using HapMap data to tag the majority of the variation (SNPs) in the proinsulin gene. Genotyping was performed using the Sequenom MassARRAY (Sequenom, San Diego, CA) at the University of Arizona.

Summary of Results: We performed association analyses for each SNP in the PBEF1 and INS genes. We conducted these analyses in the 975 Mexican American trios and in the 450 Mexican American trios. We found that the third SNP was associated with lower visfatin expression in THP-1 cells (3 fold increase at 15 mM vs 5 mM, p < 0.05).

Conclusions: Visfatin is a genetic determinant of metabolic traits. Visfatin has been shown to influence insulin sensitivity in vitro and in vivo. This study provides further evidence that visfatin is a genetic determinant of metabolic traits.

94 TOLL LIKE RECEPTORS 2 AND 4 EXPRESSION UNDER HYPERGLYCEMIA: FURTHER EVIDENCE OF INFLAMMATION IN DIABETES

Purpose of Study: Inflammation is pivotal in diabetes and monocytes (Mo) are crucial in orchestrating these effects. Toll like receptors (TLRs) contribute to the signal transduction induced by endogenous damage signals generated at sites of inflammation. Hyperglycemia contributes to vascular inflammation of diabetes complications. Among the TLRs, TLR2 and TLR4 play important role in atherosclerosis. However, there is a paucity of data examining the role of TLRs in hyperglycemic conditions. Thus, in the present study, we examined the expression of TLR2 and TLR4 expression in mononuclear cells under high glucose conditions.

Methods Used: For in vitro studies, THP-1 cells were stimulated with glucose (5, 15, 25 mM) or 14.5 mM mannitol for 24hrs, washed and surface labeled with TLR2 & TLR4 antibodies with suitable IgG isotype controls and analyzed using BD FACS array. To further establish the stimulation of TLR2 and TLR4 levels in high glucose (HG) conditions, down stream signaling molecules of TLR activation i.e. MyD88 protein expression and IRAK-1 protein phosphorylation were determined by Western blot analysis.

Summary of Results: HG treatment activated TLR2 and TLR4 expression in THP-1 cells (3 fold at 15 mM vs 5 mM, p < 0.05). MyD88 protein expression and IRAK-1 phosphorylation, and NF-κB p65 dependent activation increased under HG conditions dose dependently compared to low glucose. Inhibition of TLR2 and TLR4 expression using gene specific siRNAs abrogated HG induced IL-1B, IL-6, TNF-α, MCP-1 expression and NF-κB activation. In vitro TLR2 and TLR4 expressions were further examined using monocytes isolated from age and gender matched normal volunteers and Type 2 diabetic patients (n = 6). TLR2 and TLR4 expression is increased in T2D patients compared to controls (2 fold, P < 0.05) corroborating in vitro data.

Conclusions: Thus, we provide first evidence that in human Mo, hyperglycemia significantly activates TLR2 and TLR4 expression both in vitro and in vivo.

95 LACK OF AMYLOIDOGENICITY OF PIG ISLET AMYLloid POLYPEPTIDE MAY CONTRIBUTE TO IMPROVED FUNCTION AND SURVIVAL OF PIG ISLET XENOGRAFTS
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Purpose of Study: Islet transplantation has great promise as a treatment for insulin-dependent diabetes. However, long-term success of human islet transplants has been limited. We propose that aggregation of the beta cell peptide islet amyloid polypeptide (IAPP), which rapidly forms...
Summary of Results: Sequencing of pig IAPP confirmed notable differences from the human IAPP (hIAPP) sequence, particularly a proline substitution within the amyloidogenic region of hIAPP. While hIAPP rapidly formed fibrils, synthetic pIAPP required 6 weeks to form fibrils detectable by electron microscopy. EM results were supported by thioflavin T fluorescence and immunostaining.

Methods Used: Methods used included sequencing, western blot, thioflavin T fluorescence and immunostaining.

Conclusions: Decreased fibrillogenicity and cytotoxicity of porcine compared to human IAPP may underlie the apparent enhanced survival and function of pig islet xenografts.

96 ACCELERATED LOSS OF BETA CELL FUNCTION IN TYPE 2 DIABETES IS PARTIALLY REVERSIBLE IN KETOSIS-PRONE, NOT KETOSIS-RESISTANT DM-2

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Purpose of Study: Progression of Type 2 Diabetes (DM-2) is thought to be due to gradual progressive deterioration of beta cell function, often leading to late insulin treatment. However, some patients require insulin earlier in their course, suggesting early insulin deficiency.

Methods Used: We therefore evaluated beta cell function in DM-2 subjects at 2 different stages of diagnosed disease, short duration (SD, <1y) and long duration (LD, >5y), admitted to hospital for insulin treatment due to metabolic decompensation.

Summary of Results: Mean (±SEM) plasma glucose was 493 ± 22 mg/dl (n = 93) at presentation. All were non-ketotic (betahydroxybutyrate < 0.5 mmol/L) and GAD antibody negative. Mean DM duration in SD (n = 22) and LD (n = 71) was 0.1 ± 0.06 and 14.6 ± 0.9 y; BMI 32.2 ± 2 and 29.5 ± 1 kg/m², and age at presentation 50 ± 3 and 54.4 ± 1 y. Mean fasting plasma C-peptide (FPC) the morning after admission was 0.87±0.15 and 0.89 ± 0.1 pmol/ml in SD and LD, for similar mean plasma glucose (~200mg/dl). Thus, insulin-treated DM-2, whether SD or LD, were equally beta cell deficient - demonstrating that beta cell deficiency is more critical than duration at time of decompensation. We also studied patients in whom we anticipated more severe beta cell dysfunction, i.e., ketosis-prone (KPDM-2) patients, with SD (0.01 ± 0.01 years; n = 77), also GAD antibody negative. BMI was 32.9 ± 0.9 Kg/m², similar to the SD DM-2 group. Mean FPC in SD KPDM-2 was 3-fold lower, 0.29 ± 0.03 pmol/ml, than the non-ketotic SD DM-2 group (p < 0.0001). There was a 50% increase in FPC over the next 2 days (p < 0.02) in the SD KPDM-2, not observed in either the SD or LD non-ketotic groups, or a comparable LD KPDM-2 group (n = 38), indicating greater beta cell reserve in SD KPDM-2.

Conclusions: We conclude that in DM-2 beta cell dysfunction may occur early in the course of diagnosed disease, and this is often severe. Furthermore, there are subgroups with recent onset DM-2 that have the capacity for rapid beta cell recovery not seen in DM-2 of longer duration. Quantitation of beta cell function in recent onset patients, identification of subgroups that demonstrate rapid recovery and strategies for preservation of beta cell reserves may be important in management of recent onset DM-2.

97 HIGH-SENSITIVITY C-REACTIVE PROTEIN LEVELS AND FASTING OR 2-HOUR POST LOAD GLUCOSE LEVELS IN SUB-OPTIMALLY TREATED TYPE 2 DIABETES

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Purpose of Study: High-sensitive C-reactive protein (hs-CRP), an inflammatory biomarker, is predictive of cardiovascular events. Recent studies suggest that elevated levels of CRP is associated with abnormal glucose intolerance, new onset and poorly controlled type 2 diabetes (DM2)(HbA1c > 9). However, little is known about the correlation of hs-CRP to sub-optimal glycemia (HbA1c < 9) or post prandial hyperglycemic in DM2.

Methods Used: 53 diabetic outpatients in a VA clinic were recruited. Exclusion criteria were symptomatic heart diseases, chronic inflammatory conditions, BP > 140/90 mmHg, fasting blood glucose > 250 mg/dl, and HbA1c > 9%. Hs-CRP was measured at fasting and 6 hours after 75 grams of oral glucose load. Other clinical parameters included demographic data, smoking status, BP, body mass index(BMI), serum glucose, HbA1c, lipid profile, and current medications. Correlation and linear regression model were used for statistical analysis.

Summary of Results: 46 patients with complete data were included for final analysis. There were 12 (26%) smokers and 44 (96%) males with a mean age of 67 years, BMI of 28.5 kg/m², systolic BP of 128/74 mmHg, and HbA1c of 7.4%. Sixty-eight percent of the patients were on statin, 68% on angiotensin converting enzyme inhibitor or angiotensin receptor blocker, and 10% on insulin: There was no statistically significant correlation between hs-CRP levels and HbA1c, or serum glucose levels before and after glucose load. Patients on statin therapy had lower hs-CRP levels while smokers had higher hs-CRP levels after controlling for other known confounding variables.

Conclusions: We confirmed the positive correlation of hs-CRP to smoking status and inverse correlation of hs-CRP with statin use. However, our study did not found any statistically significant correlation between hs-CRP and glycemic levels as reported by others in patients with poorly controlled DM2(HbA1c > 11). Possible explanations are that our patients had better glycemic control (HbA1c < 9), or hs-CRP, as a down stream marker of systemic inflammation, may be affected by other medications or co-morbid conditions in an interrelated fashion. Due to the predominantly male gender, relatively old age, small sample size, larger study with more representative subjects is warranted.

98 THE ASSOCIATION OF HBAIC, BMI, AGE, AND PULSE PRESSURE WITH POST PRANDIAL HYPERGLYCEMIA IN TYPE 2 DIABETES

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Purpose of Study: Clinical evidence suggests that postprandial hyperglycemia is an independent risk for increased mortality from cardiovascular disease (CVD). Control of post prandial glucose (PPG) may play a more important role in preventing or delaying diabetic complications. However, clinicians frequently focus on fasting plasma glucose(FPG) and glycated hemoglobin (HbA1c) and overlook PPG control partly due to lack of accurate lab measurement and reliable home monitoring. Little information is available regarding factors associated with PPG in type 2 diabetes. We sought to identify other clinical parameters that are easily obtainable and may be useful in the estimation of PPG.

Methods Used: 52 VA outpatients with type 2 diabetes were recruited. Exclusion criteria were BP > 140/90 mmHg, FPG > 250 mg/dl; and HbA1c > 9%. Serum glucose was measured at fasting and 2 hours after 75 gm oral glucose load. Other lab tests included HbA1c, lipid panel, renal, urine albumin, hs-CRP. Additional clinical data included body mass index (BMI), BP, age, and medications. Correlation and multiple regression model were used for statistical analysis.

Summary of Results: There were 48(92%) males with mean age of 67 years, BMI of 28.5 kg/m2, BP of 128/74 mmHg, pulse BP (PBP) of 54 mmHg, FPG of 162.7 mg/dl, PPG of 247.7 mg/dl, and HbA1c of 7.4%. Ten percent of patients were on insulin alone or in combination with oral agents. The difference of plasma glucose concentrations between fasting and 2-hour post oral glucose load (ΔPG) was found to be positively correlated with HbA1c, age, and PBP, and negatively correlated with BMI. There was no significant association of ΔPG with other parameters.

Conclusions: Our results suggest that, in type 2 diabetes, higher HbA1c, lower BMI, older age, and higher PBP are associated with worse glucose tolerance and therefore more vulnerable to higher postprandial hyperglycemia. Although the underlying mechanism remains to be defined, it is clear that those patients have less insulin response to oral glucose challenge and can benefit from more aggressive treatment targeting post prandial hyperglycemia. Due to the study population of predominantly male gender, relatively old age, small sample size, and low baseline HbA1c level, large study with more representative subjects is warranted.

GENDER DIFFERENCES IN INSULIN SENSITIVITY AMONG YOUTH WITH TYPE 1 DIABETES MELLITUS (DM)

K.J. Nadeau1, J. Reusch2,3, and J. Regensteiner2. 1University of Colorado Health Sciences Center, Aurora, CO; 2Denver Veterans Affairs Medical Center, Denver, CO.

Purpose of Study: Type 1 (T1DM) and type 2 diabetes mellitus both increase the risk of cardiovascular disease (CVD). In T1DM, traditional CVD risk factors alone do not explain the increased risk of CVD. Insulin resistance, while not commonly considered a feature of T1DM, is linked to CVD in other populations, and thus may also contribute to CVD risk in T1DM. Interestingly, non-diabetic premenopausal women typically have lower CVD risk than similarly aged men, but the protective effect of gender is negated when women have DM. We hypothesized that T1DM is associated with decreased insulin sensitivity (IS), and that this abnormality is more pronounced in girls than boys.

Methods Used: To address this hypothesis, we measured IS in boys and girls with T1DM vs. control subjects. Subjects included 38 adolescents with T1DM (18 boys and 20 girls and 10 non-diabetic controls (5 girls and 5 boys) with similar BMI, age and physical activity levels. Primary end-points included IS by hyperinsulinemic euglycemic clamp and body composition by DEXA.

Summary of Results: IS, expressed as the glucose disposal rate (mg/kg/min) at steady state, was significantly lower in subjects with T1DM (8.5 ± 3.3) vs. controls (15.9 ± 4.8, p < 0.0001). Control girls (12 ± 2.2) had significantly lower IS than control boys (18.6 ± 4.9, p < 0.03). T1DM girls (7.3 ± 3) also had significantly lower IS than T1DM boys (9.7 ± 2.7, p = 0.03). After correcting for differences in body composition by expressing IS as mg/kg lean tissue/min derived from DEXA, subjects with T1DM (11.8 ± 4.3) still had significantly reduced IS vs. controls (20.6 ± 4.3, p < 0.0001). However, when expressed as mg/lean kg/min, differences between genders in both the T1DM and controls were no longer significant.

Conclusions: T1DM subjects of both genders had decreased IS compared to controls, not explained by differences in body composition. In contrast, the lower IS in control and T1DM girls was at least partly explained by differences in body composition. Girls with T1DM have the lowest IS, which may contribute to increased risk of CVD. Further research is needed to clarify the importance of decreased IS in T1DM and CVD risk, and examine gender differences in a larger population.

100 IMPROVED GLYCEMIC CONTROL WITH REAL LIFE USE OF CONTINUOUS HOME MONITORING OF GLUCOSE (CHMG)

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Purpose of Study: To evaluate changes in glycemic control seen with the new technology of CHMG that is currently in clinical use.

Methods Used: This study includes 24 subjects who used CHMG at 11, 6, 12 weeks as well as 23 computer matched controls for age, gender, duration of diabetes, and baseline A1c values (p = NS). Before using CHMG subjects attended a class that covered glucose trends, features of the CHMG receiver, insertion techniques, and the importance of confirming glucose values with self-monitored blood glucose.

Summary of Results: Each sensor was used for 6.8 ± 1.6 days (mean ± SD), despite approval for 3 days, and each subject used their sensor a mean of 17.6 ± 8.4 days per month. The CHMG group showed a decrease at 12 weeks in mean A1c values with no change in insulin dose (figure 1a). In addition the number of subjects achieving A1c values < 7.5% was higher in the CHMG group at 12 weeks (OR = 7.229, p = 0.0234). Mixed model repeated measures analysis showed an increase in mean percent of glucose readings within the target range (60-150 mg/dL), a decrease in glucose readings above the target range (>150 mg/dL), without any increase in glucose readings below the target range (<60 mg/dL) at 12 weeks compared to baseline (figure 1b).

Conclusions: This is the first real life study which demonstrates that clinical use of CHMG improves A1c values, with a significant increase in target range values, without increasing hypoglycemia.
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**EFFECT OF CARELINK, AN INTERNET-BASED INSULIN PUMP MONITORING SYSTEM, ON GLYCEMIC CONTROL IN RURAL AND URBAN CHILDREN WITH TYPE 1 DIABETES MELLITUS**

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**Purpose of Study:** To determine whether use of the Internet-based insulin pump monitoring system, Carelink, improved glycemic control in rural and urban children treated with insulin pump therapy.

**Methods Used:** We reviewed records of 94 children treated with insulin pump therapy between the years 2004–2007. Records were divided into 3 groups: No Access consisted of patients without Carelink access due to software incompatibilities (33 patients). Non-Users consisted of patients who had access to Carelink but did not use it (20 patients). Carelink Users consisted of patients who used Carelink to upload and review their pump and glucometer data (41 patients). Through record review, we also assessed glycemic control, diabetes self-care measures, frequency of clinic visits and patient geographic location associated with Carelink use.

**Summary of Results:** Carelink Users showed significant improvement in HbA1c levels after initiation of Carelink use (8.0 ± 0.1 (SE) vs. 7.7 ± 0.1 (SE), p = 0.002). No Access patients followed in a conventional manner, showed no change in HbA1c (8.0 ± 0.2 (SE) vs. 8.1 ± 0.2 (SE), p = 0.17) during the study period. Carelink Non-Users had a higher HbA1c level than Carelink but did not use it (20 patients). Carelink Users consisted of patients who used Carelink to upload and review their pump and glucometer data (41 patients). Through record review, we also assessed glycemic control, diabetes self-care measures, frequency of clinic visits and patient geographic location associated with Carelink use.

Carelink Users showed significant improvement in HbA1c levels after initiation of Carelink use (8.0 ± 0.1 (SE) vs. 7.7 ± 0.1 (SE), p = 0.002). No Access patients followed in a conventional manner, showed no change in HbA1c (8.0 ± 0.2 (SE) vs. 8.1 ± 0.2 (SE), p = 0.17) during the study period. Carelink Non-Users had a higher HbA1c level than Carelink Users and No Access patients at the start of the study and did not change over the study period (8.9 ± 0.2 (SE) vs. 9.0 ± 0.3 (SE), p = 0.82). Rural Carelink Users (patients who live >1 hour drive from clinic) showed significant improvement in HbA1c levels following Carelink use (7.9 ± 0.2 (SE) vs. 7.4 ± 0.2 (SE), p = 0.001) compared to urban Carelink Users (patients who live within 1 hour drive from clinic) (8.1 ± 0.2 (SE) vs. 7.9 ± 0.1 (SE), p = 0.15), yet had significantly fewer clinic visits per year as compared to urban patients (2.8 ± 0.2 (SE) vs. 3.5 ± 0.1 (SE), p = 0.001).

**Conclusions:** Carelink use was significantly associated with improved glycemic control in children with Type 1 diabetes on insulin pump therapy. Carelink may be an especially useful tool to help improve glycemic control in patients who do not live in close proximity to their diabetes care clinic.

**Neonatal—Pulmonary**

**Concurrent Session**

8:30 AM

Friday, February 1, 2008

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**GROWTH RESTRICTION DYSREGULATES P53-DEPENDENT REGULATION OF CELLULAR PROLIFERATION AND ANGIogensis**


**Purpose of Study:** Intratierine growth restriction (IUGR) predisposes both humans and rats to chronic lung disease (CLD) which is characterized by mesenchymal thickening of distal airways. We have shown that IUGR increases p53 mRNA in association with histone hypoacetylation and mesenchymal thickening in developing rat lungs. Evidence suggests that acetylation of the transcription factor p53 affects its regulation of p21 (an inhibitor of cellular proliferation) and vascular endothelial growth factor (VEGF) (a mediator of angiogenesis that is required for alveolar formation). While p21 and VEGF are known to play a role in CLD, the role of p21 and VEGF in IUGR-induced mesenchymal thickening remains unknown. Because the enzymes responsible for histone and p53 acetylation are the same, we hypothesized that IUGR decreases p53 acetylation in association with decreased p21 and increased VEGF expression in developing rat lung.

**Methods Used:** To test this hypothesis, bilateral uterine artery ligation surgery was performed on rats at E19. Pups were killed and lungs were harvested at term (D0). mRNA and protein were isolated. Real-time RT-PCR was performed to assess mRNA levels of p21, VEGF, and the VEGF receptor Flk-1. Western blotting was performed to assess protein levels of acetyl lysine(K)-373 p53.

**Summary of Results:** In association with IUGR-induced mesenchymal thickening, IUGR significantly decreased p21 mRNA in D0 rat lungs (IUGR % of control: Male 74.98 ± 2.82*, Female 74.67 ± 5.13*). Similarly, IUGR decreased VEGF and Flk-1 mRNA at D0 (VEGF IUGR % of control: M 72.45 ± 5.6*, F 61.09 ± 6.44, Flk-1 IUGR % control: M 57.70 ± 3.82*, F 58.07 ± 4.23*). In conjunction with decreased p21 and VEGF, IUGR significantly increased acetylated K-373 p53 (Acetyl K-373 p53 IUGR M: 0.362 ± 0.08 arbitrary units (AU) vs Sham M 0.152 ± 0.032 AU*, IUGR F 0.625 ± 0.099 AU vs Sham F 0.318 ± 0.06 AU*, p < 0.05, n = 8-11, ± SEM).

**Conclusions:** IUGR-induced mesenchymal thickening of the distal airways is associated with increased acetyl K-373 p53 and decreased expression of p21, VEGF and Flk-1. We speculate that IUGR disrupts acetylated p53-dependent inhibition of cellular proliferation while maintaining inhibition of VEGF and Flk-1 mRNA levels.

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**INTRATERTERINE GROWTH RESTRICTION ALTERS FETAL LUNG PROGRAMMING BY AFFECTING ESSENTIAL EPITHELIAL-MESENCHYMAL SIGNALING PATHWAYS**

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**Purpose of Study:** Intratierine growth restriction (IUGR) increases the risk of respiratory compromise throughout postnatal life. However, the molecular mechanism(s) underlying the respiratory compromise following IUGR is not known. Since lung development is determined by spatio-temporally specific alveolar epithelial-mesenchymal interactions, we hypothesized that IUGR would affect the key alveolar epithelial-mesenchymal signaling pathways that are essential for normal lung development.

**Methods Used:** We utilized a rodent model of 50% maternal food restriction (MFR) from day 10 of gestation to term and studied the offspring at postnatal day (PND) 1, 3 weeks, and 9 months. We used lung morphometry, pulmonary function tests (PFTs), and Western blotting to analyze the molecular markers of key alveolar epithelial-mesenchymal interactions, we hypothesized that IUGR would affect the key alveolar epithelial-mesenchymal signaling pathways that are essential for normal lung development.

**Summary of Results:** In general, MFR increased the expression of extracellular matrix proteins OSM and calpin, but decreased the...
expression of elastin at all time points examined. The expression of PPARγ, and its downstream targets, was decreased at 3 weeks, but increased at 9 months. Expression of VEGF, its receptor, and GRα also showed temporal specific changes. Further, in contrast to significant changes in lung morphology and PFTs at 9 months, there were no significant changes in these parameters at PND1 and 3 weeks.

Conclusions: IUGR due to MFR alters fetal lung programming by affecting specific epithelial-mesenchymal signaling pathways, offering the possibility for specific interventions to overcome these effects. Supported by NIH (HL75405 & HL55268) and TRDRP (14RT-0073 & 15RT-0250).

104 COMPARISON OF NITRITE METABOLISM IN FETAL AND ADULT BLOOD

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Purpose of Study: Oxyhemoglobin saturations are low in the mammalian fetus and there is less resistance throughout the circulation. This low vascular tone is maintained in part by the vasodilator, nitric oxide (NO), which is synthesized endogenously but is also formed when nitrite reacts with deoxyhemoglobin. Methemoglobin forms as a byproduct. Earlier work has shown faster nitrite conversion in fetal whole blood than in adult, but it remained unknown whether the difference relates to fetal hemoglobin itself or is caused by differences in other red cell constituents. We hypothesized that the reduction of nitrite to NO would be greater in fetal blood than in adult blood due to the intrinsic property of fetal versus adult hemoglobins.

Methods Used: Blood was collected from adult and fetal sheep. After cell lysis, purified hemoglobin was prepared by passage through G25 Sephadex columns. Hemoglobin concentration, oxyhemoglobin saturation, and pH were adjusted to be the same in whole blood and hemoglobin solutions. Sodium nitrite was added to the solutions in sealed flasks at body temperature and rates of nitrite disappearance and methemoglobin production were measured.

Summary of Results: Nitrite disappeared almost twice as fast in fetal whole blood as in adult blood with half-lives of 3.7 ± 0.5 and 7.9 ± 0.3 min, respectively (p < 0.01). NO in the form of iron-nitrosyl hemoglobin, HbFeINO, and as S-nitrosothiols, SNO-Hb, appeared rapidly and in higher concentration in fetal blood. These bound forms of NO then dissociated more quickly in the fetal blood, which may infer greater NO availability in the fetal circulation. In purified fetal hemoglobin nitrite also disappeared almost twice as fast as in adult hemoglobin with half-lives of 5.0 ± 2.4 and 9.5 ± 2.5 min, respectively. The initial rates of methemoglobin production were also faster in fetal hemoglobin.

Conclusions: Thus properties of the fetal hemoglobin molecule itself rather than other attributes of whole blood account for differences in reaction kinetics. The findings support a role for augmented NO production from nitrite in the fetal circulation that is likely intrinsic to fetal hemoglobin and partly accounts for the low resistance to blood flow that characterizes fetal life.

105 NF-κB REGULATES ALVEOLAR FORMATION DURING MOUSE LUNG MORPHOGENESIS


Purpose of Study: To determine the role of NF-κB in lung alveolar formation using a cre/loxP transgenic mouse model to selectively delete NF-κB signaling targeted to lung epithelium. Previous studies have demonstrated that NF-κB is involved in mouse limb and mammary gland organogenesis. This study is designed to delete IKK-beta, a critical enzyme necessary for NF-κB activity, in lung epithelial cells expressing Nkx2.1 during development.

Methods Used: Transgenic Nkx2.1-cre mice bred on a C57BL/6 background were crossed with strain-matched IKK-beta F/F (floxed) mice (a generous gift of M. Karin, UCSD) to generate double-transgenic (Nkx2.1-;IKK F/F) animals. The resultant progeny were sacrificed at designated embryonic and postnatal stages to obtain lung tissue for histological and mRNA analysis.

Summary of Results: Deletion of NF-κB in the lung epithelium led to fewer and widened airspaces in double transgenic (Nkx2.1-;IKK F/F) animals as compared to controls (Nkx2.1-;IKK F/F). The animals continued to survive into adulthood and the resultant phenotypic difference was most marked immediately following birth thru the first week of life. Preliminary immunohistochemical evaluation in double transgenic animals also revealed fewer cells positive for Nkx2.1 (a marker for epithelial cells) per unit area.

Conclusions: The phenotypic changes due to epithelial deletion of NF-κB in the developing mouse lung suggest that NF-κB may play an important role during lung alveolar formation. The molecular signals mediating NF-κB and alveolar signaling pathways remain to be elucidated.

106 INTRAUTERINE GROWTH RESTRICTION AFFECTS POSTNATAL RETINOIC ACID SIGNALING IN THE RAT LUNG


Purpose of Study: Intrauterine growth restriction (IUGR) increases the incidence of chronic lung disease (CLD) and preliminary data from our lab suggest a gender-specific difference with males being more vulnerable to the insult. Supplementation with retinoic acid (RA) decreases the incidence of CLD in both animal models and humans. However, it is unknown how IUGR effects the expression of genes involved in RA signaling and metabolism.

We hypothesized that IUGR would decrease expression of genes involved in signaling and synthesis of RA, and males would be more greatly affected.

Methods Used: We used an established model of IUGR in the rat. Lung tissue was harvested at day 0 (DOL0) and day 21 (DOL21) of life. We extracted mRNA, synthesized cDNA and used real-time PCR to assess mRNA levels for the following genes: RARα, RARβ, and RARγ receptors, aldh1a1 and aldh1a2 (synthesis), and Rbp1 (transport).

Summary of Results: We began by comparing mRNA levels for our genes of interest at different ages and found consistently and significantly increased levels at DOL21 vs. DOL0 in both sham and IUGR pups. We then compared IUGR pups vs. sham controls and found significantly decreased mRNA levels of RARα, RARβ, aldh1a1 and aldh1a2 for both genders at DOL0 (p < 0.001, 0.002, 0.03 and < 0.001, respectively). Of interest, in IUGR DOL0 pups, females had significantly higher levels of mRNA than males in all genes of interest except...
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HYPEROXIA AUGMENTS INCREASED EXPRESSION OF INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN-3 IN THE DEVELOPING LUNG OF GROWTH-RESTRICTED RATS


Purpose of Study: Intrauterine growth restriction (IUGR) and hyperoxia predispose infants towards chronic lung disease (CLD), and both are known to alter the expression of insulin-like growth factor binding protein-3 (IGFBP-3). IGFBP-3 exerts anti-proliferative and pro-apoptotic effects by both IGF-1 dependent and independent mechanisms. It is expressed early in lung development and its expression is increased in adult lung pathology. Therefore, we hypothesized that IUGR and hyperoxia in the perinatal rat would lead to increased levels of IGFBP-3 mRNA and protein levels within the lung during alveolarization.

Methods Used: To test our hypothesis, we used a well-established rat model of IUGR. We analyzed control and IUGR day 6 rat lungs that were exposed to hyperoxic (60% inspired oxygen for 3 days) conditions for IGFBP-3 mRNA and protein levels. For comparison, we examined these same levels in the lungs of control and IUGR rats at day 0 and day 21 of life. Immunohistochemistry localized IGFBP-3 protein within the lung.

Summary of Results: At day 0 and day 21 (beginning and end of alveolarization), IGFBP-3 mRNA and protein levels were unchanged between IUGR and controls. However, at day 6 (during alveolarization), IUGR significantly elevated both IGFBP-3 mRNA and protein levels to 259 +/- 15% (p < 0.0001) and 211 +/- 35% (p = 0.025) of controls, respectively. While hyperoxia did not independently alter IGFBP-3 levels at day 6, interaction bar plots combining the effects of IUGR and hyperoxia on IGFBP-3 protein levels revealed a significant interaction (p = 0.0012). Hyperoxia increased IGFBP-3 protein levels in IUGR rat lungs, but decreased IGFBP-3 levels in control rat lungs. Immunohistochemistry localized IGFBP-3 protein to the epithelia of large airways and mesenchymal tissue, with subjectively increased staining in IUGR lungs as compared to controls.

Conclusions: We conclude that IUGR significantly increases IGFBP-3 mRNA and protein in the developing rat lung and that exposure to relatively mild levels of hyperoxia further augments this increase. We speculate that this upsets the normal balance of cellular proliferation and apoptosis during this crucial stage of lung development and plays a role in predisposing IUGR infants towards CLD. (Supported by CHRC, HD41075).

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HYPEROXIA-INDUCED NEONATAL RAT LUNG INJURY INVOLVES ACTIVATION OF WNT AND TRANSFORMING GROWTH FACTOR-BETA SIGNALING: PROTECTION BY ROSIGLITAZONE

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Purpose of Study: Bronchopulmonary Dysplasia (BPD) is a chronic lung disorder that is consequence of abnormally repaired lung damage. Despite several therapeutic advances, significant morbidity is still associated with BPD. Hyperoxia disrupts critical signaling pathways that support normal lung development. So, we aim to characterize in vivo hyperoxia induced (a) perturbation of Wnt and TGF-β signaling, (b) aberrations in lung morphology in post-natal day (PND) 7 rat lungs and (c) tested if pretreatment of neonatal rat pups with the PPARγ agonist rosiglitazone (RGZ) can prevent such aberrations.

Methods Used: Newborn rat pups were divided into three groups, namely, normoxic (21% O2), hyperoxic (95% O2), and hyperoxic with RGZ (3 mg/kg intraperitoneally once daily during hyperoxia). At PND7, lungs were isolated for analysis by (a) immunoblotting, (b) morphometry and (c) immunohistochemistry (IHC).

Summary of Results: In hyperoxia there was significant up-regulation of Wnt signaling markers, Lef-1 and β-catenin, in lung tissues from PND7 pups, along with pSMAD3 and SMAD7, the TGF-β pathway markers (P < 0.05). IHC revealed a robust up-regulation of Lef-1 in lungs. Markers of the myogenic phenotype, α-SMA and calponin were also up regulated. Consistent with above aberrations, there was significantly (P < 0.05) reduced alveolar septal thickness, radial alveolar count, but larger alveoli. PPARγ expression, however, was significantly (P < 0.05) down regulated. Hyperoxia-induced molecular and morphometric changes were almost completely prevented by RGZ treatment.

Conclusions: We demonstrate up-regulation of Lef-1, β-catenin, pSMAD3 and SMAD7 in neonatal rat lungs in hyperoxia. Hyperoxia-induced simplified alveoli, a hallmark of BPD, along with altered molecular changes strongly implicate these in hyperoxia-induced lung injury. Administration of a PPARγ agonist could be an effective strategy to prevent the lung injury that leads to BPD. (Grant Support: NIH-HL 75405, HL55268, TRDRP-14RT-0013-1517-0250).
Conclusions: Following exposure to chronic high altitude hypoxia, the relaxation responses to NO in intrapulmonary arteries and veins of pregnant ewes are differentially altered. In veins, nitric oxide induced relaxation is decreased and is no longer mediated by activation of guanylate cyclase but occurs through activation of Na+-K+-ATPase. In arteries, NO-mediated relaxation is also decreased but is still mediated via cGMP.

110 EFFECTS OF INDOMETHACIN AND IBUPROFEN ON LUNG MORPHOMETRY AND BIOMARKERS FOR OXIDATIVE STRESS, DNA DAMAGE AND PPHN IN NEONATAL RATS

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Purpose of Study: Indomethacin (Indo) has been the drug of choice for the treatment of symptomatic PDA, however, its use is associated with renal and gastro-intestinal adverse effects. Although ibuprofen (Ibu) lysine has been shown to be a safer alternative to Indo, it was therefore compared the effects of early versus late use of Indo and Ibu on morphology and biomarkers for oxidative stress, DNA damage and pulmonary hypertension in normoxic and hypoxic rat lungs.

Methods Used: Newborn rats were treated with room air (RA) or hyperoxia (50% O2) from the first day of life (P1). For early treatment, the rat pups received IP injections of either Indo (0.2 mg/kg) on P1 and 0.1 mg/kg on P2 and P3; Ibu (10 mg/kg) on P1 and 5 mg/kg on P2 and P3; or saline (Sal) on P1, P2 and P3, then euthanized on P4. For late treatment, the rats received Indo, Ibu, or Sal on P4, P5 and P6, then euthanized on P7. Lung morphometry and biomarkers for oxidative stress (8-epi-PGF2α), DNA damage (8-hydroxy-2-deoxyguanosine) and PPHN (ET-1, big ET-1, and total NO) were assessed.

Summary of Results: Despite timing of the dose and oxygen exposure, both drugs produced oversimplified alveoli with dilated alveolar ducts, but the effect was more severe and widespread with Indo. Changes in lung morphometry with Indo were associated with higher 8-epi-PGF2α but the effect was more severe and widespread with Indo. Changes in lung morphometry with Indo were associated with higher 8-epi-PGF2α and total NO during hyperoxia (p<0.05); and higher ET-1 (p<0.05) during RA. Both drugs suppressed 8-hydroxy-2-deoxyguanosine (reflecting DNA damage) during early treatment in hyperoxia (p<0.05). Changes: These data demonstrate that postponing the use of NSAIDs had no benefit over early treatment with respect to lung morphometry. Although the effects were less severe with Ibu than Indo, treatment with NSAIDs may lead to impaired alveolar and pulmonary vascular development.

111 HISTONE ACETYLATION IN THE LUNG IS AFFECTED BY VENTILATION MODE IN PRETERM LAMBS


Purpose of Study: Chronic lung disease (CLD) of prematurity is characterized by histone 3 (H3) hypoacetylation and DNA hypermethylation. Long-term changes in gene expression and phenotypic changes suggest persistent changes in gene expression by altered determinants of chromatin structure, such as histone acetylation. In premature lambs, alveolar formation is improved by using nasal CPAP as an alternative mode to mechanical ventilation (MV), as well as by administering histone deacetylase inhibitors (valproic acid, VPA or trichostatin A, TSA) during MV. Those findings suggest that ventilation mode affects histone acetylation in the lung. We hypothesized that histone acetylation in the lung will be affected by ventilation mode in chronically ventilated preterm lambs.

Methods Used: Preterm lambs (~132d gestation; term ~147d), treated with antenatal steroids and postnatal surfactant, were managed by MV, nasal CPAP, MV+VPA, or MV+TSA. At the end of 3d, frozen lung tissue was analyzed by real-time RT-PCR for histone deacetylase 1 (HDAC1) mRNA expression, immunoblot for acetylated H3K9 and acetylated H3K14 protein abundance, and colorimetric assay for HDAC activity. Summary of Results: Relative to the internal control GAPDH, the quantity of HDAC1 mRNA was significantly greater in MV than the other groups (table; *p<0.05 by ANOVA/FPLSD). Acetylated H3K9 and acetylated H3K14 protein abundance was statistically lower in the MV group. HDAC activity was statistically greater in the MV group.

Conclusions: We conclude that ventilation mode affects HDAC1 mRNA expression, histone acetylation, and HDAC activity in the preterm lamb lung. Nasal CPAP preserves acetylation of histones whereas MV does not. We speculate that the greater degree of histone acetylation, which affects patterns of gene expression, contributes to alveolar formation during nasal CPAP ventilation of preterm neonates. (HL62875, HL56401, HD41075, CHRC).

112 POSITIVE DISTENDING PRESSURE GENERATED BY HIGH FLOW NASAL CANNULA AS COMPARED TO NASAL CONTINUOUS POSITIVE AIRWAY PRESSURE


Purpose of Study: Analyze end-expiratory mean esophageal pressures (EEEP) delivered by nasal continuous positive airway pressure at 6cm H2O (NCPAP+6) versus the Fisher & Paykel RT329 HHNC system at varying gas flows.

Methods Used: 15 patients on NCPAP+6 had a saline-filled catheter placed into the distal esophagus and verified via occlusion test. After 30 minutes, a 5-minute continuous recording of the EEEP and physiologic data were recorded. The patients were transitioned to HHNC at 6 liters per minute (L/min), then flow was decreased in 1 L/min increments. Similar measurements for each flow value were taken after 30 minutes or until the patient demonstrated intolerance. Heart rate (HR), respiratory rate (RR), Fio2, SaO2 and RDS scores were recorded each minute during the EEEP recording period; EEEP was measured from 100 selected breaths. Data were analyzed using repeated measures ANOVA.
Summary of Results: 3 patients did not tolerate 1L/m. HR, FiO2, SaO2 and RDS score were not different. RR increased significantly as flows decreased (p < 0.02). There was a trend towards decreasing EEEP as flows decreased (p = 0.08). Coefficient of variation was >100% for EEEP at all flows (figure). Linear regression analysis showed no relationship between patient weight and flow to produce NCPAP+6.

Conclusions: With this system, it appears impossible to predict EEEP for a given flow level due to inter- and intrapatient variation. Patient size does not predict pressure resulting from a given flow.

112a CARCINOEMBRYONIC CELL ADHESION MOLECULE 6: A NEW HUMAN SURFACTANT ASSOCIATED PROTEIN
C.J. Chapin1, L.W. Gonzales2, N. Bailey3, J.D. Merrill3, R.A. Ballard4, and P.L. Ballard5. 1UC San Francisco, San Francisco, CA; 2University of Pennsylvania School of Medicine, Children’s Hospital of Philadelphia, Philadelphia, PA and 3Children’s Hospital Oakland Research Institute, Oakland, CA.

Purpose of Study: To determine the location within lung type II cells and alveolar profile of Carcinoembryonic Cell Adhesion Molecule 6 (CEACAM6), a glycoprophosphatidylinositol anchor glycoprotein that is upregulated during hormone-induced lung type II cell differentiation.

Methods Used: Epithelial cells were isolated from human fetal lung and treated with dexamethasone and cAMP to induce type II cell differentiation; CEACAM6 expression was assessed by confocal immunofluorescence. Large aggregate surfactant and supernatant fractions were isolated from tracheal aspirates (TA) of intubated premature infants (mean gestational age 25.5 wk, n=30) during the second postnatal week. CEACAM6 was measured by Western and immunofluorescence, which is sensitive to 0.3 ng. Surface activity of commercial surfactant in the presence of inhibitory proteins with or without CEACAM6 (by siRNA silencing) was determined by pulsating bubble surfactometry.

Summary of Results: CEACAM6 localized to both plasma membrane and within lamellar bodies of cultured type II cells. In tracheal aspirate of premature infants, CEACAM6 was present in both large aggregate surfactant (57±4% of total) and the supernatant (43%). CEACAM6 concentration in surfactant was 2±0.4% of total protein and 2.3±0.4% of phospholipid, similar to the values for SP-B, and was primarily the 90 kDa form. Concentration of CEACAM6 decreased with increased recovery of TA surfactant. CEACAM6 in the supernatant averaged 0.5±0.1% of total protein and was primarily 60 kDa. In the presence of inhibitory proteins from conditioned culture medium, low minimum surface tension was obtained with but not without endogenous CEACAM6.

Conclusions: CEACAM6 is a surfactant-associated protein that is expressed during type II cell differentiation in vitro and in vivo after premature birth. CEACAM6 has a potential function in promoting surfactant stability as shown by its ability to reduce surface tension in the presence of inhibitory proteins.

Neonatology—General I
Concurrent Session
8:30 AM
Friday, February 1, 2008

113 PRENATAL METHAMPHETAMINE (MA) EXPOSURE AND GROWTH AT 12, 24, AND 36 MONTHS: PRELIMINARY RESULTS FROM THE INFANT DEVELOPMENT, ENVIRONMENT, AND LIFESTYLE (IDEAL) STUDY
Z. Tejani1, L.L. Lasgasse2, C. Derault2, E. Newman2, R. Shab3, L. Smith1, A. Arria2, M. Huestis2, S. DellaGrotta2, J. Liu2, and B.M. Lester2. 1Harbor-UCLA Medical Center, Torrance, CA and 2Children’s Hospital Oakland Research Institute, Oakland, CA.

Purpose of Study: Preliminary findings from the IDEAL study demonstrated that prenatal methamphetamine (MA) exposure was associated with an increased incidence of children born small for gestational age. The purpose of this study was to examine the effects of prenatal MA exposure on growth parameters from birth to 3 years.

Methods Used: Participants were selected from the IDEAL study, a multisite longitudinal study of the effects of prenatal MA exposure on childhood outcomes. 412 subjects were enrolled at birth (N = 208 comparison and N = 204 MA exposed). The MA and comparison groups were matched by race, birth weight, maternal education, and type of insurance. Both groups included prenatal alcohol, tobacco, and marijuana exposure, but excluded exposure to opiates, lysergic acid diethylamide (LSD), or phencyclidine (PCP). Physical growth data was obtained annually from age 1-3 years. Data from 351 children (N = 176 MA exposed) attending at least 2 of the annual visits were included. Individual growth models were used to examine the effects of prenatal MA exposure on weight and height trajectories after controlling for recruitment site and prematurity. Separate analyses were conducted for the total sample (N = 351) and for term subjects only (N = 301).

Summary of Results: Overall, height trajectory was lower in the MA exposed versus the comparison children (P = 0.015), with no difference in the rate of growth between the two groups (P = 0.322). Prematurity was associated with a reduction in height relative to term infants (P < 0.001). Preterm infants also had a higher growth rate than term infants (β = 0.11, P < 0.001). In term subjects, MA exposure was also associated with a lower height trajectory (P = 0.024), but there was no difference in the rate of growth between the two groups (P = 0.485). There was no difference in weight trajectories between the comparison group and the MA exposed.

Conclusions: These preliminary findings suggest children exposed prenatally to MA have a modest decrease in height during the first three years of life with no observed difference in weight.
114 Prenatal Methamphetamine (MA) Exposure and Motor Development at 12 and 36 Months: Preliminary Results of the Infant Development, Environment, and Lifestyle (IDEAL) Study

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Purpose of Study: MA use during pregnancy is an increasing problem in the United States but how prenatal MA exposure affects motor development is not known. The objective of this study was to examine the effects of prenatal MA exposure on childhood motor development at 12 and 36 months.

Methods Used: The IDEAL study enrolled 412 subjects at 4 sites. MA exposed subjects (N = 204) were identified by maternal self-report or confirmation of amphetamine/metabolites in infant meconium. Matched controls (N = 208) denied maternal amphetamine use and had negative meconium screens. Both groups included prenatal alcohol, tobacco, and marijuana exposure, but excluded exposure to opiates, lysergic acid diethylamide (LSD), or phencyclidine (PCP). Data from a subset (N = 347) of the IDEAL study with completed 12 and/or 36 month visits (N = 330 and 230, respectively) was analyzed. The Peabody Developmental Motor Scales (PDMS-2) were administered at both visits. ANOVA analysis conducted on the PDMS-2 scores included exposure group effects, heavy MA use effects (>3 days/week), and adjusted for background drugs, socioeconomic status, and site. Correlation analysis examined the association between motor scores and frequency of MA use by trimester.

Summary of Results: MA exposure was associated with lower grasp scores at 12 months relative to controls (P = 0.049). Heavy MA exposure was associated with significantly lower grasp scores than controls and those less heavily exposed at 12 months (P = 0.026). Second trimester MA exposure was correlated with a lower motor quotient at 36 months (r = −0.14, P = 0.039). There were no other differences in motor performance at 12 and 36 months.

Conclusions: There was a subtle MA exposure effect on fine motor performance at 12 months with the poorest performance observed in the most heavily exposed children. By 36 months, no differences in fine motor performance were observed, however poorer overall motor skills were related to increased MA exposure during the second trimester. These findings suggest MA exposure has modest motor effects at 12 months that are mostly resolved by 36 months.

115 Hydrographic Magnetic Resonance Imaging of the Fetal Eye

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Purpose of Study: Magnetic resonance imaging (MRI) is being used more frequently in diagnosis and perinatal management for an increasing variety of fetal abnormalities. The most commonly employed MR images are single-shot fast spin-echoes. These T2-weighted images demonstrate contrast well between water and solid structures, which is very useful in the fetus. Heavily weighted T2 images, also called hydrographic images, provide even greater contrast at these interfaces. Previous work has shown that additional detailed diagnostic information may be gained from hydrographic imaging of the fetus. We hypothesized that hydrographic images would provide for better resolution of the fetal eye.

Methods Used: Both standard T2 single-shot fast spin-echo and heavily weighted T2 single-shot fast spin-echo imaging (hydrographic technique) were retrospectively examined in 38 studies of patients gestational age 19-30 weeks with abnormal sonographic findings referred for fetal MR imaging. The high-resolution hydrographic technique included the following parameters: TE = 488 ms, TR = 4000 ms, Matrix = 256 × 192 and thickness of 3 mm. Synapse PACS was used for image analysis. Density values were obtained for intraorbital and immediately extraorbital regions on both standard T2 and hydrographic MR images. Intraorbital to extraorbital density ratios were calculated for each image and compared to determine if any quantifiable difference in image contrast exists.

Summary of Results: In all images, the density differences detected by hydrographic technique were greater than those of the standard T2 densities. This difference was 1.06–3.49 times greater in the hydrographic images, with an average of two. There appears to be no correlation between gestational age and resolution for hydrographic images.

Conclusions: Hydrographic MRI imaging of the fetal eye can provide up to twice greater contrast of the fetal orbit than standard T2 imaging. This may enhance prenatal diagnosis of congenital eye anomalies (i.e., cataracts) and/or retinoblastoma tumor detection. Further study, including that of more advanced gestational age subjects, should be done to confirm whether gestational age affects clarity of the hydrographic images.

116 Effects of Early Versus Late Ibuprofen and Indomethacin on Pulmonary Prostanoids in Neonatal Rats

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Purpose of Study: Ibuprofen (Ibu) lysine has been shown to be as effective as indomethacin (Indo) for closure of patent ductus arteriosus in very low birth weight (VLBW) infants with fewer renal side effects. However, Ibu has been found to result in pulmonary hypertension which resolved with inhaled nitric oxide. Pulmonary hypertension post Ibu may be due to inhibition of pulmonary vasodilator prostanoids, such as prostacyclin (PGI2). We investigated the effects of early versus late administration of Ibu and Indo on systemic and pulmonary prostanoids in normoxic and hypoxic neonatal rats.

Methods Used: Newborn rats were exposed to room air (RA) or hyperoxia (50% O2) from the first day of life (P1). For early treatment, the rat pups received IP injections of either Indo (0.2 mg/kg) on P1 and 0.1 mg/kg on P2 and P3; Ibu (10 mg/kg) on P1 and 5 mg/kg on P2 and P3; or saline (Sal) on P1, P2 and P3, then euthanized on P4. For late treatment, the rats received Indo, Ibu, or saline on P4, P5 and P6, then euthanized on P7. Plasma and lung tissue homogenates were assessed for PGE2, PGF2α, 6-ketoPGF1α (stable metabolite of PGI2), and TxB2 (stable metabolite of TxA2).

Summary of Results: As expected, early and late treatment with Ibu and Indo suppressed plasma prostanoids in RA (p < 0.01), but the effect was less remarkable in the lungs. Hyperoxia alone suppressed all plasma prostanoids (p < 0.01), except TxB2, while the combination of hyperoxia and Indo or Ibu failed to have a similar effect. In the lungs, hyperoxia alone had no significant effects on prostanoids, but the combination of hyperoxia and early Ibu suppressed 6-ketoPGF1α (p < 0.05).

Conclusions: These data demonstrate that the suppressive effect on pulmonary PGI2 may explain in part, the development of pulmonary hypertension seen in VLBW infants treated with Ibu during the first few days of life.
117 ECONOMIC EVALUATION OF INHALED NITRIC OXIDE IN VENTILATED PRETERM INFANTS

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Purpose of Study: In the recently reported NO-CLD trial (New Engl J Med 2006;355:343), ventilated preterm infants who received a course of inhaled nitric oxide between 7 and 21 days of life had a significant improvement in survival without bronchopulmonary dysplasia (BPD), as well as a shorter duration of admission and ventilation. However, the high price for the drug itself may be a barrier to its use. We sought to estimate the incremental cost-effectiveness of inhaled nitric oxide (iNO) therapy to prevent BPD in infants < 1250 grams.

Methods Used: This retrospective economic evaluation used patient-level data from the NO-CLD randomized trial. The study took a third-party payer perspective and measured costs and effects through hospital discharge. We applied previously reported hospital per diem costs stratified by intensity of ventilatory support, nitric oxide costs from standard market prices and professional (physician) fees from the Medicare fee schedule. We compared log-transformed costs using multivariable modeling, and performed incremental cost-effectiveness analysis with estimation of uncertainty through non-parametric bootstrapping.

Summary of Results: The mean cost per infant was $193,125 in the placebo group and $194,702 in the iNO group (adjusted p = 0.17). The point estimate for the incremental cost per additional survivor without BPD was $19,700. For infants in whom iNO was initiated between 7 and 14 days, the mean cost per infant was $187,407 in the placebo group and $181,525 in the iNO group (adjusted p = 0.46). In this group of early treated infants, there was a 71% probability that iNO actually decreased costs and improved outcomes.

Conclusions: Despite its price relative to other neonatal therapies, inhaled nitric oxide in this trial was not associated with higher costs of care, an effect that is likely due to its impact on length of stay and ventilation. Indeed, for infants who receive nitric oxide between 7 and 14 days, the therapy appeared to lower costs while improving outcomes.

118 TIMING OF PRENATAL STEROIDS AND ITS EFFECT ON RESPIRATORY DISTRESS SYNDROME

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Purpose of Study: To evaluate when the incidence of RDS is lowest according to the time of prenatal steroid administration.

Methods Used: Using the Pediatric Babysteps computerized clinical data system, medical records of singletons, twins, triplets, & quadruplets born between Feb. 2005 & Feb. 2007 were reviewed. Of 125 infants who did not receive prenatal steroids had a 54.4% rate of RDS. The 125 infants who did not receive prenatal steroids had a 54.4% rate of RDS.

Summary of Results: A Stepwise Linear Regression analysis showed the two most powerful predictors of RDS were birth weight (p = 0.001) & timing of prenatal steroids (p < 0.002). Conclusions: Based on the 241 medical records studied, only 214 records contained the steroid administration time. When comparing Group II to any other group, there is an obviously significant change. However, between Groups I, III, IV, and V, there is minimal variation in the % RDS occurrence, ranging only from 57% to 64%. The above graph shows our results of % RDS occurrence based on steroid administration time. Group II had a much lower rate of RDS than any of the other 4 groups. This study shows that prenatal steroids decrease the incidence of RDS in premature babies. However, the benefit of prenatal steroids seems to be lost if delivery occurs outside of the 24–48 hour window post delivery.

119 A RANDOMIZED, MASKED STUDY OF WEEKLY ERYTHROPOIETIN DOSING IN NEONATES

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Purpose of Study: Erythropoietin (Epo) has been shown to be effective in increasing and maintaining hematocrits using once weekly dosing in adults with anemia due to end stage renal disease. Epo has been used in preterm infants to treat the anemia of prematurity, and is usually given three times a week as a subcutaneous injection. While the pharmacokinetics of Epo in preterm infants would suggest more frequent dosing, the erythropoiesis, as evidenced by increased absolute reticulocyte counts (ARC), transfusions, phlebotomy losses, and adverse events were recorded.

Summary of Results: Fourteen preterm infants (971 ± 56 grams, 27.5 ± 0.5 weeks gestation, 16 ± 2 days of age) were enrolled. There were no differences between groups in baseline characteristics. Infants randomized to the once weekly dose achieved a significant increase in ARC by day 14 of the study (p = 0.03 versus baseline). Infants randomized to the thrice weekly dose increased ARC by day 28 of the study, however the difference did not reach statistical significance (p = 0.08 versus baseline). Hematocrits remained stable throughout the 28 day study period in both treatment groups. Phlebotomy losses were inversely proportional to gestational age, and trended higher in the thrice weekly Epo group (p = 0.07). A total of 4 transfusions were administered to the thrice weekly Epo group, while two transfusions were administered to the once weekly Epo group. No adverse effects of either dosing schedule were noted.

Conclusions: Preterm infants respond to weekly Epo by increasing erythropoiesis, as evidenced by increased absolute reticulocyte counts
and maintenance of hematocrit. We speculate that once weekly Epo dosing might be beneficial to those preterm infants requiring increased erythropoiesis, especially those transferred or discharged while still receiving Epo therapy.

120 INOSITOL AND MANNOSE UTILIZATION IN LATE PRETERM NEWBORNS


Purpose of Study: Studies in human pregnancy demonstrate umbilical uptake of mannose, suggesting fetal dependence on an external mannose supply, whereas inositol is taken up from the fetal circulation by the placenta, consistent with fetal production of inositol. Both are present in breast milk, and inositol is widely supplemented in formulas. Inositol supplementation in preterm neonates has been shown to reduce retinopathy of prematurity and chronic lung disease. These studies suggest that mannose and inositol are potentially important to the neonate, though little is known about their metabolism. Therefore, the purpose of our study was to determine the plasma disposal rates (DR) of mannose and inositol in newborns, as an index of utilization and as an improved guide to more accurate supplementation practices.

Methods Used: Late preterm infants 33 to 37 weeks gestation (n = 9) were studied. After baseline blood samples, stable isotopes of mannose\textsubscript{m+1} and inositol\textsubscript{m+1} were infused for 3 hours. Mannose\textsubscript{m+6} and inositol\textsubscript{m+6} were also infused beginning 30 min later. Near the end of the infusion period, two more blood samples were obtained 15 min apart (representing 4 time points) to establish steady state enrichment conditions for each carbohydrate. Isotopic enrichments and corresponding plasma concentrations were measured using GCMS.

Summary of Results: Neonates were studied at median (range) 33 (32–36) weeks gestation with birthweight of 2.01 (1.80–2.29) kg and postnatal age of 3.9 (1-24) days. Molar percent enrichments determined by isotope m+1 or m+6 for each sugar were not different. The mannose DR for mannose and inositol m+1 were also infused beginning 30 min later. Near the end of the infusion period, two more blood samples were obtained 15 min apart (representing 4 time points) to establish steady state enrichment conditions for each carbohydrate. Isotopic enrichments and corresponding plasma concentrations were measured using GCMS.

Conclusions: DR for mannose and inositol are 230-fold and 15-fold higher, respectively, than predicted average enteral intake calculated from their free sugar concentrations in breast milk, indicating a requirement for de novo production of these carbohydrates from glucose in the late preterm infant. There was no evidence of extensive futile cycling for either carbohydrate. Correlations with plasma concentrations suggest that dietary supplementation will increase neonatal utilization of these carbohydrates.

121 EARLY POSTNATAL BODY FAT DEPOSITION IN INTRAUTERINE GROWTH RESTRICTED (IUGR) TERM INFANTS

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Purpose of Study: To compare early postnatal fat deposition by air displacement plethysmography (ADP) in IUGR and AGA infants from birth to 6 weeks of age.

Methods Used: Term IUGR (Birth Wt <10th%ile) and appropriately for gestational age (AGA) infants were studied. Maternal consent was obtained during the 2nd trimester to assess fetal growth by 2-D ultrasound at 20 and ~35 wks gestation. Maternal and infant medical histories and serum glucose (mg/dL) were obtained. Percentage infant body fat (%BF) by ADP (PEA POD, Life Measurement, Inc.) and dual energy x-ray absorptiometry (DXA, QDR4500, Hologic), and anthropometrics (body Wt, length, and head and regional circumferences) were measured in at 48 hrs and 6 wks of age. Derived anthropometric measurements included weight/length, body mass index (BMI, Wt/length\textsuperscript{2}), ponderal index (PI, wt/length\textsuperscript{3}), and regional to head circumference (HC) ratios. ANOVA and correlations and were performed with p < 0.05.

Summary of Results: A total of 33 infants (IUGR= 13, 3M; AGA =20, 7M) were studied. Asymmetrical growth (Birth Wt only <10th%ile) was present in 9/13 (70%) of IUGR. Feeding source was distributed equally (60% breast-fed, 20% breast+ formula, 20% formula only) from birth to 6 wks. Gestational age and length and head circumferences at birth and 6 wks were similar. Wt, BMI, and circumference ratios (abdominal, mid-arm and -thigh) at birth and 6 wks and %BF at birth were significantly lower in IUGR (p < 0.001). Wt %ile and %BF gains were greater and wt/length %ile gain lower in IUGR infants from birth to 6 wks (p < 0.05; Table). Greater postnatal %BF gain was positively related to greater fetal abdominal circumference %ile loss from 20 wk to 36 wk in 2-D ultrasound (R = 0.45, p = 0.01). Postnatal growth changes were greatest in asymmetrical IUGR infants while 75% (3/4) of symmetrical IUGR infants remained <10th%ile for growth measures at 6 wks.

Conclusions: These findings demonstrate that body fat is the major component of "catch-up" growth observed in asymmetrical IUGR infants during the first six weeks of life.

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<th>Wt,%BF Change</th>
<th>%BF Change</th>
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<tr>
<td>IUGR N=13</td>
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<td>13.5±6.5</td>
<td>0.4±0.7</td>
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<td>11.5±4.5</td>
<td>5.5±0.5</td>
<td>9.1±1.1</td>
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122 USE OF ECG FOR INITIAL NEWBORN HEART RATE ASSESSMENT: A PILOT/FEASIBILITY STUDY


Purpose of Study: Heart rate (HR) is used for newborn management in the delivery room. Measurement of newborn HR by pulse-oximetry (PO), palpation or auscultation, and O\textsubscript{2} saturation (O\textsubscript{2}Sat, by PO) have not been rigorously validated. We hypothesized that HR measurement by ECG is feasible and that PO HR and O\textsubscript{2}Sat are valid in newborns.

Methods Used: This study was approved by UCSD’s IRB. Informed consent was obtained before delivery. We used a modified ECG in 10 newborns (gestation 36–40 wk, 50% male, birth weight 2.8–4.3 kg, apgar 8 at 1 & 5 min); electrodes were attached immediately after babies’ placement on the radiant warmer. A PO sensor was placed per standard procedures. ECG and PO signals were digitized and recorded for approximately 5 min. Statistical comparisons were done by Student’s t-test for two time epochs (relative to time after placement of both devices): “epoch 1” defined as 0–120 sec, and “epoch 2” defined as 120 sec until end of recording. HR data from ECG and PO were also compared by Bland-Altman plot and frequency histogram.

Summary of Results: Relative to placement on the warmer, 39 ± 27 sec (mean ± SD; range 10–91 sec) was needed to connect the PO sensor to the baby and PO base unit, and 20 ± 6 sec (range 11–29 sec) was needed to attach ECG electrodes. An additional 123 ± 86 sec (range 46–306 sec) was needed for PO to give a “correct” HR (defined as ±60, 60–100, or >100 bpm by ECG). ECG required 1.7 ± 0.7 sec (p = 0.002 compared to PO; there was 85–90% power to detect a difference of 1 SD in time to “correct” HR by ECG vs. PO). 16 ± 11 sec was required for “correct” HR by palpation or auscultation. In epoch 1, PO and ECG HR agreed by
123 LENGTH OF UMBILICAL CORD AND FETAL BONE MINERALIZATION

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Purpose of Study: Activity promotes bone mass in infants and adults. However, the effect of in utero activity on the fetal bone mass has not been studied. One of the major restrictions to fetal activity may be the length of the umbilical cord. We hypothesized that the fetus with a shortened umbilical cord may have low bone mineralization because of decreased movement or activity. We investigated seven healthy term infants with shortened umbilical cords and 15 control term infants.

Methods Used: Mothers with pre-eclampsia, hypertension, choorioamnionitis or prolonged ruptured of membranes were excluded from the study as well as infants with congenital anomalies. Mother’s age, parity, infants’ gender and birth and placenta weights were recorded. The umbilical cord length and diameter were measured as well as the newborn’s tibial speed of sound (SOS). SOS measurements (Sunlight Omnisense 7000P instrument) were obtained at the tibial midshaft and reflect the tibial cortical thickness and bone mineral density.

Summary of Results: Measurement reproducibility was 0.8 ± 0.6% (mean ± SD) for repeat SOS measures in the same leg (n = 20). There were no differences between the experimental and control groups in mother’s age (24.6 ± 5.8 v 25.8 ± 3.3 years), parity (2 ± 1 v 2 ± 1), infant’s gender (29% females v 47% females), birth weights (3320 ± 451 v 3409 ± 452 g) or placental weights (521 ± 69 v 588 ± 105 g). Umbilical cord diameters were also similar, 1.1 ± 0.3 v 1.1 ± 0.2 cm. However, there was a difference in the cord length between the two groups, 46 ± 2 v 57 ± 4 cm (Mann Whitney, P < 0.001). The newborn infants with the short umbilical cord also had lower tibial SOS compared to controls, 3006 ± 96 v 3235 ± 304 m/sec (Mann Whitney, P < 0.05). Tibial SOS was related to the infant’s umbilical cord length (r = 0.57, P < 0.01) but not to infant’s birth weight, gender, umbilical cord diameter, maternal age, or placenta weight.

Conclusions: Infants with a short umbilical cord length have lower bone mineralization that may predispose to future bone problems.

Neuroscience I
Concurrent Session
8:30 AM
Friday, February 1, 2008

124 HISTOLOGICAL ANALYSIS OF POST MORTEM ALZHEIMER’S DISEASE AND CEREBRAL AMYLOID ANGIOPATHIC BRAIN AT POINT OF SMALL HYPOINTENSITIES IN SUSCEPTIBILITY WEIGHTED MR IMAGES

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Purpose of Study: The etiology of sporadic Alzheimer’s dementia (AD) is a subject which has been subjected to a great deal of research but despite the volumes we now know about the disease, its origins remain enigmatic. The hope for a cure or effective treatment must lie in early detection and diagnosis, as damage to mature neural pathways is all-too-often irreversible. A few key pieces of evidence have emerged that are consistent with vascular degeneration as a major finding in AD. Extracellular and intracellular iron deposits are present in the AD brain. Whether this is due to endogenous dyshomeostasis or exogenous deposition from the blood, or both, is not yet clearly established. The blood brain barrier is also frequently compromised in AD, leading to the extravasation of plasma components. The complete significance of this finding is not yet known. Finally, cerebral amyloid angiopathy (CAA) which results from the deposition of amyloid-beta peptide (Aβ) in the walls of cerebral vessels is found in as many as 95% of confirmed AD brains. Aβ is toxic to vascular smooth muscle cells and has been shown to cause a thinning of the vessel wall. Hemorrhaging from the microvasculature is a frequent finding in CAA. In light of these observations, we seek to implicate vascular degeneration as a factor in the etiology of AD.

Methods Used: Ongoing in vivo studies of mildly cognitive-impaired human subjects with an MR imaging technique that is sensitive to iron, namely susceptibility weighted imaging (SWI), have detected small hypointensities (SH) which may prove to correlate with the risk of conversion to AD. The current study seeks to clarify the identity and significance of SHs in confirmed CAA and AD post mortem brain by histological and immunological techniques.

Summary of Results: Hypointense residues on SWI correlate with microvascular hemorrhages and iron deposition accompanied by significant apoptotic neuronal loss and HO-1 dysregulation in the perifocal zone.

Conclusions: No previous study has provided systematic confirmation that SHs detected in dementia patients are related to bleeding, so our work represents an important step in the study of vascular degeneration’s role in neurodegeneration.

125 TISSUE LOSS IN PATIENTS WITH MILD COGNITIVE IMPAIRMENT AND ALZHEIMER’S DISEASE: A LONGITUDINAL VOXEL-BASED MORPHOMETRY STUDY

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Purpose of Study: Regional brain volume and gray matter (GM) loss is associated with Alzheimer’s disease (AD). The goal of this longitudinal study was to measure changes in GM, white matter (WM), and cerebrospinal fluid (CSF) volume using voxel-based morphometry to determine associations between cognitive impairment and tissue volume loss in subjects with mild cognitive impairment (MCI) and those that convert to AD.

Methods Used: 34 subjects were categorized as MCI (n = 11), AD-converters (n = 9) or controls (n = 14) using the Mini Mental State Exam (MMSE) and Clinical Dementia Rating (CDR) scale. Neurocognitive testing of executive function, word fluency, and episodic memory was evaluated every 6 months over a 3-year period. Following cognitive testing, MRI was performed on a 1.5T Siemens Vision MR scanner using a T1 MP-RAGE sequence (TR/TE = 20:5 ms, 2 mm slice thickness, 2562 matrix). Statistical Parametric Mapping software was used to measure total brain volume and density of each tissue type. To account for variations in inter-study duration, cognitive and imaging measures were analyzed as the annual percent change (APC). Statistical significance was
measured using one-way ANOVA. Correlations between MRI and cognitive data were determined using Pearson partial correlations.

**Summary of Results:** All subjects showed a significant increase in CSF volume over time. MCI subjects had a significant decrease in WM and AD converters had a significant decrease in GM volume. Only the AD converters showed a significant (p = 0.011) increase in the APC of CSF volume. However, only MCI subjects showed significant associations between the APC of WM loss and changes in episodic memory and executive function.

**Conclusions:** The conversion to AD is associated with an increased rate of change in tissue loss and CSF volume increase, compared to cognitively normal aging adults. Interestingly, MCI subjects showed a decrease in WM volume that was significantly associated with changes in neurocognitive function, findings not seen in the AD converter group, which may reflect compromised neuronal circuitry.

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**126 EFFECT OF D3 STEROISOMER OF A CARBOXY DERIVATIVE OF BUCKMENTERFULLERENE ON ACTION POTENTIALS OF GIANT AXONS OF THE CRAYFISH VENTRAL NERVE CORD**

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**Purpose of Study:** Following spinal cord injury, glutamate excitotoxicity can damage nerves by calcium entry via NMDA glutamate receptors. D3, a stereoisomer of a carboxyderivative of buckminsterfullerene that antagonizes NMDA receptors can depress electrically-evoked excitatory junctional potentials (EJPs) at neuromuscular junctions (NMJs) in the dactyl opener muscle of crayfish (Onconectes rusticus) walking limbs. To determine if D3 effects on presynaptic action potentials (APs) contribute to the EJP depression, we examined the effect of D3 on AP amplitude and conduction velocity (CV).

**Methods Used:** The ventral nerve cord was exposed in the cephalothorax and abdomen. A cord segment (approximately 3 cm) was dissected and placed on an electrode grid in a nerve recording chamber. The cord was kept moist with van Haarvevd's (VH) solution (pH 7.2± 0.1). The nerve was stimulated with a superthreshold, 0.4 ms bipolar pulse at 0.1 Hz. Data were recorded with a digital storage oscilloscope. Following a pretreatment control, 50μM D3 in VH was applied to the nerve between the stimulating and recording electrodes. APs were recorded immediately following application and at 5 min post treatment. The D3 application area was then washed with VH and a posttreatment control AP was recorded.

**Summary of Results:** Changes in AP amplitude (≤ 0.2 mV) and CV (0 – +5% of CV) following 50μM D3 treatment were not significant. Qualitatively similar results were obtained with 4 preparations.

**Conclusions:** The 50μM D3 concentration that depressed EJP amplitude did not have significant effects on AP amplitude or CV. It is unlikely that a D3 effect on the presynaptic AP contributed to D2-induced depression of EJPs in this preparation, unless the effect differs at terminal branches of the axon.

Supported by the Arnold C., Barbara M. and Georgianna Fossa Spinal Cord Injury Research Fund and the IU School of Medicine Brain and Spinal Cord Injury Research Program.

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**127 ENHANCEMENT OF EXCITATORY JUNCTIONAL POTENTIALS BY HOMOQUINOLINIC ACID ON NMDA GLUTAMATE RECEPTORS**

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**Purpose of Study:** Glutamate excitotoxicity mediated by NMDA glutamate receptors causes secondary injury to neurons in spinal cord injury (SCI). Crayfish (Orconectes rusticus) dactyl opener muscle neuromuscular junctions (NMJs) provide a model for studying NMDA glutamate receptor activity. A potent NMDA glutamate receptor agonist, homoquinolinic acid (HQA), was studied to identify its effect on glutamate-mediated excitatory junctional potentials (EJPs) of NMJs at the dactyl opener muscle of crayfish walking limbs.

**Methods Used:** A 1st or 2nd walking limb was removed. The meropodite was dissected to isolate the single excitatory axon. The carpopodite was dissected to expose the opener muscle. The preparation was bathed in Van Haarvevd’s (VH) solution (pH 7.2 ± 0.1) for collection of pre- and posttreatment data. Following a pretreatment control, HQA diluted in VH was applied at the NMJ. Using standard intracellular electrophysiological techniques, EJPs were recorded immediately following application, at 1 minute of HQA exposure, and following washing. Post-treatment data were collected following washing, defined as three complete bath changes using VH. Short-term facilitated (10 second stimulus at 30 Hz) EJPs were averaged over 10 seconds. All data collections were initiated following 10s of stimulation.

**Summary of Results:** Data were reported as percent change from control amplitude for each preparation. Results from 3 to 6 preparations per concentration were reported. 2 mM HQA enhanced EJP amplitude 13.4%; 1 mM HQA enhanced EJP amplitude 16.5%; 0.1 mM HQA enhanced EJP 17.0%; 0.05 mM HQA enhanced EJP amplitude 21.7%.

**Conclusions:** HQA enhances EJP amplitude over a range of concentrations. Further research needs to establish the ED50 HQA enhancement of EJP amplitude. Finally, this study confirms the presence of NMDA glutamate receptors in the crayfish dactyl opener muscle preparation.

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**128 DIFFERENTIAL ACTIVATION OF CASPASE-3 IN HIPPOCAMPAL GLIAL CELLS BY ASSEMBLY FORMS OF Aβ1-42**

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**Purpose of Study:** To examine the ability of different assembly forms of Aβ1-42 to cause activation of caspase-3 in hippocampal glial cells that have been either co-cultured with neurons or cultured alone.

**Methods Used:** Fibrillar Aβ42 was prepared by aggregation of HIFP-disaggregated peptide in dilute HCl, ADDLs were prepared following a published protocol, and smaller, less aggregated forms were prepared by aggregation in Neurobasal medium. Activated caspase-3 was detected in individual cells using a specific, fluorogenic substrate or antibodies for the enzyme, and individual GFAP-positive glial cells were scored for the presence or absence of activated enzyme by fluorescence microscopy and flow cytometry. Western blotting was also employed to test quantitative levels of caspase-3 activation.

**Summary of Results:** Results to date have shown significant activation, p<0.01, of caspase-3 in the fibrillar Aβ42 exposed co-cultured GFAP-positive glial cells, by means of fluorescence microscopy. Initial flow cytometry data supports the scoring percentages performed by fluorescence microscopy. Western blotting data is in progress.

**Conclusions:** Amyloid-beta (Aβ) peptides are the primary constituents of amyloid plaques in the brain in Alzheimer’s disease (AD). Significant evidence indicates that Aβ peptides cause AD, but how they do so is not clear. Aβ peptides spontaneously aggregate, or self-assemble, to
generate distinct macromolecular forms that can differ significantly in their effects on cells. We showed previously that 1) more aggregated forms of Aβ1-42 (Aβ42), including both highly enriched preparations of fibrils and a mixture of assembly forms that has been termed Aβ-derived diffusible ligands (ADDLs), bind to neurons much more abundantly than preparations that contain smaller forms and lack visible fibrils, 2) fibrillar Aβ42 binds almost exclusively at extrasynaptic sites, and 3) fibrillar Aβ42 consistently causes activation of caspase-3, a marker of neurodegeneration in AD, in neurons, while other assembly forms tested, including ADDLs, do not. This project attempts to tie these findings to hippocampal glial cells. If supported by further data, the results to date would suggest that a specific form of Aβ42 triggers apoptosis in hippocampal glial cells.

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FUNCTIONAL EFFECTS OF CHLOROTOXIN ON HUMAN GLIOMA CELLS IN VITRO
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Purpose of Study: Chlorotoxin (CITx), named for its suspected action as a chloride channel blocker, is a 36-amino acid peptide purified from the Leiurus quinquestriatus scorpion which has a unique specificity for malignant versus normal cells. Importantly, the native protein has been shown to reduce glioma growth in animal models suggesting CITx has intrinsic anti-glioma effects. However, the mode of action for CITx has not been well characterized and its binding partner(s) are not known. Therefore in this study we sought to elucidate the mechanisms of CITx anti-tumor activity and its protein binding partners through functional assays of invasion, cell growth, migration, radiosensitivity, and angiogenesis as well as a proteomic analysis of CITx binding partners.
Methods Used: CITx tumor specificity was studied by direct immuno-histochemical detection of biotinylated-CITx in tissue sections. Assays to characterize the biological action of CITx included cell growth (MTT assay), invasion (matrigel filter), migration (wound assay), in vitro radiosensitivity (colony forming assay after irradiation), and angiogenesis (endothelial cell culture on confluent glioma cells or co-culture on matrigel substrate). To identify CITx binding partners, proteins from glioma cell lysates treated with biotinylated-CITx were eluted and identified by GCMS.
Summary of Results: Similar to previous reports, CITx demonstrated specific binding to glioma tumor tissues. Contrary to prior reports, CITx exposure in 2 cell lines did not decrease invasion. CITx treatment had no significant impact on cell growth, migration or radiosensitivity. Preliminary analysis indicated a possible modest effect on endothelial cell morphology and tube formation. Candidate binding proteins are under analysis to validate their specificity for CITx binding in vitro.
Conclusions: These studies confirm the unique tumor specific binding properties of CITx. The present study indicates that CITx action may possibly function to abrogate angiogenesis rather than through mechanisms related to tumor cell growth, invasion, or radiosensitivity. Further study is warranted to confirm this finding and establish other anti-neoplastic actions in vivo. Ultimately, the validation of CITx binding partners will provide important insight into mechanisms of CITx anti-glioma activity and new therapeutic applications.

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THE ROLE OF PROOPIOMELANOCORTIN NEURONS IN THE HYPERPHAGIC EFFECT OF ORPHANIN FQ
B. Farhang1, K. Lutfy2, and E.J. Wagner1. 1Western University of Health Sciences, Pomona, CA and 2Western University of Health Sciences, Pomona, CA.

Purpose of Study: The specific aim of the research is to show that Orphanin FQ (OFQ) OFQ stimulation of feeding is due to pre- and postsynaptic effects on proopiomelanocortin (POMC) neurons, which are a critical anorectic component of the hypothalamic feeding circuitry.
Methods Used: Feeding studies were conducted over the course of 5–6 days in ORL1 receptor knockout (KO) mice and their wildtype (WT) littermate controls that were fasted 18 hr prior to initiating the six-hr observation window. We also performed whole-cell patch clamp recordings in hypothalamic slices from ORL1 KO mice and their corresponding WT littermates.
Summary of Results: We found that ORL1 KO mice exhibited a comparatively blunted hyperphagic response; consuming less food over the first three hrs of the observation window. Meal consumption occurred at a higher frequency, but the meals were of shorter duration and less food was eaten per meal as compared to the WT littermates. In addition, the average daily weight of the ORL1 KO animals was significantly less than their WT littermates. Whole-cell patch clamp recordings of arcuate neurons obtained from WT controls, revealed that OFQ (1μM) induced a decrease in the frequency but not amplitude of miniature excitatory postsynaptic currents that were antagonized by the ionotropic glutamate receptor antagonists NBQX (3μM) and CGS 19755 (10μM). OFQ also elicited an reversible outward current that reversed polarity near the Nernst equilibrium potential for K+. Exhibited increased conductance at membrane potentials more hyperpolarized than the reversal potential, and was attenuated by the G protein-gated inwardly-rectifying K+ (GIRK) channel blocker tertiapin (10 nM). Cells showing these responses expressed both the posttranslational POMC hyproduct α-melanocoyte stimulating hormone and the GIRK1 channel subtype. By contrast, arcuate neurons from ORL1 receptor KO animals did not express the ORL1 receptor, and as a result the actions of OFQ were negated.
Conclusion: These findings indicate that the hyperphagic effect of OFQ is ORL1 receptor-mediated and due, at least in part, to a combination of presynaptic inhibition of glutamate synaptic input onto anorexigenic POMC neurons, and the postsynaptic activation of GIRK1 channels in these cells.

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SEX DIFFERENCES IN THE APPETITE-MODULATING EFFECTS OF CANNABINOIDS UNDER DIFFERENT MOTIVATIONAL CONDITIONS
S.V. Diaz and E.J. Wagner. Western University of Health Sciences, Pomona, CA.

Purpose of Study: Convincing evidence indicates that cannabinoid regulation of reproduction and other homeostatic functions such as appetite occurs via the hypothalamus. There are also reports of sexually differentiated physiological effects of cannabinoids, including hemodynamics, locomotion, and nociception. This study sought to determine the effect of cannabinoid agonists and antagonists administered under different motivational conditions (e.g., ad libitum vs. restricted food access), as well as any sex differences that affect feeding behavior under these different motivational conditions. The working hypothesis is that both endogenous and exogenous cannabinoids increase food intake in a sexually differentiated manner.

Methods Used: The study was conducted using castrated male and female Topeka guinea pigs. Feeding behavior was monitored under food restricted and ad libitum feeding conditions, using a Comprehensive Lab Animal Monitoring System (CLAMS) from Columbus Instruments. For seven consecutive days, the animals were weighed, injected with a CB1 receptor agonist or antagonist, and their feeding behavior monitored. Food restricted animals were monitored for a six hour period in which they had access to food, while ad libitum animals were monitored around the clock.
Summary of Results: Under ad libitum conditions, the CB1 receptor agonist WIN55,212-2 (1 mg/kg; s.c.) increased absolute food intake for both males and females. However, the response was more prominent in males, and was associated with increased meal duration, as well as an increase in the amount eaten per meal and per gram body weight in males but not females. The CB1 receptor antagonist AM251 caused a decrease in meal frequency, a decrease in the amount of food per gram body weight and, paradoxically, an increase in the amount eaten per meal for males but not females. Similar trends in feeding behavior were also seen in food restricted conditions.

Conclusions: The data from these behavioral studies demonstrate that males are much more responsive to cannabinoid modulation of feeding behavior. Our findings indicate that males would be more responsive to the therapeutic potential of cannabinoids in the treatment of AIDS and cancer related cachexia, as well as obesity.

132 ESTRADIOL ATTENUATES OXYTOCIN-INDUCED CALCIUM SIGNALING IN HYPOTHALAMIC ASTROCYTES

O.R. Hariri, J. Kuo, and P.E. Micevych. David Geffen School of Medicine at UCLA, Los Angeles, CA.

Purpose of Study: Astrocytes mediate important functions of the nervous system. Gonadal steroids in general and estradiol in particular have emerged as a key regulator of astrocytes. Another important signal in the hypothalamus is oxytocin (OT). Since estradiol and OT interact to regulate a number of neuroendocrine functions that may involve nervous system. Gonadal steroids in general and estradiol in particular may involve

Methods Used: We investigated the interaction of estradiol and OT stimulation of hypothalamic astrocytes. Methods Used: We investigated the interaction of estradiol and OT stimulation of hypothalamic astrocytes by examining their effects on free cytoplasmic calcium concentration ([Ca2+]i) monitored with the calcium indicator, Fluo-4.

Summary of Results: The estrogen receptor (ER) antagonist, ICI 182,780 (2 μM), blocked the rapid [Ca2+]i flux (ΔF Ca2+ 518.18 ± 70 vs. 208.69 ± 50; p < 0.05). OT also produced [Ca2+]i flux in 90% of astrocytes. Interestingly, the astrocyte response to OT, up to a concentration of 400 nM, was not as dramatic as that induced by estradiol (ΔF Ca2+ = 576.7 ± 70 vs. 282.2 ± 40). As it has been demonstrated in neurons, rapid actions of estradiol on [Ca2+]i flux in astrocytes are mediated by group I metabotropic glutamate receptors, specifically the mGlur1a. The mGlur1a antagonist, LY367385 (20 nM), decreased the estradiol-induced [Ca2+]i flux from 580.3 ± 45 to 271.45 ± 66 (p < 0.05). Moreover, LY367385 also blocked the OT-induced [Ca2+]i flux (559.86 ± 70 vs. 92.15 ± 30; p < 0.05). These results point to a convergence of estradiol and OT signaling at the mGlur1a. While OT did not interfere with estradiol signaling, estradiol severely attenuated OT signaling in astrocytes. In astrocytes treated with OT (400 nM) and then estradiol (2 nM) + OT, the [Ca2+]i flux was comparable (ΔF Ca2+ = 310 ± 50 vs. 550 ± 45; p < 0.05).

Conclusions: When astrocytes were first treated with estradiol and then OT, the [Ca2+]i flux was blocked. These results indicate that estradiol can block the actions of OT on Ca2+ signaling in astrocytes, and suggest another point of estradiol regulation of the OT system.

133 CHARACTERIZATION OF BRAIN DAMAGE AND TAUOPATHIES IN A MOUSE MODEL OF HYPOXIA-ISCHEMIA

N.H. Nguyen, G. Liao, and X. Bi. Western University of Health Sciences, Pomona, CA.

Purpose of Study: Stroke is leading neurological disorders in elderly population. In this study, we characterized the brain damage induced by a modified Levine model in C57Bl6 mice using Nissl staining. Previously Dr. Guanghong Liao and colleagues have shown that the modified Levine model combines hypoxia-ischemia thus gives the advantage of controlling brain damage by manipulating levels of hypoxia. Our results showed that consistent brain damage was induced using the modified model, which confirms that the modified model is an excellent stroke model. Recent studies also show that truncation of tau at site D421 is associated with cell death in cultured neurons following various insults, including amyloid-beta peptide treatment. Therefore, the relation between stroke-induced neuronal cell death and tau phosphorylation and tau truncation was also investigated. We found that there was a marked increase in tau truncation in the C57Bl6 mice at 24 hours and 5 days after hypoxia-ischemia. In term of tau phosphorylation, stroke increased phosphorylation at sites recognized by AT-8 antibody but reduced phosphorylation at sites recognized by AT-231 antibody in C57Bl6 mice. Interestingly, double immunostaining with AT-231 and Tau-C revealed a "complementary" staining pattern, suggesting that phosphorylation at AT231 sites may interact with caspase-3 mediated truncation. Together, these findings suggest that tau truncation rather than phosphorylation is probably related to stroke-induced brain damage, at least in C57Bl6 mice.

Methods Used: Modified Levine model combines hypoxia-ischemia. Summary of Results: Nissl staining showed that damaged brain areas accounted for about 80% of the hemisphere in wildtype mice. We measured the phosphorylation of tau protein using the antibody AT-8 and AT-231. AT-8 immunoreactivity was higher on the lesion side. AT-231 decreased 30% compared to the contralateral side. We measured the commitment of apoptosis in neuronal cells using the antibody cleaved caspase-3 which increased on the lesion side. We found that truncated tau increased in the beginning and spread in the whole region.

Conclusions: Results from double immunostaining with AT231 and Tau-C antibodies indicate an interaction between tau phosphorylation and truncation, and tau truncation may play critical roles in stroke-induced acute brain damage.

134 INHIBITION OF CALCIUM/ CALMODULIN-DEPENDENT PROTEIN KINASE II (CaMKII) PROTECTS AGAINST HYPOGLYCEMIA-INDUCED NEURONAL CELL DEATH

R.J. McMurtrey, R. Vest, L. McMurtrey, and K.U. Bayer. UCHSC. Denver, CO.

Purpose of Study: The calcium/calmodulin-dependent protein kinase II (CaMKII) is a major mediator of calcium signaling in neurons. This enzyme plays an important role in modulating synaptic plasticity and long-term potentiation. Interestingly, there is some evidence that CaMKII regulates neuronal cell death, but the mechanisms by which it does so are presently not known. The aim of this study is to determine the role of CaMKII in the death of hippocampal neurons in dissociated culture.

Methods Used: Rat hippocampal cultures were subjected to a variety of conditions at 7 days-in-vitro, including glucose deprivation (GD) alone, GD with tacCN21 (a CaMKII-inhibitor designed by the lab, which, unlike other available inhibitors, has thus far proven to be entirely specific to CaMKII) added at insult or at 90 minutes, GD with KN93 (a non-specific CaMKII inhibitor) added at insult or at 90 minutes, GD with APV (a glutamate antagonist) added at insult, or excitotoxic glutamate exposure. Analysis of cell death was done using LDH assays at 24 hours post-insult. Glutamate antagonist) added at insult, or excitotoxic glutamate exposure. Analysis of cell death was done using LDH assays at 24 hours post-insult. Conditions at 7 days-in-vitro, including glucose deprivation (GD) alone, GD with tacCN21 (a CaMKII-inhibitor designed by the lab, which, unlike other available inhibitors, has thus far proven to be entirely specific to CaMKII) added at insult or at 90 minutes, GD with APV (a glutamate antagonist) added at insult, or excitotoxic glutamate exposure. Analysis of cell death was done using LDH assays at 24 hours post-insult. Conditions at 7 days-in-vitro, including glucose deprivation (GD) alone, GD with tacCN21 (a CaMKII-inhibitor designed by the lab, which, unlike other available inhibitors, has thus far proven to be entirely specific to CaMKII) added at insult or at 90 minutes, GD with APV (a glutamate antagonist) added at insult, or excitotoxic glutamate exposure. Analysis of cell death was done using LDH assays at 24 hours post-insult. Conditions at 7 days-in-vitro, including glucose deprivation (GD) alone, GD with tacCN21 (a CaMKII-inhibitor designed by the lab, which, unlike other available inhibitors, has thus far proven to be entirely specific to CaMKII) added at insult or at 90 minutes, GD with APV (a glutamate antagonist) added at insult, or excitotoxic glutamate exposure. Analysis of cell death was done using LDH assays at 24 hours post-insult. Conditions at 7 days-in-vitro, including glucose deprivation (GD) alone, GD with tacCN21 (a CaMKII-inhibitor designed by the lab, which, unlike other available inhibitors, has thus far proven to be entirely specific to CaMKII) added at insult or at 90 minutes, GD with APV (a glutamate antagonist) added at insult, or excitotoxic glutamate exposure. Analysis of cell death was done using LDH assays at 24 hours post-insult.
Summary of Results: Preliminary data from both cell death assays and morphological analysis show that CaMKII inhibition with tapCN21 can provide significant protection against neuronal death induced by glucose deprivation, remarkably even when administered 90 minutes after exposure to glucose-free conditions. The non-specific CaMKII-inhibitor KN93 also significantly protects against cell death when administered at 90 minutes, but not when administered at 90 minutes. In all glucose deprived conditions the predominant mechanism of cell death was apoptotic. Apoptotic morphology was also reduced in all conditions that protected against cell death.

Conclusions: This research further elucidates the role of CaMKII in neuronal cell death, and suggests that CaMKII is an important pathway for apoptotic cell death. Much more research will be done with this new CaMKII-specific inhibitor. This research holds implications for stroke, CNS trauma, and the role of glucose management in diseases of the nervous system.

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THE EFFECTS OF EXPERIMENTALLY-INDUCED CONDUCTIVE HEARING LOSS ON SPECTRAL AND TEMPORAL ASPECTS OF SOUND TRANSMISSION THROUGH THE EAR
J.E. Lupo, K. Koka, and D.J. Tollin. University of Colorado at Denver and Health Sciences Center, Aurora, CO.

Purpose of Study: Conductive hearing loss (CHL) is known to produce hearing deficits, including sound localization ability. The differences in sound intensities and timing experienced between the two tympanic membranes are important cues to sound localization. Although much is known about the effect of CHL on hearing level, little investigation has been done into the effects on timing and subsequent effects on the cues to location. This study investigated effects of earplugs on cochlear microphonic (CM) amplitude and timing and their corresponding effect on two localization cues, interaural level difference (ILD) and interaural time difference (ITD).

Methods Used: Acoustic and CM measurements were made in 5 chinchillas before, after earplug insertion and after earplug removal using pure tones (500 Hz to 24 kHz).

Summary of Results: Occlusion resulted in a variable mild hearing loss of 20–33 dB (mean 27 ± 4.9 dB) depending on frequency. In the normal and occluded situation, ILDs increased with increasing frequency (5.1 dB and 26 dB at 500 Hz and 20 kHz, respectively). Across all positions in azimuth, with the ear occluded, ILD magnitude was increased. At 0°, with the ear occluded, timing delays varied from −400 to −1500 μs (mean −800 ± 0.5 μs) with 500 Hz demonstrating the greatest delays. ITDs in the normal and occluded cases decreased with increasing frequency across all positions in azimuth. ITD magnitude with the ear occluded increased as a result of conduction delays at the occluded ear.

Conclusions: CHL leads to substantial changes in the magnitudes of both the ITD and ILD cues to sound location, which results in a shifted auditory space. This may be the basis for the difficulties in sound localization seen in patients with CHLs.

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SPIROMETRIC CHANGES IN PATIENTS UNDERGOING HYPERBARIC OXYGEN THERAPY

Purpose of Study: To determine if long-term hyperbaric oxygen therapy is associated with changes in lung functions.

Methods Used: Spirometry was performed on patients undergoing hyperbaric oxygen (HBO) therapy. Tests were performed before, during, and up to a year after HBO treatment. Patients underwent hyperbaric oxygen therapy for the following indications: compromised skin graft/ flap, diabetic foot ulcer, refractory osteomyelitis and soft tissue radiation damage.

Summary of Results: 13 patients (10 males and 3 females) with an average age of 62 were enrolled. Spirometric measurements pre- and post-HBO treatment did not differ. [See table]
Conclusions: There are no statistical significant differences in spirometric measurement of patients undergoing long-term HBO therapy. Further investigation on a larger patient population is ongoing.

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<th>P-value</th>
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PNEUMONITIS IN LIVER TRANSPLANT RECIPIENTS RECEIVING SIROLIMUS

K. Zeschin, and J.F. Trotter. University of Colorado Health Sciences Center, Denver, CO.

Purpose of Study: Sirolimus (SIR) has become an increasingly popular method of immunosuppression in liver transplantation. Pneumonitis is an uncommon side effect of this drug and has rarely been reported after liver transplantation. Because we have one of the largest cohorts of liver transplant recipients receiving SIR, we reviewed our cohort to determine the incidence of SIR-induced pneumonitis.

Methods Used: The charts of liver transplant recipients transplanted between 1988 and 2007 at the University of Colorado Health Sciences Center were reviewed to determine exposure to SIR. Of all our transplant patients, 414 received SIR at some point after transplant. These patients were retrospectively analyzed for pneumonitis which was confirmed histologically by bronchoscopy in all cases.

Summary of Results: Of 414 liver transplant patients receiving SIR, 4 (0.97%) developed pneumonitis. These patients’ symptoms included shortness of breath and wheezing with occasional instances of dry cough and fever. Three of the four patients were male, and all were over the age of 62 (range 62–75, average 67.5). The average time after transplant until SIR initiation was 50.75 months. The average time of SIR usage until presentation of symptoms was 9.5 months, with three of the four patients receiving SIR for less than a year before symptoms occurred. Three of the four patients received 2 mg SIR per day, with the fourth patient receiving 1 mg. The average SIR level at the time of symptoms was 14.4 ng/ml. Three patients were using cyclosporine as immunosuppression at the time of symptoms.

Conclusions: 1) Although rare, SIR-induced pneumonitis occurs in 1% of all patients. 2) Male patients over 60 years of age may be at particular risk for this complication. 3) With SIR being administered to an increasing number of liver transplant recipients, clinicians should be aware of this important side effect.

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PROGRESSION OF EARLY AIRWAY EFFECTS OF ½ MUSTARD EXPOSURE DOCUMENTED BY OPTICAL COHERENCE TOMOGRAPHY

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Purpose of Study: Optical Coherence Tomography (OCT) is a non-invasive, high resolution imaging technology capable of delivering real-time, near histologic images of tissues. Mustard gas is categorized as a vesicant/blistering agent used in wartime. The primary consequences of exposure include severe damage to airway, lungs, internal organs, eyes and skin. The ability to assess airway injury in this clinical setting is limited. The purpose of this study is to assess the ability of OCT to detect and monitor progression of ½ mustard airway injuries with minimally invasive techniques.

Methods Used: A ventilated New Zealand white rabbit mustard exposure airway injury model was developed. A 1 mm diameter flexible fiberoptic OCT probe was introduced into the distal trachea to image airway epithelium and mucosa. Progression of injury and edema was observed over eight hours with OCT using a prototype superluminescent diode OCT system we constructed in our laboratory. OCT tracheal images from the mustard exposed animals were compared to control rabbits for mucosal thickening and other changes. Histological examination of the treated versus control tracheal tissue was completed.

Summary of Results: OCT documented the early occurrence and progression of dramatic changes including thickening and sloughing of epithelium and mucosa in the experimental group, consistent with tracheal edema after exposure to ½ mustard. Histology confirmed significant hemorrhage, epithelial and mucosal changes.

Conclusions: OCT has the potential to be a valuable, high resolution imaging modality capable of evaluating, assessing and directing treatment for airway injury following mustard exposure with high resolution after acute vesicating agent injury.

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EFFECTIVENESS OF MEDICAL SIMULATION ON KNOWLEDGE IN SEPTIC SHOCK MANAGEMENT DURING PRE-CLINICAL MEDICAL TRAINING

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Purpose of Study: Evaluate the use of simulation-based teaching during the pre-clinical years of medical training to increase future clinician knowledge in the treatment of septic shock.

Methods Used: This is a prospective interventional study at a university-based medical simulation center. A 5-hour curriculum including didactic lectures, skill workshops on central line insertion and intubation, and simulated case scenario of a septic shock patient were administered to incoming first-year and second-year medical students. A skills checklist including 21 tasks was completed during the case scenario. An 18-question pre-test, post-test and 2-week post-test were given to evaluate the effectiveness of the curriculum on student knowledge and knowledge retention. The students also completed a
Conclusions: Medical simulation is an effective method of educating medical students regarding septic shock management prior to their clinical training. From a scale of 1–5, first-year students noted their pre-curriculum and post-curriculum confidence levels in managing septic shock patients were 1.0+/−0.0 and 2.9+/−0.6, respectively, p < 0.01. Second-year students noted their confidence levels as 1.3+/−1.0 and 3.3+/−0.7, respectively, p < 0.01. First and second-year students agreed or strongly agreed that the curriculum should be a requirement during medical school training, 4.5+/−0.7 and 4.7+/−0.7, respectively.

Conclusions: Medical simulation is an effective method of educating medical students regarding septic shock management prior to their clinical training.

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**DEFICIENCY OF MAP KINASE PHOSPHATASE-1, A CRITICAL INFLAMMATORY MODULATOR, INDUCES DEVELOPMENT OF PULMONARY HYPERTENSION IN MICE**

K.M. Shields1, J. Harral1, M. Oka2, N. Honma2, N. Burns1, J. West2, and M. Das1. 1University of CO Denver Health Sciences Center School of Medicine, Denver, CO; 2UCDHSC, Denver, CO and 3UCDHSC, Denver, CO.

**Purpose of Study:** MAP Kinase Phosphatase-1 (MKP-1), a deactivator of MAP kinases, is an important inflammatory regulator. However, the role of MKP-1 in pulmonary hypertension is unknown. We hypothesized that MKP-1 will contribute to development of pulmonary hypertension by dephosphorylating the MAP kinase pathway.

**Methods Used:** MKP-1 null (MKP-1−/−), heterozygous (MKP-1 +/−), and wild type (MKP-1+/+) mice (4–5 weeks old) (Lexicon Genetics Incorporated) were exposed to sea level (SL), Denver altitude (DA; 5280 feet), or severe altitude (HYP; 17,000 feet) for 6 weeks and then assessed for weight gain, cardiac hypertrophy measured by right ventricle to left ventricle + septum wet weight ratio (RV/LV+S), and right ventricular systolic pressure (RVSP) via cardiac catheterization. Lung sections were stained for alpha smooth muscle actin (SMA), phosphoERK1/2, phosphoJNK, phospho-p38, and COX-2. Lung sections were stained for alpha smooth muscle actin (SMA), phosphoERK1/2, phosphoJNK, phospho-p38, and COX-2.

**Summary of Results:** MKP-1−/− had the least weight gain and increased weight gain in MKP-1−/− and MKP-1+/−. RV/LV+S was increased by HYP in all groups (MKP-1−/−: SL 0.27, DA 0.29, HYP 0.40; MKP-1+/−: SL 0.28, DA 0.27, HYP 0.40; MKP-1+/+: SL 0.26, DA 0.29, HYP 0.42). RVSP at all altitudes correlated with the corresponding RV/LV+S. SMA staining was increased in the HYP lungs of MKP-1−/− and MKP-1+/− mice. Vessel to alveoli ratio was decreased in MKP-1−/− lungs of MKP-1−/− and MKP-1+/− mice. Vessel to alveoli ratio was decreased in HYP lungs of MKP-1−/− and MKP-1+/− mice. Vessel (150μm) wall thickness to diameter ratio was not affected by hypoxia in MKP-1−/− lungs (DA: 0.14 vs. HYP: 0.12), but increased in MKP-1+/− (DA: 0.12 vs. HYP: 0.18) and MKP-1+/+ (DA: 0.12 vs. HYP: 0.2) lungs. Immunoreactivity against phosphoERK1/2 and phosphoJNK was similar in all groups. Phospho-p38 staining intensity was highest in the MKP-1−/− lungs with positive reaction in vessels, respiratory epithelium, and interstitial cells. COX-2, which is induced during inflammation, also had strongest staining in the MKP-1−/− lungs. Conclusions: Lack of MKP-1 causes sustained p38 activation in the lung, which may be a major contributor to the development of pulmonary hypertension.

## 142
**POLYMORPHISMS IN THE ACUTE LUNG INJURY CANDIDATE GENE PRE-B-CELL COLONY ENHANCING FACTOR ARE ASSOCIATED WITH HUMAN NEUTROPHIL RESPONSES**

J.B. Stansberry1, J. Nick1, K. Poch2, E. Abraham3, M. Tripp-Addison3, D. Calabrese2, and J.P. Maloney3. 1St Joseph Hospital, Denver, CO; 2University of Colorado, Denver, CO and 3National Jewish Medical and Research Center, Denver, CO.

**Purpose of Study:** Sepsis is associated with significant morbidty, mortality, and end-organ dysfunction including Acute Lung Injury (ALI). The neutrophil (PMN) plays a primary role in the pathogenesis of sepsis. Inherited factors appear to determine sepsis risk and outcomes. Single nucleotide polymorphisms (SNP) in Pre-B-Cell Colony Enhancing Factor (PBEF) have been associated with human ALI. Interestingly, PBEF functions as a pro-survival factor for PMN. We hypothesized that common SNP in PBEF determine heritable variations in PMN activity relevant to ALI risk.

**Methods Used:** PMN activity was studied ex vivo in 154 samples from 46 healthy volunteers (1–6 separate isolations each) for lipopolysaccharide (LPS)-induced PMN necrosis (nonapoptotic, uncoordinated cell death) and TNF-alpha release at 4hr; and superoxide (O2−) release to phorbol esters (PMA) at 15 min. All genotyping was by Taqman. We genotyped 10 common (frequency >5%) single nucleotide polymorphisms (SNP) in 100 healthy controls to define linkage disequilibrium (LD) and the “tagged” SNP genotyping set. We determined the association of 6 tagged SNP with PMN activation parameters. Statistical analysis was by ANOVA and student’s t-testing between genotype groups. Significance was defined as p < 0.05.

**Summary of Results:** The C-1545T promoter SNP (T allele previously associated with decreased ALI risk) was significantly associated with less LPS-mediated necrosis (34% CC, 27% CT, 23% TT: p = 0.005). The G-948T promoter SNP was associated with higher necrosis and higher TNF-alpha release (GG vs. GT: p < 0.01; no TT carriers). A SNP in intron 6 (IVS6+ 4760T/C) in full LD with an exon 7 SNP was associated with large variations in LPS-mediated necrosis (TT 27%, CT 33%, and CC 42%; p < 0.01) and in superoxide release (TT 1.5 uM, CT 2.3 uM; p = 0.001).

**Conclusions:** These genetic differences in bacterial lipopolysaccharide-stimulated necrosis, cytokine release, and superoxide release from human neutrophils represent crucial functional evidence to support PBEF genetic variation as an ALI risk factor.

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**DIFFUSE OPTICAL SPECTROSCOPY (DOS) MEASURED TISSUE SCATTERING IN RABBIT HEMORRHAGE MODEL**

R. Goldberg1, R. Patino1, J. Lee2, S. Mahan2, K. Kreuter2, D. Mukai2, J. Kim2, B. Tromberg2, and M. Brenner1. 1UC Irvine, Orange, CA and 2UC Irvine, Irvine, CA.

**Purpose of Study:** Hemorrhage is a potentially lethal event that can occur after trauma. Methods for evaluating degree of hemorrhage and resuscitation include measurement of hemoglobin in blood. However, changes in hemoglobin levels associated with acute hemorrhage are often delayed. Broadband DOS provides the potential for accurate measurement of changes in hemoglobin levels associated with acute hemorrhage.
THE MODULATION OF PROINFLAMMATORY CHEMOKINE EXPRESSION IN HUMAN AIRWAY EPITHELIAL CELLS BY SIMVASTATIN

A.A. Zeki1,2, P. Thai1,2, and R. Wu1,2. 1 U.C. Davis Medical Center, Sacramento, CA and 2 Center for Comparative Respiratory Biology & Medicine, Davis, CA.


Methods Used: Immortalized human bronchial epithelial cells (hBEC-1) were grown under air-liquid interface conditions. Cells were treated with simvastatin (Sim) 5 μM & 25 μM 90 min before cytokine treatment. Proinflammatory cytokines TNF-α, IL-17, & IL-13 were incubated with simvastatin for 24 hrs in cell culture. RT-PCR of chemokines Gro-β, CCL20, & eotaxin-2 mRNA expression was performed (mRNA normalized to β-actin). DMSO was the drug vehicle & drug control.

Summary of Results: In the simvastatin treated groups, there was a decrease in TNF-α-induced Gro-β expression, IL-17-induced Gro-β expression, and IL-17-induced CCL20 expression. There was no attenuation of IL-13-induced eotaxin-2 expression (data not shown).

Conclusions: These preliminary data suggest that simvastatin attenuates airway epithelial cell chemokine expression, specifically Gro-β and CCL20, but not eotaxin-2. Since TNF-α and IL-17 activate NF-κB, the observed attenuation may occur through inhibition of the NF-κB pathway. Ongoing work will test if simvastatin reduces chemokine expression via inhibition of NF-κB signaling.

145 PULMONARY CHARACTERISTICS OF HEPATOPULMONARY SYNDROME AT A HIGH ALTITUDE PULMONARY LABORATORY

H. Gallo, R.O. Crapo, and R.L. Jensen. LDS Hospital, Salt Lake City, UT.

Purpose of Study: Hepatopulmonary syndrome is a fairly common finding in patients awaiting liver transplantation and is defined by the presence of liver disease, an increased A-a gradient, and increased platypnea and orthodeoxia in an upright position. The reported frequency of hepatopulmonary syndrome varies to a great extent. The goal of this analysis was to assess the characteristics and frequency of hepatopulmonary syndrome at a location approximately 4500 feet above sea level.

Methods Used: Between 1994 and 2007, 655 liver failure patients awaiting liver transplantation were evaluated for hepatopulmonary syndrome at LDS Hospital in Salt Lake City, UT. Each patient was evaluated in three positions. They were asked to sit for 10 minutes, stand for 10 minutes, and then lie down for 10 minutes. At the end of each 10 minute interval, oxygen saturation was recorded and the patient was asked to score their shortness of breath using a BORG scale. An arterial blood gas sample was collected after the patient had been sitting for 10 minutes. Single breath carbon monoxide diffusing capacity (DLCO) was measured to complete the hepatopulmonary evaluation. Data were collected and entered into a database for analysis.

Summary of Results: Of the 655 patients studied, 236 (36%) were female and 419 (64%) were male. The mean age of all patients was 49.5 years old. Data were analyzed with ANOVA statistics using STATISTICA 6.0, Statsoft, Inc. An increased A-a gradient was associated with a decrease in percent predicted DLCO and an increased sitting dyspnea score. Increased dyspnea while standing was also correlated with a decreased percent predicted DLCO. However, sitting and supine dyspnea scores were not correlated with the DLCO measurement. All dyspnea scores, regardless of position, were significantly correlated to a small decrease in SpO2. Many of the patients screened had characteristics associated with...
hepatopulmonary syndrome. However, only one patient at LDS Hospital fulfilled all criteria and was diagnosed with hepatopulmonary syndrome. 

Conclusions: LDS Hospital has a low prevalence of hepatopulmonary syndrome in liver failure patients awaiting liver transplantation. The high altitude may be a factor in this low rate of hepatopulmonary syndrome. Further analysis is needed to clarify the role that altitude may have in hepatopulmonary syndrome.

Surgery I Concurrent Session
8:30 AM Friday, February 1, 2008

146 FUNCTIONAL EVALUATION OF A NOVEL ACELLULAR PERIPHERAL NERVE GRAFT

S. Lundy1,2, S. Chen2, and C. Schmidt2. 1University of Washington, Seattle, WA and 2University of Texas, Austin, TX.

Purpose of Study: The current clinical standard of care for traumatic peripheral nerve damage is the autograft, which results in donor site morbidity and suffers from lack of recipient universality. To address these limitations, our lab has created an acellular nerve graft composed of cadaveric nerve tissue chemically treated to remove cellular debris. This chemical treatment process was optimized to preserve microstructural integrity while maintaining nonimmunogenicity. In order to functionally characterize this novel optimized acellular (OA) graft, we compared it to the autograft and several other previous decellularization techniques (Termal and Sondell treatments).

Methods Used: Grafts were surgically implanted across transected sciatic nerves in Lewis rats. Animals were functionally evaluated for a period of 1 year via digital sciatic function index analysis. At the conclusion of the study, nerve grafts were recovered, fixed, and paraffinized. Fixed nerve tissue was sectioned and stained with RT97 axonal antibody with DAB visualization. Stained sections were digitally analyzed for axon number 100 micron2.

Summary of Results: The OA graft exhibits a notably higher axon density when compared to previous decellularization methods (.98 vs .5 and .69 axons/100 um2, p < 0.05). Sciatic Functional Index (SFI) evaluation (0 is normal, –100 is fully impaired) suggests statistically similar regenerative capacity to autograft and superior capacity to alternative decellularized grafts (Figure 1, p < 0.05).

Conclusions: When compared to the current autologous tissue graft, the optimized acellular graft exhibits statistically axon regeneration and functional regenerative capacity. These results suggest that the OA graft may be a viable clinical alternative in the future.

147 DETERMINATION OF THE YIELD OF ADIPOSE DERIVED STEM CELLS FROM DIFFERENT LIPOSUCTION METHODS

J. Tan1,2, J. Thompson1, J. Lynes2, and S. Gupta1. 1Loma Linda University Medical Center, Loma Linda, CA and 2California State University San Bernardino, San Bernardino, CA.

Purpose of Study: Adipose derived stem cells (ADSCs) are adult stem cells of mesodermal origin capable of differentiation into muscle, fat, cartilage, bone, and possibly more cell lines. They can be easily harvested through liposuction techniques and cultured in vitro. Fat aspirates were analyzed to determine what effect different liposuction techniques have on stem cell yield.

Methods Used: ADSCs were obtained by in vivo suction-assisted liposuction (SAL), back-table suction-assisted liposuction (bSAL), and two types of in vivo ultrasound-assisted liposcopy (UAL): the LySonix® 3000 Soft Tissue Aspirator and the Vaser®. Back-table SAL was performed on flaps of adipose removed from the patient during a pancreatectomy or abdominoplasty, then steriley liposuctioned. The area of liposuction and the catheter bore were then taken into account. ADSCs were isolated by washing the liposuction aspirate multiple times with phosphate buffered saline and then incubating with collagenase. Cells were grown in a medium of RPMI, 10% fetal calf serum, and 2% antibiotic/antimycotic in T25 flasks. Cells were counted 24 hours after being plated, and the time to confluence was recorded.

Summary of Results: The number of ADSCs/mL was significantly higher in SAL then in any other type of liposuction procedure. LySonix® UAL had the second highest amount of ADSCs/mL, bSAL had the third highest number of ADSCs/mL, and Vaser® UAL had considerably lower numbers of ADSCs/mL then any other type of liposuction. SAL cells were the first to reach confluence, followed by bSAL and LySonix® UAL. Vaser® UAL stem cells did not achieve confluence for several weeks. Catheter bore and the area liposuctioned had little effect on ADSC yields.

Conclusions: The results of this small study have clinical relevance as to which type of liposuction technique is ideally suited for stem cell harvest as technological advances allow for the use of autologous transplantation of ADSCs for plastic and reconstructive purposes. Direct SAL of the donor appears to be the superior method of procurement and holds promise as the quickest way to culture ADSCs in vitro for subsequent transplantation back into the patient.

148 THE EFFECT OF HYPERBARIC OXYGEN ON INFLAMMATORY MEDIATOR PRODUCTION IN ISCHEMIA-REPERFUSION INJURY

D.A. Scott1, L.L. Stephenson2, W.Z. Wang2, K.T. Khani2, and W.A. Zamboni2. 1University of Nevada School of Medicine, Reno, NV and 2University of Nevada School of Medicine, Las Vegas, NV.

Purpose of Study: Hyperbaric oxygen (HBO) has been shown to have beneficial effects on the complex process known as ischemia/reperfusion (I/R) injury. There is limited information about inflammatory mediators, HBO, and I/R injury. We designed this experiment to examine the early effects of HBO on the production of inflammatory cytokines, including the protective cytokine IL-6, in the setting of I/R injury.

Methods Used: The gracilis muscle flap in male Wistar rats (n = 30) was isolated on its vascular pedicle. The rats were randomly divided into 3 experimental groups: (1) Sham, no ischemia, no HBO (n = 10); (2) 4 hours of ischemia, no HBO (n = 10); and (3) 4 hours of ischemia, HBO treatment –100% oxygen at 2.5 ATA during the last 90 minutes of ischemia (n = 10). Following 15 minutes of reperfusion, 1 mL of...
blood was drawn from the gracilis muscle flap. The blood was assayed for inflammatory cytokines by a Multiplex Bead Immunoassay and the Luminex system. Statistical analysis was by analysis of variance and a p value of < 0.05 was accepted as significant.

Summary of Results: IL-6 levels were significantly increased in rats treated with HBO when compared to both the sham group and ischemia/reperfusion alone group. There were no significant changes in the other cytokine levels between groups.

Conclusions: HBO treatment increases the anti-inflammatory cytokine IL-6, and this early phenomenon may play a role in the beneficial effects that HBO has in I/R injury.

149 ELECTRICAL GUIDANCE OF STEM CELL MIGRATION AND DIFFERENTIATION
A. Kopecky, R. Sivamani, and R. Isseroff. UC Davis School of Medicine, Davis, CA.

Purpose of Study: Endogenous electrical gradients are known to guide cell migration during embryogenesis. In skin wounds, wounding generates a local electric field of about 100 mV/mm that also guides keratinocyte migration and contributes to repair. Indeed, the application of exogenous electromagnetic fields to sites of tissue injury to enhance healing is currently being studied. Previously, disruption of endogenous electrical currents in wounded amphibian limbs has been shown to disrupt limb regeneration. Thus, we hypothesized that endogenous electrical currents may play a role in adult human mesenchymal stem cell (HMSC) recruitment, differentiation and migration. We propose that imposing an exogenous electric field of physiologic strength may directly and observably influence adult mesenchymal stem cell migration and differentiation.

Methods Used: Cultured adult HMSCs were plated on collagen I-coated glass coverslips (2,000 cells/sq cm) and placed in custom built glass galvanotaxis chambers. Current applied to silver electrodes flows through agar bridges placed in wells with free flow across the chamber delivering an electric field of 100 mV/mm across the cells on the coverslip. In control chambers, no current was set. Images taken at 10-minute intervals during the cell migration period were analyzed. Cell tracking was performed using the Openlab imaging program, and migration speed and cosine value of movement direction were calculated; results were subject to statistical data analysis.

Summary of Results: Preliminary results demonstrate that HMSCs migrate at speeds of approximately 23.4 microns/hour in the presence or absence of an applied electric field. A subpopulation of the cells moves preferentially to the cathode, and another subpopulation moves to the anode. While trends are evident, the number of cells analyzed to date is too small to determine if these directional migratory paths significantly differ from those of cells not exposed to electric fields.

Conclusions: The influence of electrical gradients on mesenchymal stem cells is an area that requires further study. Planned work on this project includes tracking larger numbers of cells, and genome microchip array for mRNA expression analysis of laser captured cells displaying anodal and cathodal migration.

150 BIOMECHANICAL CHARACTERISTICS OF 9 ARTHROSCOPIC KNOTS
D.J. Patton, K.A. Dahl, M.D. Wongworawat, and Q. Dai. Loma Linda University Medical College, Loma Linda, CA.

Purpose of Study: To determine the optimal arthroscopic slip knot through comparison of ease of placement, loop security, knot security, and amount of suture material needed using a new suture material.

Methods Used: Nine commonly used arthroscopic knots (Dines knot, Field knot, Nicky knot, Hu knot, San Diego knot, Snyder knot, Tennessee slider, Triad knot, Tuckahoe) were tested. Nine knots were tied with a modern suture material, Fiberwire, for each testing mode trial (n = 10). Each knot was tested with the Instron materials testing machine (Instron, Norwood, MA) for ease of knot placement, loop security, knot security, and suture mass. Ease of placement is assessed by force needed to slide the knot as well as low resistance to forward sliding and high resistance to backsliding. Loop security is evaluated by force and resistance necessary to elongate the slip-knot loop. Final knot security is measured in terms of force and resistance necessary to elongate the knot to failure after placement of three reversing half-hitches. The distance of loop elongation is measured. The amount of suture material needed to create the knot is compared using the knot weight. ANOVA with Kruskal-Wallis analysis and Bonferroni correction (alpha < 0.01) was used to compare different knots; the knot with superior characteristics in most categories was selected as the best knot.

Summary of Results: For each parameter measured, knots with superior characteristics in each category are designated (*). The Snyder knot achieved superior results in the most categories (6 of 9).

Conclusions: The Snyder knot provides superior knot characteristics with use of less suture material.

151 KNOCKDOWN OF THE PROSTAGLANDIN TRANSPORTER IN SMOOTH MUSCLE CELLS ENHANCED EXPRESSION OF INFLAMMATORY CYTOKINES
E.P. Cheng1, R.D. Kenagy2, and A.W. Clowes2. 1University of Washington School of Medicine, Seattle, WA and 2University of Washington School of Medicine, Seattle, WA.

Purpose of Study: Prostaglandins, especially PGE2, play an important role in the shift to a proliferative/pro-inflammatory state in vascular smooth muscle cells (VSMC) following injury. While newly synthesized PGE2 is secreted into the peri-cellular environment via simple diffusion, the prostaglandin transporter (PGT) is responsible for the re-uptake of PGE2 for degradation and intra-cellular signaling. We initiated this study to answer whether PGT enhanced the development of an inflammatory phenotype in VSMC.

Methods Used: In this study, we treated cultured baboon VSMC to the pro-apoptotic stimulus Fas ligand plus cycloheximide (Fas-L/CHX). After 24 hours, we extracted mRNA, and gene expression was quantified using Taqman® qRT-PCR. We knocked down PGT expression in baboon VSMC via the transfection of siRNA designed against human PGT, and we transfected VSMC with medium-GC content scrambled
achieve emmetropia. Simulated keratometry values alone were found to cause an induced spherical equivalent refractive change and the measured post-cataract spherical equivalent error was graphed against the predicted refractive error for the chosen IOL, yielding the post-emmetropic postoperative result.

Methods Used: This was a retrospective review of the medical records of a consecutive series of patients who underwent cataract surgery after LASIK. Permission to perform this study was obtained from the Human Subjects Protection Committee at UCLA. Only the most recent refraction and IOL power data were used if the patient had a postoperative IOL exchange (because of a large refractive error) after cataract surgery. The difference between the LASIK-induced spherical equivalent refractive change and the post-cataract spherical equivalent error was graphed against the predicted refractive error for the chosen IOL, yielding the post-cataract spherical equivalent error to target to achieve emmetropia. Preoperative simulated keratometry values were also compared graphically to postoperative spherical equivalent refractive errors.

Summary of Results: When we treated VSMC with Fas-L/CHX, we observed an up-regulation of PGT in VSMC treated with FAS-L/CHX, we observed increased up-regulation of the inflammatory cytokines II-1α and MCP-1 compared to the negative control. We verified PGT expression knock-down using Taqman®.

Summary of Results: When we treated VSMC with Fas-L/CHX, we observed an up-regulation of PGT, II-1α, and MCP-1. When we knocked down the expression of PGT in VSMC treated with FAS-L/CHX, we observed increased up-regulation of the inflammatory cytokines II-1α and MCP-1 compared to the negative control.

Conclusions: This study suggests that PGT may enhance the development of a pro-inflammatory phenotype in VSMC via the up-regulation of inflammatory cytokines, and furthermore, VSMC PGT may have clinical implications as a pharmacologic target since inflammation is intimately linked to the pathogenesis of atherosclerosis and neointimal hyperplasia.

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muscles, mid-scapular line and posterior axillary line, respectively. The IC nerve was exposed to injury from supracostal access in 85%, 100% and 100% of IC spaces at the same locations as before. The IC artery was exposed below the 11th rib 30%, 60% and 70% of the time and was up to 6 mm below the 11th rib in 25% of dissected IC spaces. The IC vein, exposed in 0%, 5% and 10% of spaces at these same locations was least likely to be exposed below the rib.

Conclusions: The IC vessels are exposed to potential injury during PCNL in a significant number of patients. Access placement immediately above the 12th rib and at the lateral border of the erector spine muscle decreases the chance of IC vessel injury. Injury to these vessels may be one potential cause of the increased blood loss and transfusion rate associated with upper pole renal access.

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INCIDENCE AND PREVENTION OF IATROGENIC URETHRAL INJURIES
C. Kashefi1, K. Messer2, R. Barden2, C. Sexton2, and J. Parsons1.
1UCSD Medical Center, San Diego, CA; and 2UCSD Medical Center, San Diego, CA.

Purpose of Study: Although improper urethral catheter insertion is a source of preventable injury in male patients, the extent of this problem is not known. We studied the incidence and mechanism of iatrogenic urinary catheter injuries occurring in adult male inpatients at a single institution and designed and implemented an intervention to prevent these injuries.

Methods Used: This study was conducted over a 13-month period. During months 1 through 6, all catheter-related injuries occurring among all adult male admissions at a single academic tertiary care center were prospectively tracked. Incidence data were calculated, injury severity analyzed, and injury mechanism identified. During month 7, based on injury mechanism data, a nursing education program was designed and implemented by Urology staff that included basic urologic anatomy, urethral catheter insertion techniques, and catheter safety. During months 8 through 13, catheter-related injuries were again tracked. Pre- and post-intervention incidence was compared.

Summary of Results: During the pre-intervention period, iatrogenic urethral catheter injuries occurred in 14 of 4310 consecutive adult male admissions, an incidence of 3.2 injuries per 1000. Penile and/or perineal pain occurred in 14/14 (100%) and penile bleeding in 12/14 (86%) of affected patients. One patient required cystoscopy for catheter placement and one developed recurrent urethral strictures requiring multiple dilations. During the post-intervention period, 3 injuries occurred among 4523 consecutive patients, a statistically significant decrease in risk by a factor of 4.9 (p<0.006, Fisher’s exact test; incidence rate 0.7 per 1000 adult male admissions). Of the 3 post-intervention injuries, 2 (67%) occurred in the operating room, an area not targeted as part of the intervention.

Conclusions: Iatrogenic urethral injuries are a substantial source of preventable morbidity in hospitalized male patients. Implementation of a nursing education program may significantly reduce the incidence of iatrogenic urethral injury and thereby improve patient safety.

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PRESURGICAL LANGUAGE fMRI: A COMPARISON BETWEEN TASKS AND METHODS OF ANALYSIS
A.F. Alexander-Bloch1,2, and S.J. Price. 1David Geffen School of Medicine at UCLA, Los Angeles, CA; 2Cambridge University, Cambridge, United Kingdom and 3Cambridge University, Cambridge, United Kingdom.

Purpose of Study: Functional magnetic resonance imaging (fMRI) has presurgical potential for language tumors, as a way to distinguish eloquent tissue. At present, there is no standard language task(s) with which to test patients, nor standard method of analyzing data. This study compared different tasks in a control population. Also, this study tested the reliability of real-time, inline fMRI analysis, as performed by many modern MR imagers.

Methods Used: Six audio tasks were developed using DMDX software, including repetition, generation, and judgment tasks. Echoplaner imaging was accomplished with a 3T Siemens Tim Trio. The inline results were validated against a standard manual analysis, using the SPM package. The two methods of analysis were compared visually and with statistical measures, such as the percent identity of thresholded t-maps and the correlation coefficients of the maps. The language tasks were compared both with a random effects analysis at the group level and also by clustering the data using hierarchical methods and partitioning around medoids (PAM).

Summary of Results: Five consecutive subjects had known language areas activated by some tasks. The SPM and inline results showed activation in the same anatomical areas. The correlation coefficients for the t-maps generated by the two modes of analysis ranged from .5 to .8. At a t-value threshold of 5, all of the maps were more than 80% identical, and all except 3 were more than 90% identical. The random effects analysis was uninformative because of high intersubject variability. The data formed natural clusters by subject, rather than by task. However, task-wise clustering within subjects demonstrated high similarity between certain tasks.

Conclusions: The between subject variability, in terms of language activity provoked by a given task, implies that efforts to find a single, standard fMRI language task could be misguided. The similarity between the inline and the SPM results justifies future use of the inline analysis on its own. A conjunction of 2–3 tasks, with dissimilar patterns of activation, was recommended for future, clinical work. Since the completion of this study, plans to begin testing on a patient population have moved forward.

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THE BAKULI BOYS’ BRIGADE: EXPANDING HUMANITARIAN INITIATIVES WITHIN A CANADIAN GLOBAL HEALTH PROGRAM

Purpose of Study: Meaningful international relationships and a true understanding of social responsibility may evolve from supporting local community efforts during global health initiatives. In 2007, a team of Canadian university students from Vancouver, BC traveled to Uganda, Africa to deliver the Brighter Smiles Africa Program. They undertook an additional humanitarian intuitive to develop trust and form a partnership with a local marching band consisting of male street youth in Kampala. The students met to discuss and better understand the band’s mandate: to empower street youth to be self-reliant. Support from the student team was requested and the students met the boys to establish areas of common interest. The students attended Brigade performances, tutored the boys in academic subjects, engaged in sports and encouraged the boys to realize their full potential. In turn, the boys shared life experiences and local customs as well as identifying nutritional needs and other health issues. Interactions and interviews with the brigade were captured on video and demographic data was collected.
Summary of Results: 7 Canadian university students interacted with “The Bakuli Boys Brigade” to identify their needs and health issues. The band director’s interview about rising from delinquency to playing the trumpet, to a healthy lifestyle, forming the band and rescuing street youth into a meaningful existence is a compelling story. Video material collected will assist with project development, fundraising and to develop a new global health university curriculum.

Conclusions: University students engaged in a global health program gained a better understanding about the issues of Ugandan youth by simultaneously adopting a new humanitarian initiative. Students gained trust and developed a partnership with a worthy, local community group while appreciating hardships that street youth and orphans face. This new partnership will open doors for other humanitarian initiatives, broaden the educational experience of university students and enhance their awareness of social responsibility to become global citizens.

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PALS AT UNIVERSITY OF CALIFORNIA, IRVINE SCHOOL OF MEDICINE
E.M. Alvarez, K. Trangsrud, S. Chung, E. Andrade-Kitching, P. Murata, and F. Waffarn. UCI School of Medicine, Irvine, CA.

Purpose of Study: PALS is a student-initiated curriculum fostering long-term relationships between medical students and chronically ill children. Medical students increase their understanding of chronic illness and its impact on the patients and their families. It also serves as a medium for patients’ families to build their confidence in health care professionals and gain a support system.

Methods Used: PALS was initiated by three UCI medical students in 2003 as a pilot program of 10 medical students. Students range from 6 to 19 years old and have a wide range of diagnoses. Each student interacts with their assigned child through visits to the home, clinic, and PALS group events. Students attend discussion sessions, with physician speakers and they complete a pre- and post-program surveys. Post-program interviews were conducted with family representatives.

Summary of Results: 100% of the medical students reported improved understanding of the effects of chronic illness, 100% reported a beneficial relationship with the child, 75% reported a greater interest in working with chronically ill children in their clerkship.

Conclusions: Patients, their families, and medical students report having a valuable experience through PALS which through preliminary data, is being shown to continue into their third year pediatric clerkship. PALS has 63 participants.

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INFLUENZA VACCINE IN CHILDREN: BARRIERS IN TRANSLATION

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Purpose of Study: Although multiple studies have shown that influenza vaccination substantially reduces the incidence of influenza-like illnesses in both vaccinated children and their family members, the implementation of guidelines for routine influenza vaccination faces several barriers, and annual vaccine coverage remains low.

The purpose of this study was to perform a systematic review of the literature available on barriers to influenza vaccination in children and to identify interventions that improve vaccination rates.

Methods Used: Studies relating to barriers of influenza vaccination were identified by searching electronic databases and citation tracking using keywords “influenza vaccine”, “pediatrics”, “barriers”, and “intervention”.

Summary of Results: The search strategy identified 23 articles which evaluated barriers and/or interventional methods for influenza vaccination in children. The vaccine coverage ranged from 9% to 71% in different practice settings. The most common barrier identified was the lack of a physician recommendation to receive the vaccine. Other common barriers reported include parental misconceptions regarding side effects and benefits of the vaccine. Studies reporting lower vaccination rates identified the cost of the vaccine and the lack of insurance as the main barriers to vaccination. Of the 23 studies, 8 evaluated the effect of interventional programs on the rate of influenza vaccination. These programs increased vaccination rate by 3 to 42% in different settings. Combinations of standing orders, walk-in clinics, Saturday vaccination clinics and reminders to providers and patients were the most effective interventional strategies.

Conclusions: Our study demonstrates that many barriers for influenza vaccination exist in various practice settings. To improve translation of influenza vaccine guidelines into practice, interventional programs should be tailored to the barriers identified in that particular setting.
Greater agreement was observed between parents and children than parents and adolescents and between parents and prosthesis non-wearers than parents and wearers. **Conclusions:** Children with UCBED report better upper extremity function and quality of life than parents perceive, but may also be experiencing more pain. Parent reports of function may provide a helpful counterbalance to child and adolescent reports, but because quality of life is subjective by nature, the child or adolescent’s report is the gold standard. Due to variability in agreement, the PODCI and PedsQL parent reports cannot be considered true proxies for the child or adolescent with UCBED.

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**SMOKING EDUCATION & CESSATION IN ANAKTUVUK PASS, ALASKA**

C.R. Baldwin. University of Washington, Seattle, WA.

**Purpose of Study:** Anaktuvuk Pass was selected as the site for a presentation that sought to provide education for the community of Anaktuvuk Pass on the negative health effects of smoking tobacco and to provide connection for the community with the Chief Andrew Isaac Health Center’s (CAIHC) Tobacco Cessation program in Fairbanks, Alaska.

**Methods Used:** After coordinating with the village mayor and health aide, a flyer was sent to advertise a presentation on the health effects of smoking. The local school was also informed. The town hall was reserved as the site. Information for the presentation was gathered from journal articles, anti-smoking groups including the American Lung Association, literature available through the CAIHC Tobacco Cessation Program and other sources.

The presentation described the adverse effects of cigarette smoking upon the lungs, heart, circulation and reproductive systems. Pictures of tissue damage caused by cigarette smoking were shown because several local health care professionals and community members suggested that that strategy would have a greater impact. The presentation concluded by providing contact information for the local tobacco cessation counselor. There were many brochures available afterwards provided by the counselor as well that gave more information on a broad variety of subjects related to tobacco use.

**Summary of Results:** The presentation was given at the city hall to a group of approximately 30 high school students. Afterwards, eight students decided to contact the CAIHC tobacco cessation counselor about quitting cigarettes. Their names were given to the health aide for follow-up. The tobacco cessation counselor was informed about the positive response in the community and agreed that further education in other villages would be beneficial.

**Conclusions:** Students were open to talking about their struggles with tobacco and how it had affected their lives. Several expressed the desire to quit, but indicated they had little support. They seemed hopeful about the possibility of getting medications and counseling through CAIHC to help them quit. Further evaluation would determine if those students succeeded in quitting and if others decided to quit as well. Overall, the presentation accomplished its purposes both in providing previously unrealized information and connecting community members with an infrastructure to help them quit.

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**CHILDHOOD OBESITY IN METLAKATLA, ALASKA**

J.H. Rinker. University of Washington, Seattle, WA.

**Purpose of Study:** Metlakatla, Alaska has a very high rate of obesity among the general population, and an increasing number of obese children aged 5–12 years. A project was developed to introduce children and their parents to healthy eating and exercise habits to help combat childhood obesity and its detrimental side effects.

**Methods Used:** PubMed literature research was reviewed to determine what research had been conducted on this area of health, and how this information could be incorporated into the Metlakatla Project. A program was developed for the children to try to walk 10,000 steps in a day, tracking their progress with a pedometer. The focus of the program was for the children to have fun, involve parents so the children would have guidance at home, and get good exercise as well.

**Summary of Results:** The parents were very eager and receptive to ideas that would help their children get some exercise instead of watching television or playing video games. The children were delighted with the pedometers and were immediately eager to get out and use them. Both the children and parents liked the idea of having tangible results after exercising, which led to immediate excitement about exercising. The group was also very interested in ways to cut down on unhealthy eating and took an active part in our discussions of such matters.

**Conclusions:** With the help of the children’s parents, local children will use this project as a strategy to attain their daily recommended amount of exercise, as well as eat in a healthier manner. By giving them attainable and sustainable goals for exercise and nutrition, hopefully they will develop good habits that will last a lifetime.

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**PROMOTING NUTRITION AMONG LOW-INCOME RURAL FAMILIES**

M. Hoane. University of Washington, Seattle, WA.

**Purpose of Study:** The aim of this project was to promote healthy eating habits by distributing a cookbook that encompassed several nutritious, inexpensive, kid-friendly meal options that are easy to prepare. Although the main goal was to target low-income families with children in Sheridan, MT, the cookbook was made available to any individual who would benefit from this resource.

**Methods Used:** The restrictions and resources that dictate the assortment of foods available to low-income families in Sheridan were researched by interviewing the local grocery store manager and by obtaining the Montana WIC program approved foods list. To keep the cost of each meal minimal, recipes were formulated to include at least one or two ingredients available through the WIC program. The cookbook was designed to be user-friendly, as well as economical to produce. It included hints for the chef, a list of common ingredients (all inexpensive), cooking definitions, tips for guiding healthy eating habits in children and a total of 35 recipes. A literature research was conducted to determine the best strategies for success.
Summary of Results: The Grant Office of Madison County funded production of 100 copies, which were distributed for free as follows: 25 copies to the local daycare, 20 copies to the WIC clinic and 55 copies to the CHC clinic. Hardcopies were left with multiple healthcare providers in both Sheridan and surrounding communities, including Butte, to ensure availability of this resource in the future.

Conclusions: This cookbook achieved and surpassed its aim. Not only did members from all corners of the Sheridan community eagerly accept this collection of recipes, but the appeal of this resource was so great that other healthcare providers requested permission to distribute copies to patients in neighboring towns, as well as Butte.

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XYLITOL GUM USE FOR THE PREVENTION OF EARLY CHILDHOOD CARIES ON THE BLACKFEET RESERVATION, MT

D. Tarby. University of Washington, Seattle, WA.

Purpose of Study: Early Childhood Caries (ECC) of the primary teeth has been implicated in subsequent increased rates of tooth decay as an adult. Furthermore, rates of ECC among Native American children have been documented to be as much as 600% higher than children of other ethnicities. Given the high rates of ECC on the Blackfeet Reservation a project was designed to increase awareness amongst mothers in the community as well as the medical staff about the preventative effects of xylitol gum.

Methods Used: Gum sweetened with 100% xylitol sugar has been shown to significantly reduce the rates of ECC in children when their mothers chew two pieces a day starting before the child is born. Meetings were held with the dental staff, obstetricians, and pediatricians to coordinate methods of prevention amongst the three fields. Expectant mothers arriving in the prenatal clinic were given a brief discussion about xylitol gum along with a flyer outlining dosage, benefits, and availability. A literature search using the PubMed database searching for xylitol gum with an emphasis on studies directed towards expectant mothers and prevention.

Summary of Results: The medical staff was interested to learn what other fields were doing as far as prevention of ECC. Prior to the project only the dental staff was aware of the effects of xylitol on Strep. Mutans and the associated rate of caries. The expectant mothers arriving in the prenatal clinic seemed interested in ways to help their new child and several were already using the commercially available gum that contains xylitol sugar. 15 mothers heard the discussion about the gum and more flyers were printed and placed in the prenatal clinic waiting room.

Conclusions: The project increased awareness about the preventative effects of xylitol gum amongst both the medical staff and mothers in the community. The special order gum is also available through the dental clinic if requested and is free to Indian Health Service beneficiaries as well as at larger stores in the area. A continuation of the project might include pushing for the gum to be available through the USDA’s Women, Infant, and Children special nutritional supplement program.

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HUMAN PAPILLOMAVIRUS AND HPV VACCINE AWARENESS AND EDUCATION IN TORRINGTON, WYOMING

C.J. Pederson. University of Washington, Seattle, WA.

Purpose of Study: Very little information was available in Torrington, WY regarding human papillomavirus or HPV immunization. As a conservative, rural community, citizens of Torrington and surrounding communities are very cautious about what health information is made available to adolescents. A community project was designed to provide both young females and parents with information promoting awareness and education about human papillomavirus, consequences of infection, and vaccination options.

Methods Used: Information about current available services and information regarding sexual and reproductive health was gathered from the public health office as well as the medical clinic. Healthcare providers were informally interviewed to understand general community attitudes, needs, and potential barriers to HPV educational material. A literature search was conducted to review HPV vaccine administration and efficacy as well as public response to HPV vaccination.

Summary of Results: Potential community resistance to HPV vaccine promotion due to conservative political and religious views was identified. A brochure was designed and approved by practicing community physicians. This brochure will be handed out to middle and high school girls at female athletic physicals and was made available to the public health offices. An article was sent to the local newspaper providing more information about human papillomavirus and state-supported vaccination programs.

Conclusions: Understanding a community’s culture and general views about sexual health information is critical in determining how to deliver health information regarding human papillomavirus infection. Targeted, group-specific written materials can be used to provide important information about a sensitive topic (human papillomavirus vaccine) to relatively large populations.
COMMUNITY EDUCATION AND PREVENTION OF ANAPHYLAXIS IN MCCALL, IDAHO

K.L. De Niro. University of Washington School of Medicine, Seattle, WA.

Purpose of Study: Anaphylaxis is an allergic reaction that is both life-threatening and treatable. Compared to the national average, anaphylaxis accounts for a much greater percentage of the total emergency room visits in McCall, ID. A community project was designed address the high incidence of anaphylaxis through education and training of childcare providers.

Methods Used: An extensive literature review suggested the education of childcare providers to be an effective method to reducing overall incidence of anaphylaxis in a community. In addition to the education of childcare providers, evidence-based sources emphasize the importance of developing an action plan for treatment of anaphylaxis, and the need for hands-on training with an injectable epinephrine training device to ensure proper operation during an anaphylactic event. To meet the above objectives, a seminar was conducted at Camp Ida-Haven, a local summer camp in McCall, ID. The hour-long seminar included information about the physiology of anaphylaxis, recognition of anaphylaxis, how and when to treat, prevention of the biphasic reaction, and general anaphylaxis preparedness. In addition to the educational lecture, an injectable epinephrine training session provided hands-on training of the camp staff.

Summary of Results: Forty childcare providers were educated about the prevention and treatment of anaphylaxis.

Conclusions: In order to explore the process of affecting community-oriented change in a rural setting, a community need was identified, evidence-based sources were consulted for planning purposes, and a community-based education project about the prevention of anaphylaxis in the childcare setting was implemented.

GLENOHUMERAL MUSCLE ARCHITECTURE DIFFERS WITH AGE

G. Altobelli¹, C. Eng¹, D. Gokhin¹, A. Taylor², R. Lieber¹, and S. Ward¹. ¹University of California, San Diego, La Jolla, CA and ²Duke University, Durham, NC.

Purpose of Study: The purpose was to provide a detailed architectural comparison of middle-aged and elderly rotator cuff and deltoid muscles.

Methods Used: Forty-one cadaveric shoulders were used and were split into middle-aged (12) and elderly (29) specimens. Mass (M) and muscle length (Lₘ) were measured for each muscle. Length of predetermined fiber bundles was measured, and sarcomere length (Lₛ) was determined using laser diffraction. Lₛ was used to normalize muscle fiber length (Lₘ) to 2.7 μm. Physiological cross-sectional area (PCSA), a measure of a muscle’s capacity for force generation, was calculated according as follows: PCSA = (M x cosθ)(ρ x Lₛ) where θ is pennation angle and ρ is muscle density, 1.055 g/cm³.

Summary of Results: The PCSA of middle-aged deltoids (24.0 ± 3.2 cm²) was greater than elderly deltoids (16.0 ± 1.8 cm²) (P < 0.001), the PCSA of middle-aged subscapularis (19.85 ± 2.11 cm²) was greater than elderly subscapularis (14.7 ± 1.0 cm²) (P < 0.005), and middle-aged infraspinatus (10.4 ± 0.7 cm²) (P = 0.136). There was no main effect of age on fiber lengths (Fig. 1), and fiber length variability due to age differences was small (2 –10%).

Conclusions: The rotator cuff muscles have relatively short fiber lengths and large PCSAs. As these muscles age, their force-generating capacity is reduced, while their excursion range remains constant.
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DEPRESSED MOTHER’S PERCEPTIONS OF THEIR INFANTS’ MASTERY MOTIVATION
T. Sparks1, S. Hunter2, and T. Backman3. 1University of Colorado at Denver Health Sciences Center, Denver, CO; 2University of Colorado at Denver Health Sciences Center, Denver, CO; and 3University of Colorado at Denver Health Sciences Center, Denver, CO.

Purpose of Study: Maternal depression has been shown to influence child behavior and development, including aspects of mastery motivation. Mastery motivation is a “psychological force that stimulates an individual to attempt independently, in a focused and persistent manner, to solve a problem or master a skill or task that is moderately challenging for him or her.” This study will examine mastery motivation in infants of mothers with and without depressive symptoms. Additionally, as this study’s measure of mastery motivation is a parent-report measure and since maternal depression may alter perceptions of child behavior, this study will compare mothers’ ratings of their infants’ mastery motivation with the Behavior Rating Scale (BRS) of the Bayley Scales of Infant Development, which is completed by a trained examiner and has demonstrated discriminate validity in a number of groups of high-risk infants.

Methods Used: Infants’ mastery motivation will be assessed by having the mother complete the Dimensions of Mastery Questionnaire (DMQ) at six, twelve, and eighteen months of age. Mother’s1 current level of parental distress will be assessed at each of these visits using the Parenting Stress Index Short Form (PSI-SF). Additionally the BRS will be completed at each of the three visits.

Summary of Results: Correlation analyses will be used to evaluate the relationship between mothers’ level of parental distress (PSI-SF), mothers’ assessment of infants’ mastery motivation (DMQ), and a rater’s assessment of infants’ performance (BRS).

Conclusions: We hypothesize that higher parental distress subscale scores on the PSI will be negatively correlated with infant DMQ scores. We further hypothesize that observer ratings will be higher than maternal ratings of infant behavior.

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STORY STEM RESPONSES IN PRESCHOOLERS WITH MOOD DISTURBANCES OR ATTENTION-DEFICIT/HYPERACTIVITY DISORDER
A.K. Hutchison, C. Beresford, and R. Ross. University of Colorado School of Medicine, Denver, CO.

Purpose of Study: This study is investigating potential diagnostic tools for evaluating bipolar disorder in preschool-aged children (5 years old and younger). Since, the Diagnostic and Statistical Manual -IV (DSM-IV) does not contain criteria for diagnosing bipolar disorder in this age group, evaluation of the usefulness of these diagnostic tools that have been established for use in different populations (for disorders other than bipolar disorder), or in different age groups (6 years and older) began. The Washington University Schedule for Schizophrenia and Affective Disorders (WASH-U-KSADS) (Geller et al. 1998) has been used to assess for bipolar disorder in children ages 6–12. Additionally the Preschool Age Psychiatric Assessment (PAPA) (Egger et al., 1999) has been used to assess for some psychiatric disorders in preschoolers, but has not explored bipolar disorder. The WASH-U-KSADS and the PAPA both rely on parent interview to assess the child’s symptoms. Recently, another measure, the MacArthur Story Stem Narratives (MSSB) was added to explore the child’s symptoms through role-playing. The MSSB narratives begin with the experimenter starting a “story stem” that leads to a conflict that the child has to resolve through role-playing. The MSSB has proved to be a structured way to interview the child without parent report (Luby et al. 2006).

Methods Used: Three different groups of children will be defined through parent interviews using the PAPA: mood disorder (with or without comorbid Attention-Deficit/Hyperactivity Disorder (ADHD)), ADHD, and typical children. These children will then participate in the MSSB and two subtests of the Wechsler Preschool and Primary Scale of Intelligence (WPPSI) to rule out mental retardation.

Summary of Results: Preliminary evidence shows that the MSSB is an effective method of revealing the worlds of the children in the preschool mood disorders study. This study indicated that a difference between mood-disordered and typically developing children could be identified with the MSSB (Beresford et al., 2007).

Conclusions: We hypothesize that the MSSB will show differences between mood-disordered, ADHD and typically developing children that are consistent with the diagnostic groups formed based on the PAPA. Long-term follow-up will be necessary to verify diagnostic findings of the study.

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COGNITIVE-BEHAVIORAL GROUP THERAPY FOR ADOLESCENTS WITH AUTISM SPECTRUM DISORDERS AND ANXIETY—FINDINGS OF A PILOT STUDY
L. Zapapas, A. Blakeley-Smith, and J. Reaven. University of Colorado-Denver Health Sciences Center, Denver, CO.

Purpose of Study: Cognitive-behavioral therapy (CBT) has become the standard of psychosocial treatment for anxiety. Rates of anxiety in autism spectrum disorders (ASD) surpass that of the general population, yet patients with ASD present with challenges in social and language areas, in addition to behavioral oddities, that may interfere with their receptivity to CBT approaches. Further, adolescents remain an understudied population both on the autism spectrum and in the general population, compared to adults and children. We conducted a pilot study of adolescents with ASD and anxiety, to 1) determine the initial efficacy of CBT for this population and 2) to describe modifications that might be necessary in the implementation of CBT for this population.

Methods Used: Four adolescents (age 14–17) with a diagnosis of an autism spectrum disorder and presenting with clinical anxiety symptoms participated in a 12-week original, manualized, group CBT intervention, along with their parents. The treatment for adolescents with ASD and anxiety was based on an intervention originally developed for children 8–14 with ASD and anxiety (Coping Group: Fighting Worries and Facing Fears). Preliminary findings from the initial study (children 8–14) indicated statistically significant reductions in anxiety symptoms according to parent report, post treatment (Reaven et al., in press). Pre-treatment and post-treatment measures of anxiety were obtained. In addition, basic components were conserved from the “Face Your Fears” manual, while care was taken to modify activities appropriately for the adolescents and to note any such modifications.

Summary of Results: Pre-treatment and post-treatment results will be presented. Modifications necessary for working with adolescents with ASD and anxiety will be discussed. Anxiety symptoms identified by this group were similar to children with ASD, although the teens presented with more social and academic anxiety symptoms.

Conclusions: CBT may be an appropriate intervention for adolescents with ASD and anxiety symptoms. Modifications to traditional CBT protocols are necessary for use with teens with ASD, given the core deficits of ASD. This study provides an initial first step in creating an adolescent version of a CBT intervention for teens with ASD and anxiety.
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INCREASING PATIENT INVOLVEMENT IN HEALTHCARE IN LEAVENWORTH, WASHINGTON
D.A. Williams. 1University of Washington, Seattle, WA and 2Cascade Medical Center, Leavenworth, WA.
Purpose of Study: Leavenworth, WA has an increasingly aging population and many of the older patients in the clinic have chronic and complex health problems. Furthermore, the isolated community necessitates large numbers of referrals to distant locations such as Wenatchee or Seattle making clear patient-physician communication absolutely necessary. An intervention was developed to assist patients in tracking and subsequently communicating to health care providers their past medical histories and medication regimens.
Methods Used: A community health assessment was carried out by interviewing local physicians, health administrators, pharmacists, and patients to accurately evaluate the health needs of the community. A literature review was performed using Medline to discover national rates of medical non-adherence, factors influencing non-adherence, effective methods for encouraging adherence, and the effect of personal medical records. A Personal Health Record and Medication List were designed considering the discovered needs of the community.
Summary of Results: Simple and highly portable Personal Health Records and Medication Lists were developed and left with the health care providers at the clinic which could be easily distributed by health care providers to patients at the clinic or people in the community.
Conclusions: Specifically designed for older individuals in isolated communities, the Personal Health Record and Medication List are small, highly portable tools that have the potential to assist patients in organizing and better understanding their overall health. Additional time and research must be invested to distribute the developed tools and properly assess their effectiveness.

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NATIVE AMERICAN WOMEN AND EDUCATION ABOUT YEARLY EXAMS AND PREVENTATIVE MEASURES ON THE CROW INDIAN RESERVATION
J.R. Guffin, and C.L. Corrigan. University of Washington, Seattle, WA.
Purpose of Study: Native American women living on the Crow Indian Reservation rarely have a yearly exam done and often do not see a regular physician. Incidence of and death from breast and cervical cancer often exceed national averages in Native American women. A community presentation was designed to educate native women at a women’s health fair in Pryor, MT of the importance of preventative health measures, specifically regarding breast and cervical cancer.
Methods Used: Community members requested a health education offering focused on breast and cervical cancer prevention be provided at the annual women’s health fair in Pryor, MT. Cultural information from tribal elders was solicited regarding their understanding of why tribal women delay and skip yearly exams. Physicians, nurses, and other community members were consulted about information to be presented. Posters and pamphlets were obtained from the local health department. A literature search was conducted using PubMed to review the current knowledge about women’s health in tribal communities as well as the incidence of breast and cervical cancer.
Summary of Results: Cultural beliefs, not feeling immediately sick, being shy, lack of transportation, time, and effort, as well as minimal health education were identified as possible reasons for missing yearly exams. A women’s health prevention discussion was advertised with the annual women’s health fair all over the Crow Indian Reservation. A little over a dozen middle-aged women attended the presentation, many of whom took the available brochures and pamphlets.
Conclusions: The presentation was informal and given in a friendly setting that allowed women to come and go at their leisure. There was also no pressure on attendees to participate in any discussion or talk about topics that might make them uncomfortable. The fact that many women remained at the clinic to get a yearly exam speaks to the success of the presentation. Women’s health fairs are focused on raising awareness of community members about health promotion. It is especially important to educate those whose culture might deter them from otherwise sufficient health maintenance. Ideally, these Native women now have a better understanding of the importance of a yearly exam and screening procedures, and will share this information in the community.

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DIAGNOSING AND PREVENTING OSTEOPOROSIS IN CUT BANK, MT
S.L. Abraham1, and C.L. Corrigan2. 1University of Washington, Seattle, WA and 2University of Washington, Seattle, WA.
Purpose of Study: Cut Bank, MT has a population that is 52% female and 17.6% aged 65 years and older. These are two important risk factors for developing osteoporosis. A community presentation was given to educate participants on bone health and DEXA scans.
Methods Used: Several informal interviews with Cut Bank residents were conducted in order to learn what the general population knew about bone health and bone density scans. A literature search on PubMed was conducted to obtain the latest research on osteoporosis prevention, diagnosis, and treatment options. The results of the search were put into a PowerPoint presentation that was open to the community.
Summary of Results: Twenty-five community members attended the presentation and were given the opportunity to ask questions. The presentation was positively received.
Conclusions: Attendees of the presentation were able to learn if they may be at risk of developing osteoporosis. PowerPoint was an effective method to accurately explain medical terminology to people at any education level. Participants learned that early prevention and treatment of osteoporosis can prevent further weakening of bones as well as costly and painful fractures. Importantly, they were able to discover that there is a resource for DEXA scans in their own community.

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RECOGNIZING AND PREVENTING ADOLESCENT DEPRESSION IN MILES CITY, MT
H.A. Kerins. University of Washington, Seattle, WA.
Purpose of Study: Miles City, Montana has one of the highest suicide rates in the state. Many of the community’s physicians feel part of the reason for the high rate is the undiagnosed and untreated depression among adolescents. A community project was designed to increase awareness of the issue and provide simple tools of prevention.
Methods Used: An interactive workshop was implemented with two local 4-H clubs. The workshop included: a discussion on what depression is and the risk of suicide; a group drawing activity emphasizing that not everyone with depression looks the same; a discussion on the signs to look for in themselves or peers; what to do if they suspect depression in someone they know; and a small discussion on gun safety. Safety plans and journals were distributed as a plan for each person to cope with difficult times in their lives and included: personal triggers for sadness, coping skills, a list of people to talk to in times of crisis, and a list of suicide hotlines.
Summary of Results: In the first workshop, the participants included 11 4-H club members and 4 adults; and in the second, the participants
SKIN CANCER PREVENTION EDUCATION IN THE RURAL TOWN OF CHEWELAH, WASHINGTON  
M.H. Nasry. University of Washington, Seattle, WA.  
Purpose of Study: Skin cancer is the most prevalent form of cancer in the United States today with more than one million new cases diagnosed annually. It has been estimated that one in every five Americans and one in every three Caucasians will develop skin cancer in the course of a lifetime and that more than 90 percent of all skin cancers are caused by sun exposure. Through direct clinical observation, skin cancer prevention education was identified as a major deficit in the rural town of Chewelah, Washington. With logging and farming being the most prevalent industries, this community is at an increased risk for developing various skin cancers. Consequently, an effective, evidence-based community project was designed to raise awareness and knowledge concerning skin cancer prevention.  
Methods Used: A literature search was conducted to evaluate the appropriate method for creating motivating educational material. Secondly, educational material was developed specifically targeting three groups, which included males, females, and parents of children. Skin cancer education and prevention discussions were conducted at the local Youth Center, Nursing Home, and Pharmacy. In addition, extra educational handouts were given to the staff at each of these locations for continued use.  
Summary of Results: Educational brochures and presentations were well-received by the community and clinic staff. Awareness regarding the risks of skin cancer and prevention methods was increased within the community.  
Conclusions: The level of participation demonstrated that the local advertisement was successful for the program. The walking participants were encouraged by the first walk and were interested in continuing the weekly program. The walking program was purposefully designed to eventually become self-sustaining and run by the community itself. The wide amount of community support that was gained leading up to the walk gave the program a great amount of momentum. With persistent support and excitement of the community, this program will continue to thrive.
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GENERATION RX: ADDRESSING THE INCREASING PREVALENCE OF PRESCRIPTION DRUG ABUSE AMONG THE YOUTH OF CHEWELAH, WA

T. Dyamenahalli. University of Washington, Seattle, WA.

Purpose of Study: Prescriptions for Life, a year-old grassroots community group in Chewelah, WA, has identified prescription drug abuse, notably of opioids, as a growing problem in their youth population. The group’s mission is to implement a plan that will help eliminate or reduce the abuse of prescription drugs in their community. A project was developed to educate the group further about the problem and potential interventions, as well as create educational materials for their use.

Methods Used: A review of recent publications was done to identify information that would be appropriate for the education literature and for the Prescriptions for Life committee. The inpatient treatment director and a group of students at Daybreak, a drug rehabilitation center in Spokane, WA, were interviewed to gain knowledge about the treatment options as well as hear first-hand experiences with prescription drug abuse. The Policy Analyst from the Office of national Drug Control Policy funded Drug-free Communities grant program was contacted to learn about potential funding sources for community groups such as Prescriptions for Life. Attendance at weekly Prescriptions for Life meetings provided insight into the group’s progress, needs and future goals.

Summary of Results: A binder was assembled that included several fundamental research studies, a profile of Daybreak, a parent pamphlet and information sheet for physicians, a student substance abuse survey template, a resource sheet and contact information. Parents, family and physicians were identified as key access points for prescription drugs, and were the main targets of the educational materials.

Conclusions: Prescription drug abuse has only recently been identified as a big concern; it will take the combined efforts of concerned citizens, researchers and the governments to successfully address and eliminate the problem.

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HEPATOCELLULAR CARCINOMA IN KUMASI, GHANA

J.J. Keller. University of Washington, Seattle, WA.

Purpose of Study: Kumasi, Ghana has a high rate of hepatocellular carcinoma (HCC) due to the aggregation of key risk factors in the community and lack of widespread Hepatitis B vaccination programs. A community service project was designed to look into the causes of the disease and design an intervention aimed at reducing its incidence.

Methods Used: A literature search was performed using Medline to learn more about the epidemiology of HCC in Sub-Saharan Africa and the efficacies of possible interventions. Physicians at Komfo Anokye Teaching Hospital (KATH) in Kumasi were interviewed to gather insight into the epidemiology of the disease in the community. The Public Health Department at KATH was consulted to arrange an educational lecture targeting youths, since mandatory vaccination of newborns has begun, while current generations remain at risk.

Summary of Results: Public awareness of the disease and its risk factors, proper wound care, safe sex, diet, alcohol consumption, and the use of affordable screening tools were all deemed necessary focal points of an educational intervention. A powerpoint presentation was designed for high school students and a lecture delivered to approximately 650 students at Garrison Secondary Armed Forces School in Kumasi, aged fourteen to eighteen. Students readily engaged in post-lecture discussion. Electronic copies of the presentation were made available on all computers in the school’s library.

Conclusions: Review of medical literature and focused public health education lectures can increase public awareness of a disease and empower people to take advantage of affordable, accessible and effective screening tools.

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EDUCATION AND TUBERCULOSIS INTERVENTION IN THE YUKON-KUSKOKWIM DELTA, ALASKA

S.A. Bowerman. University of Washington, Seattle, WA.

Purpose of Study: Tuberculosis is endemic in the Yukon-Kuskokwim Delta of Southwest Alaska with a history of high mortality in the Yupik population. A recent outbreak of active tuberculosis in the village of Napakiak prompted development of a community project to counter spread of tuberculosis utilizing education in addition to traditional tuberculosis sweep methods.

Methods Used: Discussions with Napakiak community leaders were conducted to identify the areas of education they felt were important, as well as cultural and seasonal barriers to education. A literature review was performed to examine both tuberculosis history in the delta as well as health education methods. Plans were made to pair educational seminars with PPD placement at both the fish camps by boat and within the main village of Napakiak. Worksheets covering the specific topics identified by the focus group were designed for the educational seminar, as well as diagrams and pictures to alleviate possible language barriers.

Summary of Results: Education was identified as a high priority for the village of Napakiak, focusing on where TB came from, how it spreads, different treatments & PPD meaning. Low tide and a staff medical emergency precluded fish camp visits by boat, but a TB educational seminar was held for local youth and adults. Information was also presented to patients in clinic with questions. Participation was active and enthusiastic. Presentation diagrams and information were retained by the community center for further education.

Conclusions: Tuberculosis education is an effective tool to reduce disease spread. Community input is essential to effective tuberculosis intervention and education. The Napakiak community shows strong desire for tuberculosis education and seems receptive to further information.
PD-1 (programmed death-1) is a constitently expressed receptor, shown to be associated with peripheral T cell exhaustion in chronic viral infections such as HIV. Blockade of the PD-1 expression on T cells restricted by protective HLA alleles (B57 or B27) correlates with LTNP status and earlier work from our group has demonstrated that CD8+ T cells restricted by these alleles in LTNP maintain proliferative capacity. This study was designed to determine if epitope-specific CD8+ T cells restricted by protective HLA alleles (B57 or B27) correlate with LTNP status and earlier work from our group has demonstrated that CD8+ T cells restricted by these alleles in LTNP maintain proliferative capacity. This study was designed to determine if epitope-specific CD8+ T cells restricted by B27/B57 have reduced proliferative capacity and lose their functional capability to control the infection. Long term non-progressors (LTNP) are able to control HIV and remain AIDS free >10 years post infection without anti-retroviral therapy (ART). Certain protective HLA alleles (B57 or B27) correlate with LTNP status and earlier work from our group has demonstrated that CD8+ T cells restricted by these alleles in LTNP maintain proliferative capacity. This study was designed to determine if epitope-specific CD8+ T cells restricted by B27/B57 have reduced expression of PD-1 compared to epitope-specific CD8+ T cells restricted by non-protective alleles.

**Methods Used:** Using flow cytometry we analyzed Peripheral Blood Mononuclear Cells (PBMC) from LTNP, TP, and individuals with acute infection (AI). CD8+ T cells specific for epitopes restricted by protective HLA alleles (B57 and B27) vs. non-protective alleles (A02 and A03) were analyzed by the National Institutes of Health. The kinetoplastid parasite Trypanosoma cruzi causes a debilitating chronic disease throughout large portions of Latin America. Current treatment of Chagas’ disease with nifurtimox or benznidazole is insufficient because the drugs are poorly tolerated and effective only in the acute stage of infection. Nevertheless, American trypanosomiasis remains a largely neglected disease, and drug development is not currently a major commercial focus. We are screening the MicroSource Spectrum Collection to determine if it contains compounds with activity against T. cruzi at sub-micromolar concentrations.

**Summary of Results:** After screening 400 compounds we have identified five (approximately 1% of the library) with sub-micromolar activity against T. cruzi and low toxicity in mammalian cells. We are screening the remaining compounds and testing the hits for synergistic activity with other established T. cruzi inhibitors.

**Conclusions:** This survey shows that semi-high-throughput screening of an approved drug library can quickly identify compounds with activity against T. cruzi suitable for further investigation. This strategy may lead to a rapid pathway for developing new treatments for Chagas’ disease.

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**Semi-high-throughput screening of a drug library reveals compounds with potent activity against Trypanosoma cruzi**

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**Purpose of Study:** The kinetoplastid parasite Trypanosoma cruzi causes debilitating chronic disease throughout large portions of Latin America. Current treatment of Chagas’ disease with nifurtimox or benznidazole is insufficient because the drugs are poorly tolerated and effective only in the acute stage of infection. Nevertheless, American trypanosomiasis remains a largely neglected disease, and drug development is not currently a major commercial focus. We are screening the MicroSource Spectrum Collection to determine if it contains compounds with activity against T. cruzi at sub-micromolar concentrations.

**Methods Used:** A semi-high-throughput assay was used to screen the library for compounds with activity against mammalian-stage T. cruzi in culture. Infection was established in 96-well microtiter plates using β-galactosidase-expressing T. cruzi grown in murine 3T3 fibroblasts. Compounds were screened in duplicate at 1 μM and parasite growth was assessed after 120 hours by spectrophotometric analysis of a colorimetric reaction with a β-galactosidase substrate. Compounds with >50% growth inhibition were followed up with repeat testing using dilutions to determine a precise IC50.

**Summary of Results:** After screening 400 compounds we have identified five (approximately 1% of the library) with sub-micromolar activity against T. cruzi and low toxicity in mammalian cells. We are screening the remaining compounds and testing the hits for synergistic activity with other established T. cruzi inhibitors.

**Conclusions:** This survey shows that semi-high-throughput screening of an approved drug library can quickly identify compounds with activity against T. cruzi suitable for further investigation. This strategy may lead to a rapid pathway for developing new treatments for Chagas’ disease.

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**Patient recognition of recurrent genital ulcers due to herpes simplex virus type 2 in HIV positive men who have sex with men**

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**Purpose of Study:** Herpes simplex virus type 2 (HSV-2) related genital ulcers increase the risk of HIV transmission. While patients with symptomatic HSV-2 are counseled to avoid sexual activity and take antiviral medication during recurrences, ulcers may be asymptomatic. We sought to determine how frequently HIV positive patients with HSV-2 noted signs or symptoms of genital recurrences in relation to clinical signs of recurrences.

**Methods Used:** HIV and HSV-2 seropositive men who have sex with men (MSM) with a history of genital herpes were enrolled in the study. Participants kept a symptom diary and collected daily oral and anogenital swabs. Participants were seen five times per week by a clinician for physical exam and oral and AG swab collection. HSV-2 was amplified from swabs by PCR.

**Summary of Results:** Ten participants were followed for a total of 529 days (median 55 days per participant). Nine (90%) participants contrarily to published correlations between PD-1 expression on bulk HIV-specific T cells and disease progression.
reported a history of symptomatic genital herpes. Three participants were seropositive for only genital HSV-2 and 7 were seropositive for both HSV-1 and HSV-2. Seven participants were receiving antiretroviral therapy (zidovudine monotherapy) and 3 were receiving no treatment. Participants noted anogenital sign or symptom on 158 of 529 days (28.9%) and herpes-typical lesions on 87 days (16.4%). Clinicians reported anogenital signs including erythema, ulcers or fissures on 202 days (34.1%) and lesions typical of HSV-2 recurrence on 192 days (36.5%). Of 192 days with clinician-recorded lesions, participants reported any anogenital sign or symptom on 119 days (62%). Patients reported being asymptomatic on 73 days (38%) when clinicians noted typical HSV-2 related anogenital lesions.

**Conclusions:** Patient self-evaluation of symptomatic herpes episodes, even in herpes-educated and examination-trained persons, is not a reliable determination of herpes lesion status. As HSV-related ulcers are a significant risk factor for the transmission of HIV, these data may support the use of suppressive therapy to prevent HSV-2 recurrences in HIV positive persons with a history of HSV-2 related disease.

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**TOLL-LIKE RECEPTOR 5 SIGNALLING CAPACITY OF CLINICAL ISOLATES ISOLATED FROM CYSTIC FIBROSIS PATIENTS CHRONICALLY INFECTED WITH *PSEUDOMONAS AERUGINOSA***

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1University of British Columbia, Vancouver, BC, Canada and 2BC Children’s Hospital and Child & Family Research Institute, UBC, Vancouver, BC, Canada.

**Purpose of Study:** We predict that inflammation in the CF lung is due in part to over-stimulation of TLR5 in chronically infected cystic fibrosis patients.

**Methods Used:** Bacterial and cell culture; TLR5 reporter assay; motility assay.

**Summary of Results:** The percentage of bacterial isolates to stimulate TLR5 in chronically infected cystic fibrosis patients was maintained in chronic disease. Motility is not a predictor of TLR5 stimulation when *P. aeruginosa* is non-motile.

**Conclusions:** *P. aeruginosa* isolates from chronically diseased CF lungs are capable of activating TLR5 despite losing motility. As such, TLR5 may be a key player in initiating the inflammatory response in the chronically infected CF lung.

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**GRANULOMATOUS ENCEPHALITIS BY FREE-LIFE AMOEBA***

VD. Hernandez Ugarte1,2, and S. Huerta3.  
1Universidad Juarez del Estado de Durango, Durango, Mexico; 2General Hospital, Durango, Mexico and 3University of Texas Southwestern Medical Center, Dallas, TX.

**Purpose of Study:** Remember rare disease that now take part in the list of disease in the increasing number of people with AIDS.

**Methods Used:** Case review.

**Summary of Results:** Our patient died without a clinicians diagnose. At the autopsy the findings were: multiple necrohemorrhagic injuries that affected the crust and the white substance. Trophozoites were identified and cysts of ameba free in the injuries, as well as acute inflammatory infiltrated, chronic and granulomatous.

**Conclusions:** GAE is a fatal central nervous system infection that affects immunocompromised patients. Unfortunately, there is no effective treatment for affected patients.

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**SPONTANEOUS PENUMOTHORAX IN A YOUNG HEALTHY CHILD FROM PULMONARY HYDATID CYST***

VD. Hernandez Ugarte1,2, and S. Huerta3.  
1Universidad Juarez of Durango, Durango, Mexico; 2General Hospital of Durango, Durango, Mexico and 3University of Texas Southwestern Medical Center, Dallas, TX.

**Purpose of Study:** Clinical correlation of hydatid disease and its complications.

**Methods Used:** Case review.

**Summary of Results:** The patient had a marked clinical improvement following surgical intervention.

**Conclusions:** Surgery is the primary mode of treatment for patients with pulmonary hydatid disease. Complicated cases have higher rates of pre-operative and postoperative complications and require longer hospitalization time and more extensive surgical procedures than uncomplicated cases.

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**ANTI-LIG ANTIBODY RESPONSE OF LEPTOSPIROSIS PATIENTS FROM A HYPERENDEMIC REGION IN THE PERUVIAN AMAZON***

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1UCSD SOM, La Jolla, CA; 2UCSD, La Jolla, CA and 3FIOCRUZ, Salvador, Brazil.

**Purpose of Study:** A new family of proteins with bacterial immunoglobulin-like domains called leptospiral Ig-like (Lig) proteins was recently characterized in pathogenic Leptospira. LigA and ligB genes were found to be expressed in pathogenic but not saprophytic strains of leptospirosis. Because anti-Lig antibody responses appear relatively early in the course of illness, Lig proteins may be ideal antigens for early diagnosis of leptospirosis.

**Methods Used:** Sera from confirmed leptospirosis cases and negative controls from an endemic area of Peru were screened for anti-Lig antibodies. Cases were diagnosed by the presence of anti-leptospiral antibodies as evidenced by positive microscopic agglutination test (MAT) results or IgM ELISA, both of which detect anti-LPS antibodies. Sera from these patients were used to determine reactivity to four recombinant Lig proteins (rLigA, rProY, rRep and rN1). Recombinant Lig proteins...
were separated by SDS-PAGE, transferred to nitrocellulose membranes and probed using 1:500 dilutions of acute and convalescent sera. Summary of Results: Our data show that rN1 and rLigA detected a higher proportion of MAT-positive sera (with high titers against L. interrogans, a pathogenic leptospire) than either rProY or rRep; this difference was not statistically significant (n=11, p-value=0.26). Conversely, the majority of sera that were MAT-positive to only serovar VAR10 (a non L. interrogans leptospire) were non-reactive with rLigA, rProY and rN1; two serum samples reacted with rRep, though this difference was not statistically significant (n=6, p-value=0.088). The PPV and NPV for the recombinant Lig proteins were 50% and 87.5%, respectively, for use in detecting anti-leptospiral antibodies in the Peruvian Amazon. The lack of reactivity between the recombinant Lig proteins and VAR10-positive sera is consistent with previous data indicating that this serovar does not express LigA-like proteins. Conclusions: While recombinant Lig proteins from L. interrogans may be useful diagnostic antigens in some endemic regions where leptospirosis is predominantly caused by this species, recombinant Lig proteins are not optimal for diagnosing leptospirosis in regions where other leptospires dominate as the cause of leptospirosis.

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COMPARISON OF PSEUDOVIRIONS PRODUCED WHEN ALTERNATING AMOUNTS OF GAG AND/OR GP41 ARE CO-EXPRESSED
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Purpose of Study: HIV-I is an enveloped virus composed of the main structural proteins gag and env. gag forms the internal structure containing the genome, while env forms several surface glycoproteins. These glycoproteins consist of gp120, the viral receptor, which is connected to gp41, the transmembrane protein. HIV gag is sufficient to form pseudovirions, of a similar size and shape to wild type virions. Here we attempt to prove using electron microscopy and anti-gp41 mAb immunogold labeling prior to fixation that similar pseudovirions can be produced with gp41 alone or by addition of gp41 to gag.

Methods Used: 293T cells were used to produce and process gag and/or gp41, or gag and gp160. Primary human antibody specific to gp41 was added. Secondary anti-human-lgG-6nm gold followed. The cells were harvested and fixed chemically, after which they were embedded in plastic. The following five conditions were observed by electron microscopy: gag only; gp41 high, gag low; gp41 low, gag high; gp41 only; and gag with gp160.

Summary of Results: We found that gag combined with w.t. (gp41 + gp120) resulted in w.t. virions. However gold was not found, possibly due to steric hindrance from gp120. Gp41 alone resulted in many gold aggregates and few virions, however virions were labeled. High gp41 with low gag produced fewer, less uniform labeled virions than w.t., but more than gp41 alone. Low gp41 with high amounts of gag produced uniform labeled virions. Lastly, gag alone formed unlabeled uniform virions.

Conclusions: More data must be collected and measurements made of the virion particles to quantitatively confirm these observations.

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FRAMINGHAM CARDIOVASCULAR RISK ASSESSMENT OF HIV INFECTED INVIDUALS IN A PREDOMINATELY MSM POPULATION
S. Khajehgirian1, J.D. Scott2,3, and R.K. Bolan1. 1Western University of Health Sciences, Pomona, CA and 2LA Gay and Lesbian Center, Los Angeles, CA.

Purpose of Study: Combination Anti-Retroviral Therapy (cARV) increases survival of HIV-infected persons. Such therapy is often associated with dyslipidemia, diabetes, and insulin resistance. These side effects may increase the risk of cardiovascular events (CVE), but direct evidence of such an association is inconsistently reported. The objective of our study was to assess the risk of CVE in HIV infected individuals using the Framingham equation (which approximates the 10yr risk of a CVE). Our secondary objective was to assess the differences in risk of CVE based on the cARV, CD4 count, and viral load (VL).

Methods Used: 424 HIV patient charts were reviewed. Patients who met the following criteria were included: ≥ 18yo, most recent CD4 count ≥ 200, lipid panel within 1 yr of chart review, and had a clinic visit within the past 6 months. Patients were predominately men who have sex with men (MSM). Framingham risk scores (FRS) were calculated as proposed by the NCEP Guidelines. T-test analysis was performed on the data.

Summary of Results: Patients with an undetectable VL had a mean FRS of 6.39 ± 6.91% while patients with a detectable VL had a FRS of 5.01 ± 6.15% (p=0.05). Patients on treatment had a higher FRS compared to patients not on treatment (6.26 ± 6.85% vs. 4.29 ± 5.44%, p=0.01). Patients taking protease inhibitor (PI) containing cARV trended toward a higher FRS compared to patients on cARV but not containing PIs (6.69 ± 7.15% vs. 5.57 ± 6.31%, p=0.062). There was no statistically significant association between CD4 count and CVE risk score.

Conclusions: HIV-infected patients on PIs trended toward a greater FRS than those not taking PIs. Patients with an undetectable VL had a greater FRS than those with a detectable VL, suggesting HIV treatment results in a higher risk of CVE. The CD4 count did not have a significant effect on FRS. These data appear to contradict a recent, large prospective HIV treatment study (SMART). Our results are limited because FRS has not been validated in an HIV population.

Western Student Medical Research Forum
Student Scientific Session IV
8:30 AM
Friday, February 1, 2008

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IDENTIFICATION OF LIMB SPECIFIC SONIC HEDGEHOG REGULATORY MODULES

Purpose of Study: Sonic hedgehog (shh) is a potent developmental morphogen and plays a pivotal role in the growth and patterning of many organ systems. Specific regulation of its temporal and spatial expression is crucial for the normal development of the vertebrate limb. Shh is normally expressed in the zone of polarizing activity (ZPA) in the posterior margin of the developing limb bud. Disruption of a region 1Mb upstream of Shh, within an intron of the adjacent Lmbr1 gene, causes either adactyl (no digits) or pre-axial polydactyl (extra digits) that are comparable to mutations in the expression of Shh in the limb. We have identified 2 regions containing sequences that are highly conserved across divergent species within this limb-specific sonic hedgehog regulatory region (LSSRR). These evolutionarily conserved regions (ECRs) may contain the regulatory modules for limb-specific Shh expression.

Methods Used: A 1.8kb DNA fragment of the LSSRR was isolated and ECR “peaks” were identified as Peak A and B; Peak B was further divided into B1, B2, and B3. Enhancer-reporter constructs were generated by inserting the LSSRR or individual ECR fragments from LSSRR (peak A and peak B) upstream of a minimal HSV-tk promoter linked to green fluorescent protein (GFP). The constructs were co-electroporated with a red fluorescent protein (RFP) reporter to document transfection efficiency. Localized GFP expression was taken as evidence of enhancer activation. Fluorescence was monitored after transfection and embryos were harvested between 36–48 hrs.
Summary of Results: Electroporation with constructs containing the complete LSSRR, peak B or peak B3 resulted in broad GFP expression in the posterior half of the limb, coincident with cells that had expressed Shh. No green fluorescence was detected with peak A, peak B1 and peak B2. In addition, when B was mutated at a potential site for NFkappaB, a transcription factor linked to Shh regulation, GFP expression was altered and markedly reduced.

Conclusions: Our data indicates that ECRs can be useful in identifying regulatory modules. Peak B3 retains the expression pattern of Shh suggesting that this region contains the limb-specific regulatory modules. Further experiments are needed to characterize these modules and identify the ZPA-related proteins responsible for their enhancer activity.

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THE EFFECTS OF PRENATAL DEPRESSION ON P50 AUDITORY SENSORY GATING IN INFANTS
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Purpose of Study: Maternal depression is an important public health concern, as it is common and has potentially detrimental effects on women and their infants. The majority of current research focuses primarily on the postpartum period, yet depression during pregnancy is at least as common as it is postpartum. The 2nd trimester is the time in which a fetus’ HPA axis is most sensitive to prenatal stress (in this case, depression). The purpose of this study is to explore the effects of prenatal depression on auditory sensory gating in early infancy.

Methods Used: This study will measure the evoked potential component P1 (i.e., P50) in 4 groups of infants during REM sleep: (1) infants whose mothers had depressive symptoms during their 2nd trimester of pregnancy, but not between 6 and 12 weeks postpartum; (2) infants whose mothers had depressive symptoms between 6 and 12 weeks postpartum, but not during their 2nd trimester; (3) infants whose mothers had depressive symptoms both during their 2nd trimester and postpartum; and, (4) infants whose mothers did not have depressive symptoms during their 2nd trimester or postnatally. The P50 is an indicator of one’s ability to filter out extraneous auditory information and is calculated by measuring the ratios of evoked potentials to a paired-click paradigm (T/C ratio). Infants whose mothers have Schizophrenia, ADHD, OCD, or Bipolar Disorder are excluded, as adults with such diagnoses and many of their family members demonstrate gating deficits. The Edinburgh Postnatal Depression Scale is our measure of depressive symptoms.

Summary of Results: The degree of response suppression (p50 T/C ratio) will be compared across groups using analysis of variance and appropriate post hoc tests.

Conclusions: Based on prior research on biochemical measures in depressed mothers and their infants, we hypothesize only those infants whose mothers had depressive symptoms during their 2nd trimester will demonstrate gating deficits, suggesting the in-utero environment has greater effect on fetal brain development in regard to sensory auditory gating than does the postnatal environment. Further research distinguishing effects of prenatal and postpartum depression on children’s physiological and cognitive development may be beneficial in determining if and when to treat maternal depression as well as possible underlying mechanisms.

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NOISE AMIDST THE SILENCE: X-LINKED MICRORNAS ARE HIGHLY EXPRESSED DURING MEIOTIC SEX CHROMOSOME INACTIVATION
J. Michaels, S. Ro, and W. Yan. University of Nevada School of Medicine, Reno, NV.

Purpose of Study: To verify our initial finding that X-linked non-coding microRNAs (miRNAs) are highly expressed in the late pachytyne stage of spermatogenesis, while all protein-coding genes on the X or Y chromosome are transcriptionally silenced through a well known process termed meiotic sex chromosome inactivation (MSCI). Therefore, messenger RNAs (mRNAs) encoded by these genes are absent in pachytyne spermatocytes in all mammalian species. The mechanism and physiologic significance of MSCI remains largely unknown. During expression profiling analysis of 122 miRNAs that we cloned from mouse testis, we found ~20% of these testicular miRNAs are encoded by genes on the X chromosome and most of them are exclusively or preferentially expressed in the testis. By further analyzing miRNA expression during testicular development, we observed that levels of these X-linked miRNAs peaked at postnatal days 14 and 21, suggesting that they are expressed mainly by pachytyne spermatocytes. The data were surprising because X-linked mRNA-coding genes are not expressed in pachytyne spermatocytes.

Methods Used: We examined expression levels of 66 known X-linked miRNAs in the developing testes (postnatal days 7, 14, 21, and adult), and 6 purified testicular cell types including Sertoli cells, type A spermatagonia, pachytyne spermatocytes, round spermatids, elongated spermatids and vas deferens spermatosa using SYBR green-based real-time quantitative PCR.

Summary of Results: Consistent with our preliminary data, 59 of 66 X-linked miRNAs (90%) displayed the highest or the 2nd highest expression levels in pachytyne spermatocytes.

Conclusions: Our data demonstrate, for the first time, that the majority of X-linked miRNA genes are highly expressed during late meiosis, while simultaneously the X-linked mRNA-coding genes remain transcriptionally silent. It is possible that these miRNAs may be synthesized before MSCI, e.g. in pre-pachytyne stages, and then stabilized, accumulated and persistently expressed throughout the entire meiotic phase of spermatogenesis. If this is true it begs the question as to how these miRNAs are stabilized over an extended period of time. Future studies are aimed at determining the mechanism of miRNA MSCI escape and its potential physiological significance.

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VASCULAR ENDOTHELIAL GROWTH FACTOR mRNA AND PROTEIN EXPRESSION IN THE DEVELOPING HUMAN EYE
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Purpose of Study: Erythropoietin (Epo) and Vascular Endothelial Growth Factor (VEGF) are involved in angiogenesis, and may be involved in retinal vascular growth in early human development. We recently reported increased Epo mRNA and protein concentrations in the vitreous of the developing human eye (Pediatric Research 2007). It is not known whether these growth factors are involved in the development of retinopathy of prematurity. To identify changes in VEGF during normal human development, we measured VEGF mRNA and protein concentrations in 10–24 week gestation fetal vitreous and serum.

Methods Used: Fetal serum and vitreous samples were obtained from 10 to 24 weeks gestation. The contents of the globe were extracted, the lens removed, and the vitreous collected from both fetal eyes. The retina was isolated and RNA extracted for quantitative VEGF mRNA determination by PRISM PCR. Fetal blood was isolated from the umbilical cord, spun, and serum collected. Serum and vitreous samples were stored at −20 C until analyzed for VEGF protein by ELISA.

Summary of Results: 80 vitreous samples and 47 serum samples between 10 and 24 weeks gestation were collected for measurement of
VEGF concentrations. VEGF mRNA concentrations increased with increasing gestational age (p<0.01, 10–14 weeks versus 18–20 weeks, 10–14 weeks versus 21–24 weeks, and 15–17 weeks versus 21–24 weeks). However, VEGF protein concentrations did not increase with increasing gestation. Protein concentrations were similar in vitreous and serum throughout the gestational ages tested.

**Conclusions:** VEGF mRNA concentrations increase with increasing gestational age, however protein concentrations do not increase in vitreous, and are similar to serum concentrations. We speculate that VEGF plays an important role in normal retinal vascular development, and that preterm delivery and changes in production of this vascular growth factor might affect retinal vascular development. The relationship between VEGF transcription and translation in the developing human eye requires further study.

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**STRUCTURAL DOMAINS AND PROTEIN-PROTEIN INTERACTIONS THAT DETERMINE THE STABILITY OF THE TRANSCRIPTION FACTOR PROTEIN HES6**

D.V. Brown. 1University of Washington, Seattle, WA and 2University of Cambridge, Cambridge, United Kingdom.

**Purpose of Study:** The developmental processes of neurogenesis, somitogenesis, and myogenesis, respectively, are crucial elements of embryological development. It has been shown that basic helix-loop-helix transcription factors including members of the highly conserved Hes family of proteins are key proteins involved in regulating these processes (Hirata et al. 2004; Kageyama et al. 2005). One member of this family, Hes6, is particularly interesting for its apparent involvement in all three of these processes. Over-expression of Hes6 has been shown to promote neurogenesis, disrupt somitogenesis, and expand the developing myome while inhibiting terminal muscle cell differentiation (Bae et al. 2000; Cossins et al. 2002). However, the molecular basis of Hes6 function remains largely unknown.

**Methods Used:** Studies conducted on other Hes proteins, such as Hes1 and Hes7, have demonstrated that the rate at which they undergo proteolytic degradation is crucially important to normal somitogenesis (Hirata et al. 2004). I therefore investigated the structural domains of Hes6 that are important to the stability of the protein and how interactions with other proteins affect Hes6 stability. I added in-vitro translated 35S-methionine-labeled Hes6 protein to cytoplasmic extracts and translated 35S-methionine-labeled Hes6 protein to cytoplasmic extracts to facilitate protein degradation. We speculate that VEGF plays an important role in normal retinal vascular development, and that preterm delivery and changes in production of this vascular growth factor might affect retinal vascular development. The relationship between VEGF transcription and translation in the developing human eye requires further study.

**Conclusions:** These studies suggest that Hes6 may be regulated at the level of protein stability. Given the key functions of Hes6 in development, this area deserves further investigation.

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**EFFECTS OF DENERVATION IN Cavernosal Vasoreactivity in Mice**

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**Purpose of Study:** The vasoreactive changes of cavernosal tissue, after removal of hemodynamic stimuli, are poorly understood. Our aim was to investigate how hemodynamic stimuli contribute to penile integrity through removal of such stimuli by nerve transection. Studies in rats have shown significant apoptosis in smooth muscle cells (SMC) following denervation (McVary 2003). The dynamics of the tissue may be expected to change due to loss of SMC’s. We hypothesize that removal of the hemodynamic stimuli would result in decreased nerve...
mediated vasoreactivity of the cavernosal tissue whereby vasorelaxation and vasoconstriction should be impaired.

Methods Used: 15 male C57BKS mice were randomized to either bilateral cavernous nerve transection (NT) or sham laprotomy. After two weeks the mice underwent penectomy and the cavernous tissue were isolated in organ baths for tension measurements. Tissues underwent phenylephrine (PE) induced contraction, cholinergic (ACh) relaxation, sodium nitroprusside (SNP) relaxation, electrical field stimulation (EFS) sympathetic contraction, and EFS parasympathetic relaxation in vitro.

Summary of Results: Frequency-dependent nerve-evoked relaxations were markedly reduced in the NT mice compared to the sham mice (% reduction of PE pre-contraction at 10 Hz: 77.67 +/- 3.40%, n=7 vs. 54.49 +/- 3.32, n=8, P<0.05). Frequency-dependent nerve-evoked contractions were increased in the NT mice compared to the sham mice (maximum at 20 Hz: 0.0705 +/- 0.0124 mN/mg, n=6 vs. 0.121 +/- 0.0157 mN/mg-1, n=8, P<0.05). No significant changes were noticed in response to PE-induced contraction, ACh induced relaxation, and SNP induced relaxation.

Conclusions: Parasympathetic vasorelaxation decreased in cavernous tissue as expected when nerves that mediate such activity had undergone degeneration. Relative decreased sympathetic vasoreactivity in the sham mice could be due to unintended parasympathetic stimulation during frequency-dependent nerve-evoked contraction. At the two week mark, though autonomic function was altered, hemodynamic loss did not significantly alter the vasoreactivity mediated specifically by SMC’s and endothelial cells of the cavernous tissue. Further investigation may examine whether such changes would occur at longer time points.

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THE ROLE OF LIFESTYLE PRACTICES IN PSORIASIS
J. Ahdoud1, M. Chiur2, and J. Kim2. 1David Geffen School of Medicine at UCLA, Los Angeles, CA and 2David Geffen School of Medicine at UCLA, Los Angeles, CA.

Purpose of Study: Psoriasis is a multifactorial inflammatory skin disease affecting 4.5 million adults in the United States. As with other chronic disorders, several lifestyle factors have been found to play a role in the development of psoriasis. This comprehensive review assesses the effects of nutrition, exercise, stress, alcohol use, smoking, and obesity on the natural history of psoriasis.

Methods Used: Comprehensive literature review.

Summary of Results: Several reports in the literature suggest the deleterious effects of emotional stress, alcohol use, smoking, and obesity as they relate to psoriasis outcome. As a chronic inflammatory disease, the induction of inflammatory mediators related to these factors may explain the pathogenetic events occurring in psoriatic skin.

Conclusions: The possibility that simple modifications in lifestyle may influence both the prevalence and severity of psoriasis offers an exciting potential adjunct to the prevention, management, and treatment of psoriasis in the future. The design of more definitive trials including interventions such as smoking cessation, weight reduction, and stress reduction are necessary to elucidate the relationship between modifiable behavioral risk factors and psoriasis.

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TRADITIONAL SMOKELESS TOBACCO (IQMIK) USE AMONG PREGNANT ALASKA NATIVE WOMEN IN DILLINGHAM, ALASKA
K.M. Groundwater. University of Washington School of Medicine, Seattle, WA.

Purpose of Study: Western Alaska experiences a high prevalence of tobacco use among its Alaska Native pregnant population. The three main forms of tobacco use are cigarettes, store purchased smokeless tobacco, and a traditional smokeless tobacco called Iqmik. Iqmik is produced by mixing and masticating smokeless tobacco with ash from the fungus found on birch trees. Iqmik is detrimental to fetal health. The purpose of this project was to explore the socio-cultural aspects of Iqmik use and to exchange knowledge about its harmful effects with pregnant Alaska Native women.

Methods Used: An analysis of the literature was performed using PubMed. Tobacco cessation resources were reviewed to gather information and to educate pregnant women in the community. Round table and one-on-one discussions were held with local and village-boarding pregnant women to understand the cultural beliefs regarding Iqmik use, and to exchange knowledge of its harmful effects on fetal development. The outcome of the discussions was shared with local health care providers.

Summary of Results: Iqmik use by pregnant women is common in Dillingham and the surrounding villages (61% in a neighboring borough), and is deeply ingrained in the local Alaska Native culture traditionally, socially, and spiritually. Misconceptions exist that Iqmik is a safe alternative to other forms of tobacco; research indicates that fetal and maternal blood nicotine levels are actually higher in Iqmik and can be considered a “free base” form of nicotine. Pregnant women involved in the discussions were surprised to learn Iqmik use was dangerous. Tobacco cessation programs currently exist, yet little attention has been directed towards Iqmik use during pregnancy or its cultural implications.

Conclusions: There is a high prevalence of tobacco use among pregnant women in western Alaska. Iqmik, a traditional form of tobacco, is deeply ingrained in the local Native culture, and believed to be a safe alternative to other forms of tobacco; research indicates that fetal and maternal blood nicotine levels are actually higher in Iqmik than in other tobacco products. Health care providers must address this concern in a culturally sensitive manner in order to educate the community to decrease Iqmik use among pregnant Alaska Native women.

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VITAMIN D DEFICIENCY IN JUNEAU, ALASKA
A.M. Tsigonis. University of Washington School of Medicine, Seattle, WA.

Purpose of Study: Infants in Alaska feeding solely on breast milk are twelve times more likely to be vitamin D deficient than any other age group because vitamin D is not in their diet and they have minimal ultraviolet exposure. According to local physicians, the high latitude combined with overcast skies for the majority of the year causes an even higher rate of vitamin D deficiency in Juneau, Alaska than in other locations around the globe at similar latitudes. In 2003, The State of Alaska Epidemiology Office found that infants fed solely breast milk are twelve times more likely to be vitamin D deficient than any other age group.

Methods Used: Research was done using PubMed. Discussions were held with patients, community members, “new mother” and baby-parent groups in small group settings, on an individual basis, and during clinical OB visits. After presentations, one-on-one surveys were done to get feedback on how the audiences perceived the information and if they would consider vitamin D supplementation. Brochures were distributed to each participant and will continue to be handed out to future patients.

Summary of Results: A multifaceted approach to patient education is the best method to decrease vitamin D deficiency in Juneau, AK. The two mothers groups provided the largest community contact. Of the mothers who attended the presentations, ten expressed surprise and said they had not heard this information before. Six mothers knew about vitamin D; one had previously chosen not to use supplements. The feedback was positive; all the mothers said they would talk to their care providers and consider vitamin D supplementation. These mothers also felt strongly enough about this new information to share it with other young mothers.
Conclusions: Infants in Alaska feeding solely on breast milk are twelve times more likely to be vitamin D deficient than any other age group because vitamin D is not in their diet and they have minimal ultraviolet exposure. A multifaceted approach to patient education is the best method to decrease vitamin D deficiency in Juneau, AK. Education of the target population of breastfeeding mothers regarding the benefits vs. risks of vitamin D encourages patient adherence. Local health care providers will continue to hand out the vitamin D brochures and discuss the added risk to infants of vitamin D deficiency.

Adolescent Medicine and General Pediatrics
Concurrent Session
1:30 PM
Friday, February 1, 2008

203 MUTATION IN THE GENE FOR EPITHELIAL CELL ADHESION MOLECULE IS RESPONSIBLE FOR CONGENITAL TUFTING ENTEROPATHY
M. Sivagnanam1, J. Mueller1, H. Lee2, Z. Chen2, S. Nelson2, O. Libiger4, N. Schork4, J. Lavine5, S. Taylor5, R. Newbury5, R. Kolodner5, and H. Hoffman1. 1University of California, San Diego, San Diego, CA; 2University of California Los Angeles, Los Angeles, CA; 3University of California San Diego, San Diego, CA; 4The Scripps Research Institute, San Diego, CA and 5Rady Children’s Hospital, san diego, CA.

Purpose of Study: Congenital Tufting Enteropathy (CTE) is a rare autosomal recessive diarrheal disorder presenting in the neonatal period. CTE is characterized by intestinal epithelial cell dysplasia leading to severe malabsorption and significant morbidity and mortality including dependence on parenteral nutrition. The pathogenesis and genetics of this disorder are not well understood. The objective of this study was to identify the gene responsible for CTE.

Methods Used: A family of Mexican-American descent with 2 children affected with CTE was identified. The affected children are double second cousins providing significant statistical power for linkage. Using Affymetrix 50K Single Nucleotide Polymorphism (SNP) chips, genotyping was performed on only two patients and one unaffected sibling. Direct DNA sequencing of candidate genes was undertaken and RT-PCR and immunohistochemistry were performed on intestinal tissue from patients and controls.

Summary of Results: SNP homozygosity mapping identified a unique 6.5 MB haplotype of homozygous SNPs on chromosome 2p21 where approximately 40 genes are located. LOD score was determined to be 4.7. Direct sequencing of genes in this region revealed homozygous G > A substitution at the donor splice site of exon 4 in Epithelial Cell Adhesion Molecule (EpCAM) of affected patients. Parents and an unaffected sibling were found to be heterozygous for this variant consistent with autosomal recessive inheritance. This mutation was not identified in 200 ethnically matched control DNAs. RT-PCR of duodenal tissue demonstrated a novel alternative splice form with deletion of exon 4 in affected patients. Immunohistochemistry of patient intestinal tissue revealed decreased expression of EpCAM.

Conclusions: Mutations in the gene for EpCAM are responsible for Congenital Tufting Enteropathy. This information will be used to gain further insight into the molecular mechanisms of this disease.

205 ATTITUDES TOWARDS COMPLEMENTARY AND ALTERNATIVE MEDICINE IN A PEDIATRIC HOSPITAL
J. Valentine1, N. Jimenez2, and A. Kundu1,2. 1University of Washington, Seattle, WA and 2Seattle Children’s Hospital, Seattle, WA.

Purpose of Study: Complementary and Alternative Medicine (CAM) has been an area of growing interest in pediatric care. In this study, we aim to assess the attitudes of physicians in a pediatric hospital in Washington State towards CAM, with a special emphasis on patterns for recommending CAM, level of trust for CAM providers, and concerns for development of CAM in a hospital setting.

Methods Used: An anonymous electronic survey with 25 items was sent to all physicians affiliated with Children’s Hospital and Regional Medical Center. Two reminder emails were sent.

Summary of Results: Response rate was 31.3% (266 of 851). Of the 67% of providers who recommended some form of CAM therapy to patients in the last six months, providers were most likely to recommend biofeedback (42.0%) and acupuncture (32.8%). Physicians who use CAM (71.0%) were more likely to recommend CAM therapies to their patients than those who do not use CAM (75.8% vs. 45.2%, p value 0.0001). Major concerns related to CAM therapies include lack of safety data (63.6%), lack of education about CAM (44.7%), adverse interactions with current therapies (41.3%) side effects related to CAM therapies (38.6%) and qualifications of CAM providers (39.8%). MD CAM providers were perceived to be most trustworthy followed by licensed massage therapists, allied health care providers with CAM training, and licensed acupuncturists. Naturopaths and chiropractors inspired the least trust in this survey. Providers indicated they would like more education in the areas of efficacy (88%), impact of CAM on health outcomes and safety (78%), interactions of CAM therapies with conventional therapies and indications for use of various CAM therapies (74%).
Conclusions: This study indicated a strong positive correlation between personal use of CAM by physicians and recommendation of CAM to their patients. Qualifications of CAM providers and limited education relating to CAM therapies negatively impact recommendations of CAM therapies by physicians. Rigorous licensing processes and more CME courses regarding CAM therapies may improve the acceptance and integration of CAM therapies in conventional health care.

206 THE CHOKING GAME (adolescent asphyxiation activity): A potentially fatal risk behaviour sometimes misdiagnosed
Purpose of Study: For decades, children in schoolyards, playgrounds and summer camps have engaged in a self-asphyxiation thrill-seeking activity known by many different names, among them “The Choking Game”. Unfortunately, the game sometimes proves to be fatal. The game may be played in groups or alone. Pressure is applied to the neck or chest to restrict oxygen flow to the brain creating a brief euphoria or “high” feeling prior to loss of consciousness. If the constriction is not removed in time, permanent brain injury or death may quickly occur. Unfortunately for the families, these deaths have often been misdiagnosed as suicide. The purpose of this study is to raise awareness of “The Choking Game” and thus prevent injury or death by discouraging youth from participating in this risk behaviour, and to inform medical examiners about the behaviour to reduce the misdiagnosis of “suicide”.
Methods Used: Questionnaires on knowledge about, and/or participation in, the Choking Game were distributed in school children in grades 4 to 12 (age 9-18 years). Two schools were in Ontario, and 6 schools were in Texas. Three of the 8 schools had had a recent death of a child while playing the Choking Game.
Summary of Results: Of the 2762 surveys distributed 2504 (90.7%) were completed and included in the study. There were 52% females, mean age 13.7 years. 68% of children had heard about the game. The average age at which they learned about it was 12.4 years, but many seemed to have heard about it as a result of the recent deaths. 57% had heard about it more than a year previously, prior to the deaths. 45% knew somebody who played it; 6.6% had tried it, almost all of those with someone else. 40% of children thought there was no risk in playing the game. Information that you could die or have brain damage, delivered by a near-victim or victim’s family member for older children, or their own parents for younger children was most likely to change behaviour.
Conclusions: Participation in self-asphyxiation behaviour is not uncommon. Knowing that the behaviour can result in death or brain damage is likely to change behaviour. Hearing the message from parents, near-victims, or family of victims is most likely to be effective.

207 NOVEL CERULOPLASMIN DETECTION METHOD BY TMS FOR NEWBORN SCREENING OF WILSON DISEASE
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Purpose of Study: Wilson disease (WD) is an inherited autosomal recessive disorder caused by a mutated WND gene, which encodes the P-type ATPase, ATP7B. ATP7B is involved in the efflux of hepatic copper into bile. Normal metabolism of copper includes hepatic incorporation into ceruloplasmin (cp), which is secreted into plasma. In WD this process is impaired and the levels of plasma cp are reduced. WD is easily treatable if it is diagnosed before irreversible tissue damage occurs. Tandem mass spectrometry (TMS) is an accurate and precise method for detecting small amounts of compounds and has become a standard method for newborn screening. The purpose of this study is to use a peptide mass fingerprinting approach by TMS to measure cp in dried blood spots for the newborn screening of WD.
Methods Used: Purified cp was digested with trypsin and subject to Q-TOF and Ion Trap MS with subsequent SEQUEST analysis to identify peptides with strong homology to cp. Three parent peptides (arbitrarily labeled A, B, and C) were selected on the basis of peak intensity, ion score, number of product ions with a higher mass than the precursor ion, absence of cys residues, and low potential for posttranslational modification. Four product ions were selected for each precursor ion and were used to design a TMS assay using a multiple reaction monitoring approach. The optimized TMS assay was used to detect cp in digested human serum (cp is abundant in human serum) to test if the assay could be used to identify cp in a large mixture of digested proteins. Finally, the proteins of newborn dried blood spots were eluted, and the digested peptides were analyzed using the TMS assay.
Summary of Results: The total ion chromatogram (TIC) for the TMS analysis of the dried blood spot digestion had a significant peak area for precursor ion A. The single ion chromatograms (SIC) of the corresponding product ions also displayed significant peaks with intensities ranging from 1.3e4–2.4e4. Precursor ions B and C each had 2 peaks in the TIC indicating that not all of the peptides were eluting from the LC column at the same time.
Conclusions: The peak intensity in the TIC of precursor ion A, and the SICs of the product ions indicate that this assay can be used to quantify the level of cp in newborn dried blood spots for the purpose of newborn screening.

208 HEPATITIS B VACCINE BIRTH DOSE DOES NOT INCREASE THE INCIDENCE OF FEVER OR RULE OUT SEPSIS EVALUATIONS DURING THE NEWBORN HOSPITAL STAY
S. Santiago1,2, S. Wu1,2, M. Lee1, and C. Chantry1. 1UC Davis Pediatrics, Sacramento, CA and 2UC Davis School of Medicine, Sacramento, CA.
Purpose of Study: The Hepatitis B Vaccine Birth Dose has been controversial since its introduction. Many have wondered if vaccine-induced fever would cause unnecessary work-ups for an infectious source. Unfortunately, the evidence to disprove this is incomplete. At our institution we encounter a subset of parents who refuse the vaccine at birth for various reasons. Using this group as our control, we sought to determine if the administration of the Hepatitis B Vaccine Birth Dose had an appreciable effect on the incidence of fever during the newborn hospital stay. Our hypothesis was that the Hepatitis B Vaccine caused fevers which would result in more ‘rule-out sepsis’ evaluations and longer hospitalizations.
Methods Used: Retrospective chart review of 1588 babies born between January 1, 2005 and December 31, 2005 at UC Davis Children’s Hospital. Data related to demographics, the Hepatitis B vaccination status and the presence of fever were abstracted from physician records and nursing flowsheets. For children who had a documented temperature $\geq38.0^\circ$C we recorded more complete information. Our study needed a sample size of 1451 children to detect an increase in fever from 1% to 4% with 80% power, assuming that 1 in 5 parents would refuse the birth dose of the vaccine.
Summary of Results: We reviewed 1588 charts and found that 19.9% of all parents declined the Hepatitis B Vaccine. The overall incidence of fever ($\geq38.0^\circ$) was 10.6%. The incidence of fever in the group that
received the vaccine was 11.0%, while the incidence was 9.1% in the group who refused the vaccine (2-tailed p-value 0.34). Additionally there was no significant difference between the frequency of lab draws in the two groups (50% vs. 55% in those who refused the vaccine, p-value 0.06).

Conclusions: The administration of the Hepatitis B vaccine birth dose does not increase the incidence of fever or subsequent rule out sepsis evaluations during the newborn hospital stay. While our study is limited by the self selection of our control group, we believe this should not affect the incidence of fever caused directly by the Hepatitis B vaccine. As we found no evidence that the Hepatitis B vaccine causes fever, the fear of inappropriate ‘rule-out sepsis’ evaluations as a sequelae is unwarranted.

209 BLOOD PRESSURE CORRELATIONS WITH CHILDREN’S LABORATORY INDUCED PAIN TOLERANCE, INTENSITY, AND UNPLEASANTNESS

K. Trangsrud1, Q. Lu2, S. Evans3, J.C. Tsao4, and L.K. Zeltzer2. 1UC Irvine School of Medicine, San Jose, CA and 2UCLA, Los Angeles, CA.

Purpose of Study: Numerous studies indicate gender differences in pain responsivity as well as gender differences in resting blood pressure levels. Adult studies demonstrate that increased resting blood pressure levels are correlated with decreased pain sensitivity. However, few studies examined the relationship between blood pressure and experimental pain sensitivity among children. This study examined the relationship between resting blood pressure levels and experimental pain tolerance, intensity, and unpleasantness in 244 healthy children (50.8% female, mean age 12.73 ± 2.98 years, range 8–18 years). The following hypotheses were tested: (1) females would have lower resting blood pressure levels than males, and (2) resting blood pressure levels would be positively correlated with experimental pain tolerance, and negatively correlated with pain intensity and pain unpleasantness.

Methods Used: Participants underwent separate 4-trial blocks of cutaneous pressure and thermal pain stimuli, and 1 trial of cold pain stimuli in counterbalanced order. Resting blood pressure was measured three times with a 1 minute interval between readings before the laboratory session began.

Summary of Results: We found that female adolescents (12–18 years) had significantly lower resting systolic blood pressure (SBP) levels than male adolescents. However, no significant sex differences in resting SBP levels were found among children (8–11 years), and no sex significant differences in resting diastolic blood pressure (DBP) were found in adolescents or children. Correlation and regression analysis revealed that (1) age was a significant predictor of pain response measures among males; (2) those with low resting DBP levels (below the mean value of 61.3) experienced greater cold intensity, thermal unpleasantness, and cold unpleasantness than those with high resting DBP levels among young females (8–11 years); this relationship was not seen among adolescent females.

Conclusions: These results suggest that resting DBP levels impact experimental pain response measures in young females.

211 CREATING A PROGRAM FOR MEASURING CHILDHOOD OBESITY IN BRITISH COLUMBIA

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Purpose of Study: Childhood obesity is a major public health concern, and despite British Columbia’s (BC) healthy reputation, we are not immune. In 2004, the Canadian Community Health Survey estimated that 26% of BC children between the ages of 2 and 17 are overweight or obese. Obesity is estimated to cost BC’s health care system between $730 million and $830 million annually. Despite this challenge, BC actually has very little data that can be used to compare schools and districts or evaluate exercise and healthy eating programs designed to address this problem. The British Columbia Medical Association called on provincial government to implement a Child & Youth Growth Index (CYGI) in order to monitor, measure and evaluate the current obesity epidemic in young people.

Methods Used: In BC, the index would track the level of childhood obesity throughout the province by anonymously measuring the body mass index (BMI) of all students in the public school system on an annual basis. This would enable the province to compare and evaluate programs on both a district and regional basis.

Summary of Results: This feasibility study generates three cost estimates of the CYGI, ranging from $500,000 to $2,000,000 per year. The major factor affecting the varying cost estimates is labour. The cost per child would range between $0.75 and $3.57 per year. Controlling obesity among children and adults boils down to encouraging individuals to eat healthier food and to get more exercise through targeted health programs. This index will be a vital tool to accurately analyze outcomes of such programs in BC.

Conclusions: While we recognize that this index is but one critical piece of a broader set of initiatives that will be needed to combat this public health challenge, if implemented, the CYGI will play a key role in the fight against childhood obesity and will help young British Columbians lead healthier lives.
212 RECURRENT VOMITING AND DIARRHEA AS A PRESENTATION OF DYSMENORRHEA
K.A. Molas-Torreblanca, and C.J. Barangan. University of Nevada School of Medicine, Las Vegas, NV.

Purpose of Study: To report a remarkable presentation of severe dysmenorrhea in a 17-year-old black female consisting of multiple hospitalizations and emergency department (ED) visits secondary to repeated episodes of severe vomiting and subsequent dehydration. An evaluation by gastroenterology during one admission resulted in a diagnosis of cyclic vomiting syndrome. This case report describes her evaluation, diagnosis and management. The pertinent literature on primary and secondary dysmenorrhea is also reviewed.

Methods Used: We describe our investigation and subsequent management. A clinical diagnosis was made based on history and the patient was treated accordingly. No imaging studies were obtained based on our high index of suspicion. A diagnostic laparoscopy was done during a subsequent presentation to the ED for recurrence of symptoms approximately one month after our diagnosis was made.

Summary of Results: The patient presented to us with sudden onset of severe vomiting, dehydration and associated abdominal pain with radiation to the back. She denied fever, diarrhea, or dysuria. She reported taking acetaminophen for her pain without relief. Her past medical history was significant for painful menstrual cramps since menarche at 11 years old. Her review of systems was unremarkable except for dyspareunia. She was treated with intravenous fluids, analgesics and anti-emetics during her four-day hospitalization. The patient was discharged home with a diagnosis of severe primary dysmenorrhea and prescribed a non-steroidal anti-inflammatory medication and combined oral contraceptive pills. She was seen in the ED one month later for continued symptoms. A gynecologic consult was obtained and diagnostic laparoscopy was performed which revealed endometriosis causing secondary dysmenorrhea.

Conclusions: Dysmenorrhea is not a benign condition and should not be disregarded in adolescents. Presentation can vary from mild to severe symptoms requiring acute hospitalization. Taking a complete history and searching for any underlying etiology causing secondary dysmenorrhea is crucial in order to make an accurate diagnosis and provide appropriate management and relief of sometimes debilitating symptoms.

213 AN UNUSUAL COMBINATION OF MULLERIAN DUCT ANOMALIES IN AN OLDER ADOLESCENT FEMALE
C.J. Barangan. University of NV School of Medicine, Las Vegas, NV.

Purpose of Study: To describe the unusual case of a 21 year old adolescent female affected by two Mullerian duct anomalies (MDA) characterized by uterus bicornuate, partial and cervical agenesis without renal anomalies. One case with these two anomalies existing concomitantly has previously been described in the literature. This patient presented with acute lower abdominal pain at 16 years old, with urinary tract infection at 18 years old and for urinary tract infection and PAP smear at 21 years old. She was asymptomatic and lost to follow-up during the interims.

Methods Used: An MRI and pelvic ultrasound was done at initial presentation. A renal ultrasound and abdominal CT were executed to further investigate the cause of the left flank pain and hematuria at second presentation. On latter presentation, a pelvic exam and trans-vaginal ultrasound were done. A follow-up MRI could not be done because of poor follow-up and lack of insurance.

Summary of Results: The first pelvic ultrasound was significant for hematocolpus. MRI of the pelvis demonstrated an abnormal uterus with a marked didelphys configuration and bilateral enlarged ovaries with cysts. Renal ultrasound showed bilateral mild pelvicalceal fullness. CT of the abdomen with contrast revealed free fluid in the pelvis, adnexal cystic changes, enlarged kidneys with pelvicalceal fullness, left greater than right. Normal secondary sexual characteristics, a vaginal pouch and absence of the cervix were appreciated on exam. Transvaginal ultrasound showed two uterine horns with a single lower uterine segment consistent with uterus bicornuate, partial, but no definite cervix. Measurements of the ovaries were similar to the first ultrasound.

Conclusions: Early and accurate diagnosis of MDAs is important, but can be difficult, due to variable clinical presentation and, in this case, poor follow-up. A combination of agenesis and incomplete fusion of the Mullerian tract can occur. Radiological studies are invaluable in the evaluation of MDAs. Technological advances made in the last 6 years are particularly pertinent in this case. Utilizing every opportunity to evaluate an adolescent with primary amenorrhea when they present for care will facilitate early diagnosis and management.

Cardiovascular II
Concurrent Session
1:30 PM
Friday, February 1, 2008

214 A NOVEL PORCINE MODEL OF DIET-INDUCED OBESITY CAUSES DERANGED MYOCARDIAL INSULIN SIGNALING
J.K. Lee, Y. Xu, L. Lu, W. Leitner, C. Greyson, B. Bergman, B. Draznin, and G.G. Schwartz. VA Medical Center and University of Colorado Health Sciences Center, Denver, CO.

Purpose of Study: Pre-diabetic systemic insulin resistance confers increased cardiovascular risk independent of its effects on atherosclerosis. However, it is uncertain whether derangements in myocardial glucose metabolism and insulin signaling occur in the setting of pre-diabetic systemic insulin resistance. We developed a porcine model of diet-induced obesity to test the hypothesis that myocardial insulin resistance develops in parallel with systemic insulin resistance, and is characterized by impaired phosphatidylinositol-3-kinase (PI3K) and Akt signaling.

Methods Used: Yucatan micropigs (n=16) were assigned to intervention diet (17% w/w simple sugars, 25% fat from coconut oil) or control diet (2% simple sugars, 4% fat). IV glucose tolerance tests (IVGTT) were performed at 0, 3, and 6 mo of assigned diet. At 7 mo, under euglycemic clamp, myocardial glucose uptake in response to graded infusion of insulin was determined by IVGTT, and by blunted insulin stimulation of skeletal muscle PI3K activity and Akt phosphorylation (each p<.05). In parallel, myocardial insulin resistance was manifest by blunted insulin-stimulation of myocardial glucose uptake, PI3K activation (p<.05), and Akt phosphorylation (p<.05).

Conclusions: Myocardial insulin resistance develops in parallel with systemic insulin resistance in pigs fed a diet high in simple sugars and saturated fat. This abnormality could potentially impair cardiac responses to ischemia/reperfusion or other physiologic stresses where insulin signaling is believed to be important.
215 MECHANISMS OF UROKINASE-INDUCED COLLAGEN PRODUCTION BY CARDIAC FIBROBLASTS

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Purpose of Study: Cardiac fibrosis, the accumulation of excess extracellular matrix in the heart, leads to worse cardiac function and arrhythmia. Mice with macrophage-specific overexpression of urokinase plasminogen activator (SR-uPA+/0) have early cardiac macrophage accumulation and develop fibrotic specific to the heart. We hypothesized that SR-uPA+/0 macrophages secrete factors with activity specific to cardiac fibroblasts that leads to increased collagen production in the heart. Additionally, we hypothesized that SR-uPA+/0 macrophages upregulate fibrosis-related genes associated with the profibrotic, alternative activation pathway stimulated by IL-4.

Methods Used: Primary cardiac fibroblasts were isolated from wild-type C57BL6 mice. Peritoneal macrophages (mΦs) were isolated from SR-uPA+/0 and SR-uPA0/0 mice and conditioned media (CM) used to treat cardiac fibroblasts for 48 hours. RNA was extracted and used to determine Collagen 1 (Col1) transcription by qPCR, and hydroxyproline content. These data suggest that SR-uPA+/0 macrophages can promote increased collagen production specifically in cardiac fibroblasts, possibly through an alternative activation pathway.

216 CHARACTERIZATION OF CALCIUM TRANSIENTS IN GENETICALLY-DRIVEN MATURATED MOUSE EMBRYONIC STEM CELL-DERIVED CARDIOMYOCYTES

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Purpose of Study: While existing efforts have mostly focused on cardiac differentiation of embryonic stem cells (ESCs), little attention has been paid to the important fact that cardiomyocytes derived from ESCs (ESC-CMs) need to exhibit the mature electrophysiological and calcium (Ca2+) -handling properties of the non-regenerative adult counterparts being replaced due to age/disease to achieve safer and more medically effective outcomes. Indeed, ESC-CMs exhibit embryonic- or fetal-like properties that include enhanced automaticity, prolonged action potential (AP) durations, and prolonged phase 4 depolarization, all of which have been shown to be arrhythmogenic and potentially lethal in vivo transplantation studies in murine and porcine models of heart disease. Recent work by our group has shown that genetically driving the expression of the inwardly rectifying potassium current (I_K1, encoded by the Kir2.1 gene family) in both mouse and human ESC-CMs using a recombinant adenovirus facilitated the maturation of their electrical properties to be almost identical to adult cardiomyocytes and far less arrhythmogenic. At the same time, such alteration of the electrical properties of ESC-CMs by driven maturation might affect excitation-contraction coupling through changes in resting membrane potential (RMP) and AP dynamics, thereby overwhelming the immature Ca2+-handling properties of ESC-CMs via an overall reduction of Ca2+ transients (that is, the rise and fall of cytosolic Ca2+). [Ca2+]i, mediated largely by the sarcoplasmic reticulum) and worsening contractile functioning.

Methods Used: A comprehensive analysis of Ca2+ transients in I_K1-modified cells was performed in the D3 line of mouse (m) ESCs using spectrofluorometric methods that measured Ca2+ transient amplitude, maximum upstroke velocity (V_max,upstroke), and maximum decay velocity (V_max,decay) under different electrophysiological/pharmacological conditions.

Summary of Results: Overexpression of I_K1, while maturing the electrical phenotype of mESC-CMs, resulted in a significant reduction of the amplitude and V_max,upstroke of their Ca2+ transients.

Conclusions: We conclude that such reductions have also been implicated in arrhythmogenesis and may provide future targets for genetic interventions that can facilitate the maturation of ESC-CMs for safer heart therapies.

217 PRO-ARRHYTHMIC EFFECT OF MONOPHASIC FIELD STIMULATION REVEALED IN A 2-D EXPERIMENTAL MODEL OF UP-REGULATED MODE 2 GATING AND CALCIUM OVERLOAD

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Purpose of Study: Calcium (Ca2+) dynamics play an important role in defibrillation success and failure; however the role of Ca2+ overload on response to shocks is unclear. Here, we investigated the effects of field shocks on reentry and focal activity due to automaticity or triggered activity in a 2D experimental model of Ca2+ overload due to up-regulated mode 2 gating of the LTCC.

Methods Used: We studied homogeneous neonatal rat ventricular myocytes (NRVM-only) cultures (n=10) and co-cultures (n=10) of electrically uncoupled skeletal myotubes and NRVMs. Optical (voltage or calcium) mapping was performed after maturation of Ca2+ handling. Monolayers were superfused with Tyrodes containing either BayK8644 (2.5μM) and (1μM) isoproterenol (BI) in order to produce Ca2+ overload. Reentries and triggered activity were induced by rapid pacing; shocks (5.5, 8.5 & 14.5 V/cm, 10 or 25 ms) were applied to reentries and focal activity (automaticity and triggered activity) with each shock separated by a ~2-sec or 3 min interval.

Summary of Results: In the absence of Ca2+ overload, field shocks at 5.5V/cm terminated reentry (n=5). However, in the presence of Ca2+ overload induced by BI, monophasic field shocks (5.5, 8.5 and 14.5 V/cm, 10ms or 25ms) to focal activity or reentry elicited complex reentries or fibrillatory-like activity that persisted despite multiple shocks (n=10) in all cultures that were mapped. This pro-arrhythmic effect was independent of the phase of the reentry cycle at which the shock was delivered and could be prevented by biphasic field shocks (5.5V/cm, 10 and 25ms pulse duration).
Conclusions: Monophasic field shocks (5.5 & 8.5 V/cm) are pro-arrhythmic during Ca2+ overload induced by up-regulated mode-2 gating of the L-type calcium channel (LTCC). These results may help explain some episodes of shock failure observed in patients suffering from diseases like Timothy syndrome and heart failure where mode 2 gating is up-regulated.

218 ROLE OF THE JAK/STAT PATHWAY IN IL-8 TRANSCRIPTION BY OXIDIZED PHOSPHOLIPIDS IN VITRO AND IN ATHEROSCLEROSIS IN VIVO

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Purpose of Study: Interleukin 8 (IL-8) plays an important role in atherosclerosis. Oxidized 1-palmitoyl-2- arachidonoyl-sn-glycero-3-phosphorylcholine (Ox-PAPC), which accumulates in atherosclerotic lesions, induces endothelial cells (EC) to synthesize IL-8. Previously, we demonstrated a role for c-Src kinase activation in Ox-PAPC-induced IL-8 transcription. Src kinases can interact with members of the Janus activated kinase (JAK)/signal transducer and activator of transcription (STAT) pathway. In the current studies, we examined the role of the JAK2/STAT3 pathway in regulating IL-8 transcription by Ox-PAPC downstream of c-Src, as well the role of STAT3 in atherosclerosis in vivo.

Methods Used: Our in vitro studies were performed in human aortic EC (HAEC) using quantitative real-time PCR, Western analyses, chromatin IP (ChIP) assays and functional studies. EC-specific STAT3 knockout mice were created and employed in our in vivo studies.

Summary of Results: Treatment of HAEC with Ox-PAPC induced a rapid, yet sustained activation of JAK2; activation of JAK2 by Ox-PAPC was dependent on c-Src kinase activity. Furthermore, pretreatment with selective JAK2 inhibitors significantly reduced Ox-PAPC-induced IL-8 transcription. In previous studies, we had demonstrated activation of STAT3 by Ox-PAPC. Here we provided evidence that STAT3 activation by Ox-PAPC is dependent on JAK2 activation and that STAT3 activation regulates IL-8 transcription by Ox-PAPC.

Transfection with STAT3 siRNA significantly reduced Ox-PAPC-induced IL-8 transcription. Using ChIP assays, we demonstrated binding of activated STAT3 to the sequence flanking the consensus gamma-interferon activation sequence (GAS) in the IL-8 promoter; site-directed mutagenesis of GAS inhibited IL-8 transcription by Ox-PAPC. Finally, these studies have demonstrated increased staining for activated STAT3 in the inflammatory regions of human atherectomy specimens, and reduced fatty streak formation in EC-specific STAT3 knockout mice on the atherogenic diet.

Conclusions: Taken together, these data demonstrate an important role for the c-Src/JAK2/STAT3 pathway in Ox-PAPC-induced IL-8 transcription in vitro and in atherosclerosis in vivo.

219 ANT1 DEFICIENCY RESULTS IN DILATED CARDIOMYOPATHY: HISTOLOGIC ASSESSMENT AND ECHOCARDIOGRAPHIC CHARACTERIZATION OF MYOCARDIAL MECHANICS IN THE ANTI1−/− MOUSE

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Purpose of Study: The mitochondrial inner membrane proteins, adenine nucleotide translocators (ANTs) play a key role in energy production and may regulate apoptosis. Since cardiomyopathy is characterized by an energy deficient state and gradual myocellular loss, we hypothesize that Ant1 deficiency results in significant cardiomyocyte dysfunction and cardiomyopathy.

Methods Used: We examined the phenotypic consequences of Ant1 deficiency on the cardiac morphology and function. We used standard echocardiography and Velocity Vector Imaging (VVI), a new method examining myocardial mechanics, to examine 130 control and Ant1 mutant (−/−) mice, ages 2 to 19 months. Of these, we examined the hearts from 73 and 69 control and mutant mice by Hematoxylin & Eosin and Masson’s Trichrome staining, respectively.

Summary of Results: Ant1 mutant mice had a greater heart to body weight ratio compared to controls. Echocardiography showed that the left ventricle (LV) cavity dimensions and wall thickness were significantly greater in mutants. We then classified the mice into 3 functional categories by LV ejection fraction: normal, borderline normal, and abnormal systolic function. Results of the VVI analysis demonstrated that even mice with borderline normal, and some with normal function, had subnormal myocardial mechanics. Histopathologic abnormalities included myocyte hypertrophy, myofibrillarlyysis, fibrosis, edema, calcification, inflammation, and binucleation. We found these features, consistent with cardiomyopathy, more commonly in Ant1 mutant hearts than controls. Mutant hearts displayed many of the features at a younger age compared to the controls which may suggest the role of mitochondrial dysfunction in the aging process.

Conclusions: Our results demonstrate echocardiographic and histological characteristics of cardiomyopathy in Ant1 mutant mice. The Ant1 mouse model facilitates understanding of the role of mitochondria in the pathogenesis of cardiomyopathy and may permit development of intervention strategies for patients.

220 HYPOTHERMIA IMPROVES LEFT VENTRICULAR FUNCTION AND ATTENUATES LV REMODELING IN ACUTE MYOCARDIAL INFARCTION

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Purpose of Study: This study was designed to determine if induction of hypothermia immediately after acute myocardial infarction (MI) improves hemodynamic parameters, preserve left ventricular (LV) function and prevent LV remodeling.

Methods Used: We ligated the left coronary artery of adult male Sprague-Dawley rats to induce MI. We then measured LV hemodynamics and assessed LV function and chamber dimensions by echocardiography immediately following MI and one hour after induction of hypothermia (temperature < 28°C). A normothermic (temperature > 34°C) group of animals was similarly studied as controls.

Summary of Results: After MI and induction of hypothermia, the LV End Diastolic Pressure (LVEDP) was increased (P < 0.05) in normothermic rats (22.2 ± 4.4 mmHg) compared to hypothermic rats (10.7 ± 1.6 mmHg). Similarly, LV systolic pressure in normothermic rats (109 ± 4.4 mmHg) was higher (P < 0.05) than hypothermic rats (86 ± 2.4 mm Hg). There were no differences in LV dp/dt in hypothermic rats (4273 ± 153 mmHg/sec) and normothermic rats (4575 ± 457), the LV relaxation constant Tau trended towards a longer delay in hypothermic rats (36 ± 6.3 msec) compared to in normothermic rats (25 ± 2.3 msec). Hypothermia preserved LVEF compared to normothermia (58 ± 5.0 vs. 37 ± 3.3 %, P < 0.05). The displacement of the anterior infarcted region was normalized in
the hypothermic animals (0.21 ± 0.01 vs 0.05 ± 0.02 cm, P < 0.005). LV remodeling (LV systolic and diastolic chamber dilation) was also attenuated in the hypothermic animals.

Conclusions: Induction of hypothermia after acute MI limits LV remodeling, preserved LV function and restored regional wall motion in the infarcted LV segment. These findings suggest that hypothermia may be a viable therapeutic option in the treatment of acute myocardial ischemic injury.

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**IMPLANTATION OF A 3 DIMENSIONAL FIBROBLAST MATRIX IMPROVES LEFT VENTRICULAR FUNCTION AND ATTENUATES LV REMODELING IN ACUTE AND CHRONIC ISCHEMIC HEART FAILURE**

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1University of Arizona, Tucson, AZ and 2SAVAHCS Medical Center, Tucson, AZ

**Purpose of Study:** This study was designed to determine if a viable biodegradable 3-dimensional fibroblast construct (3DFC) patch implanted on the left ventricle (LV) after myocardial infarction (MI) improves LV function and alters LV remodeling after acute MI and in chronic heart failure (CHF).

**Methods Used:** We ligated the left coronary artery of adult male Sprague-Dawley rats and implanted the 3DFC patch on the infarcted anterior wall acutely as well as 3 weeks after MI. Hemodynamic, echocardiographic and blood flow studies were performed 3 weeks after 3DFC implantation in both groups.

**Summary of Results:** Acute 3DFC patch implantation improved LV systolic function by increasing LV ejection fraction (LVEF) (37.1±3.3 to 61.7±4.9%) and regional systolic displacement of the infarcted wall (0.04:0.02 to 0.11:0.03 cm); increased cardiac output from 0.10±0.01 to 0.38±0.07 ml/min and shifted the passive LV diastolic pressure volume relationship to the left toward the pressure axis. The 3DFC patch also attenuated LV remodeling acutely by decreasing LV end-diastolic diameter by 19% (P<0.05) with no change in LV end-systolic diameter or LV systolic pressure. The 3DFC patch did not change LV end-diastolic pressure (LV EDP) 25±2 versus 23±2 mmHg but the addition of captopril (2mg/L drinking water) lowered (P<0.05) LV EDP to 12.9±2.5 mmHg. The 3DFC patch increased myocardial blood flow to the infarcted anterior wall after MI over 3-fold (P<0.05). In CHF, the 3DFC patch improved LV systolic function by increasing (P<0.05) LVEF (41.5±5.9% to 53.0±3.9%). LV end-diastolic diameter was not decreased with the addition of the 3DFC patch. The patch did not improve LVEDP vs CHF nor Rx (20.5±2 and 16±4.0 mmHg, respectively). The addition of captopril in CHF did not alter LVEDP.

**Conclusions:** This biodegradable 3DFC patch provides a matrix support structure that results in improved LV function and myocardial blood flow 3 weeks after MI. In CHF, the patch improved LV function but did not change LV end-diastolic diameter. Captopril had no effect on LVEDP in CHF, unlike the lowered LVEDP observed in acute MI. The 3DFC patch is a compelling new approach to cell-based therapy for acute MI and chronic heart failure.

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**ROLE OF LIVER X RECEPTOR IN CALCIFICATION OF MURINE AORTIC CELLS**

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**Purpose of Study:** Vascular calcification is prevalent in atherosclerotic lesions and correlates with an increased risk of cardiovascular events, including myocardial infarction and death. Recent studies have shown that activation of the nuclear receptor Liver X Receptor (LXR) promotes cholesterol efflux from macrophages and decreases inflammatory gene expression. Accordingly, LXR activation reduces atherosclerotic lesion size in an animal model, and has thus received great interest for its therapeutic potential in atherosclerosis. Yet its effect on vascular calcification is largely unknown. Since LXR activation is widely considered to be anti-atherogenic, we investigated its effects on in vitro vascular cell calcification.

**Methods Used:** Primary murine aortic cells were induced to calcify with forskolin, as we have shown previously in vitro, and were cotreated with T0901317, a known synthetic LXR ligand. Osteogenic markers, including alkaline phosphatase activity, intracellular phosphate levels, and matrix calcification, were assessed after 4, 7, and 10 days, respectively.

**Summary of Results:** Results showed that LXR activation with T0901317 augmented forskolin induction of matrix calcification. Treatment with T0901317 alone, however, did not induce calcification. Cotreatment with T0901317 also augmented forskolin-induced alkaline phosphatase activity. Gene expression analysis by real-time quantitative polymerase chain reaction revealed that LXR activation induced the expression of the phosphate cotransporter Pit-1 and inhibited forskolin induction of ectonucleotide pyrophosphatase/phosphodiesterase 1. Cotreatment with levamisol, an inhibitor of alkaline phosphatase, and phosphonofumaric acid, an inhibitor of Pit-1, attenuated the effects of T0901317 on matrix calcification.

**Conclusions:** Overall, these data suggest that LXR activation promotes matrix calcification of vascular cells and may pose potential adverse effects in the vasculature.

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**CYTOKINE MEDIATED RELEASE OF VASCULAR ENDOTHELIAL GROWTH FACTOR AND STROMAL-DERIVED FACTOR 1 INDUCES NEOVASCULARIZATION AND PREVENTS LEFT VENTRICULAR REMODELING IN A PORCINE MODEL OF ISCHEMIA REPERFUSION**


University of California, San Francisco, CA

**Purpose of Study:** Cytokine therapy has been suggested to improve left ventricle (LV) function after myocardial infarction (MI). The mechanisms for this benefit remain debatable. We investigated the impact of a prolonged combined therapy with Darbepoetin (DARB) and Granulocyte Colony-Stimulating Factor (G-CSF) on LV function after MI, correlating it with circulating progenitor cells (CPC), Vascular Endothelial Growth Factor (VEGF) and Stromal-Derived Factor 1 (SDF-1) release.

**Methods Used:** MI was induced in swine by a 90 minutes balloon occlusion of the left anterior descending artery. Animals were divided between treatment group with DARB-GCSF therapy (bolus of DARB 0.9 and G-CSF 10μg/kg IV at time of reperfusion, followed by 5 doses of GCSF 5μg/kg SC and DARB SC once per week starting at day 1), control group (saline, n=8). White blood cells (WBC), CPC, and circulating levels of VEGF and SDF-1 were assessed at baseline (T0), 1 (T1), 2 (T2), and 3 (T3) weeks post-MI. LV function was assessed by echocardiography at T0, T1 and T6 (6 weeks post-MI), infarct size and vascular density by histology at T6.

**Summary of Results:** MI size was the same in both groups by post-MI CK peak and LV ejection fraction (EF) at T1 (41±/−1 vs. 40±/−2%). In the treatment group only, from T0 to T1, there was an increase in WBC (16±/−2 to 42±/−2 x 106/ml, p<0.01) and CPC (5±/−1 to 9±/−1 x 105/ml, p<0.01) vs T0. Lu et al., Circulation 123, 2011.
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HDL MIMETIC PEPTIDES AND THEIR ANTI-INFLAMMATORY ROLES

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Purpose of Study: The purpose of this study is to highlight the different HDL mimetic peptides and the roles they play in antiinflammatory responses while aiming to determine if its application in atherosclerosis treatment can be measured accurately by en face as well as aortic root lesion size calculations.

Methods Used: In studying 4F, G* and the tetrapeptides peptides LDL receptor null and apoE-null mice were maintained on either a western or chow diet. Peptides were administered either via stomach gavage or placed in the drinking water of the mice. Mice were bled under anesthesia from the retroorbital venous plexus.

HDL protective capacity against LDL oxidation was determined using a cell free or cell based monocyte chemotaxis assay. Tests were done according to standard protocols that have been previously explained.

Summary of Results: Lesion regression showed a strong correlation between aortic root lesion score and en face lesion score. As the sample size increases it is predicted that correlation will follow suit and progressively become stronger.

Conclusions: The volumetric analysis of aortic root lesions as well as en face area measurements can be used individually to determine lesion regression in the murine model of atherosclerosis. Volumetric methods of measuring lesion regression, such as intravascular ultrasound and slice-by-slice modeling, are expensive, difficult and time consuming. Showing that en face lesion areas provide useful data will considerably improve productivity while retaining the power needed to make experiments viable.

Gastroenterology and Hepatology I
Concurrent Session
1:30 PM
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A RANDOMIZED CONTROLLED DOSE RESPONSE STUDY OF LANSOPRAZOLE ORAL DISTINTEGRATING TABLETS IN SUSPENSION FOR TREATMENT OF GASTRO-ESOPHAGEAL REFLUX DISEASE IN CHILDREN

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Purpose of Study: Lansoprazole is effective for treatment of gastro-esophageal reflux disease (GERD) in children. Lansoprazole oral disintegrating tablets (LODT) dispersed in water and suspended in a viscous liquid (Oraplus) was shown to make a preparation with a consistent concentration of lansoprazole per mL. The aim of this study was to assess the efficacy of LODT suspension for treatment of GERD in children in a dose-response design.

Methods Used: Children aged 1 to 5 years with clinically diagnosed GERD were randomized to receive either 0.75 or 2.5 mg/kg/day of LODT suspension daily. Outcome was assessed by the GERD Assessment Symptom Questionnaire for Young Children (GASQ-YC).

Summary of Results: Eight children (4 males) age 1 to 5 years with GERD diagnosed by GASQ-YC scoring were studied. GASQ-YC scores at 4 and 8 weeks were used to assess the response to therapy. The cumulative symptom scores from the 8-week study period were compared for children with 0.75 to those who received 2.5 mg/kg/day of LODT. The data are shown in mean ± SE.

Conclusions: The results show that LODT suspension decreases GERD symptoms in patients age 1 to 5 years. The improvement in symptoms occurred with both doses tested. We speculate that LODT suspension is as effective as oral LODT in treatment of pediatric GERD.

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<th>Dose</th>
<th>Baseline</th>
<th>4 Weeks</th>
<th>8 Weeks</th>
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<tr>
<td>0.75 mg/kg/d</td>
<td>107.5 ± 43.4</td>
<td>27.5 ± 6.6</td>
<td>25.5 ± 1.8</td>
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<tr>
<td>2.5 mg/kg/d</td>
<td>142 ± 50.5</td>
<td>20.3 ± 5.5</td>
<td>6.3 ± 1.1</td>
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CANDIDA ESOPHAGITIS IN INFANTS WITH GASTROESOPHAGEAL REFLUX DISEASE AND FEEDING REFUSAL

S. Reese¹, A. Billings², C.J. Billings¹, K.D. Crissinger², and D.A. Gremse¹. ¹University of Nevada School of Medicine, Las Vegas, NV; ²University of South Alabama College of Medicine, Mobile, AL

Purpose of Study: Candida Esophagitis has been reported in young, normal infants in association with gastroesophageal reflux disease (GERD). We have observed several infants with symptoms not only of GERD, but also significant feeding refusal who were found to have candida esophagitis. The purpose of this study is to characterize the presentation and response to treatment of immunocompetent infants with esophageal candidiasis.

Methods Used: We queried a clinical database to identify children diagnosed with candida esophagitis and GERD. We reviewed the medical records of 12 infants found to have both candida esophagitis and GERD during this time. We collected data on these patients regarding formula, medications, growth, and response to prior therapies. All of these patients underwent esophagogastroduodenoscopy (EGD) with biopsies and esophageal brushings.

Summary of Results: There were 12 infants aged 1 wk - 7mo (0.32 yr ± 0.26, x ± SD ), 75% male, 42% Caucasian, 33% African American, 25% Hispanic. All of the patients had vomiting, 83% had feeding intolerance, 50% had diarrhea, 42% had allergic colitis, and 33% had oral candidiasis. As a group, these patients were below the mean for weight for age (z-score, -1.54 ± 1.30, x ± SD). One of the infants had received recent antibiotic therapy. There had been no improvement in the above symptoms despite multiple formula changes as well as appropriate therapy with ranitidine, metoclopramide, and various proton pump inhibitors. After Candida esophagitis was diagnosed with EGD, all patients were started on fluconazole. All patients demonstrated improvement of their symptoms following treatment with fluconazole.

Conclusions: Candida esophagitis should be included in the differential diagnosis of infants presenting with symptoms of GERD and feeding intolerance not responsive to appropriate therapy and EGD should be considered. The absence of oral candidiasis does not rule out the possibility of Candida esophagitis.
227 PATIENT CHARACTERISTICS, CLINICAL FEATURES, ENDOSCOPIC FINDINGS, AND RESPONSE TO TREATMENT OF PATIENTS WITH EOSINOPHILIC ESOPHAGITIS


Purpose of Study: To analyze demographics, symptoms, endoscopic findings, and response to treatment of a group of patients with eosinophilic esophagitis.

Methods Used: This is a retrospective study of patients diagnosed with eosinophilic esophagitis at St. Paul’s Hospital in Vancouver, Canada. Esophageal biopsy results from 2005 to 2007 were reviewed. All patients whose biopsies showed an average eosinophil count of >20 per high power field were included in the study. Electronic charts were used to study patient characteristics, endoscopic findings, and response to treatment.

Summary of Results: Between 2005 and 2007, forty-three patients were identified, of whom 32 (74%) were males and 11 (26%) were females. The mean (SD) age of patients at diagnosis was 44 (13.8) and the mean (SD) age at onset of symptoms was 37 (18.9). The predominant symptom was solid food dysphagia, which occurred in all patients. Thirty-four (79%) patients had a history of esophageal food impaction, and 13 (30%) had been to the emergency department for bolus obstruction. Of those with an available history, 23 of 30 (77%) had associated allergies and/or atopy. A ringed esophagus was the most common endoscopic finding observed in 29 (67%) patients, and 6 (14%) patients had a normal looking esophagus. Medical management with topical fluticasone was used in 20 patients, and 15 (75%) of these had clinical improvement. However, symptoms recurred in 4 of the 15 patients upon discontinuation of the medication. Management with endoscopic dilation was utilized in 13 patients, all of whom experienced clinical improvement and no complications. However, symptoms recurred in all patients.

Conclusions: There was a male predominance, and many patients had associated allergies and/or atopy. The most common clinical features were dysphagia and recurring esophageal food impaction. The most common endoscopic finding was a ringed esophagus. Topical steroids were an effective treatment strategy, but some patients needed repeated treatments due to recurrence. Esophageal dilation was a safe and effective treatment, but there was a 100% rate of recurrence.

228 FREQUENCY OF BURIED BARRETT’S METAPLASIA AFTER HALO ABLATION FOR INTESTINAL METAPLASIA

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Purpose of Study: Radiofrequency ablation is a rapidly evolving therapeutic modality for Barrett’s esophagus (BE). Buried Barrett’s metaplasia (buried BM) is a known complication of all endoscopic ablative therapies for BE. The aim of this study is to assess BE eradication (with specific attention to buried BM) following HALO radiofrequency ablation.

Methods Used: Prospective trial in patients with confirmed >1 cm BE, including short and long segment BE. Patients with nondysplastic BE (ND) and BE with low or high grade dysplasia are included. Patients in the ND group receive ablation treatments at 103/cm2 and patients in the dysplastic group receive 12J/cm2 during each treatment. All patients receive lansoprazole 30 mg bid. Assessment for buried BM is performed via 3 & 12-mo follow-up EGD with 4-quadrant bx every 1 cm from the original BE region. Patients with residual BE (superficial or buried BM) on 3-mo evaluation undergo repeat treatment. Endpoints at one year evaluation include complete resolution, partial response (50–99% eradication), residual/BE (<50% response), and loss to follow-up (patients with buried BM at 3-mo will be followed as a subgroup).

Summary of Results: 38 of 50 planned patients have undergone initial ablation. Of these, 25 patients (15 male, mean age 59 years, 20 ND, mean BE length 5.7 cm, range 1 to 11 cm) have completed 3-mo evaluation. One patient (1/25; 4%) had evidence of buried BM and underwent reablation at 6-mo. Of the remaining 24 patients, 15 (62%) showed CR at 3-mo. 8 patients have completed the 12-mo evaluation with 6 (6/8; 75%) exhibiting CR.

Conclusions: The finding of buried Barrett’s metaplasia after any therapeutic intervention for BE is concerning due to the potential for malignant transformation under the healing neosquamous epithelium that would no longer be endoscopically detectable. To date, this is the first trial to detect evidence of buried Barrett’s metaplasia following HALO ablation. However, our preliminary data suggests that it occurs at lower rates compared to other BE ablative therapies. This may be due in part to the circumferential and uniform depth of ablation delivered by the HALO system. Nonetheless, even the possibility of buried BM after ablation of BE should necessitate diligent follow-up to detect for the presence of buried BM and repeated ablation as necessary.

229 MECHANISM OF INTERLEUKIN-1B MODULATION OF INTESTINAL PERMEABILITY IN-VIVO

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Purpose of Study: Defective intestinal epithelial barrier has been postulated to be an important pathogenic factor of intestinal inflammation in Crohn’s disease. Interleukin-1B (IL-1B) is a prototypical pro-inflammatory cytokine that has been shown to play a key role in intestinal inflammation in-vivo. Previous studies from our laboratory have shown that IL-1B causes a significant increase in intestinal epithelial tight junction (TJ) permeability in-vitro. However the role of IL-1B on intestinal permeability in-vivo remains unclear. The major aims of this study were to examine the effect of IL-1B on intestinal permeability in a newly developed in-vivo mouse model and to determine the mechanisms involved. As previous studies have shown that myosin light chain kinase (MLCK) protein expression and activity plays a central role in the regulation of intestinal TJ barrier function, we examined the possibility that MLCK also mediates the IL-1B modulation of intestinal permeability.

Methods Used: An in-vivo re-cycling intestinal perfusion system was used to measure intestinal permeation of the paracellular marker FITC-dextran (m.w. = 3,000 g/mol). An Ussing chamber was used to measure transepithelial resistance (TER) of small intestinal tissue.

Summary of Results: 1) Intraperitoneal (i.p.) IL-1B (2–10 μg) caused a dose dependent increase in intestinal permeability to FITC-dextran (m.w. = 3,000 g/mol). Conversely, IL-1B caused a decrease in intestinal tissue TER. These results indicated that IL-1B causes an increase in intestinal permeability in-vivo. 3) IL-1B induced increase in intestinal permeability was preceded by an increase in MLCK mRNA and protein expression in the small intestinal tissue. 4) The inhibition of MLCK activity by MLCK inhibitor ML-7 prevented the IL-1B induced increase in intestinal permeability.

Conclusions: Our data show for the first time that IL-1B causes an increase in intestinal permeability in an in-vivo mouse model system. Additionally, our data indicate that the IL-1B induced increase in intestinal permeability was mediated by an increase in MLCK protein expression and activity. These studies provide important insight into the role of IL-1B in modulation of intestinal epithelial barrier function in-vivo.
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**EFFECT OF CURCUMIN AND CURCUMIN ANALOGUES ON TNF-α INDUCED INCREASE IN INTESTINAL EPITHELIAL TIGHT JUNCTION PERMEABILITY**

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1University of New Mexico, Albuquerque, NM and 2University of New Mexico, Albuquerque, NM.

**Purpose of Study:** Patients with Crohn’s disease have an increase in intestinal epithelial tight junction (TJ) permeability which results in paracellular penetration of luminal antigens and inflammation. TNF-α, a pro-inflammatory cytokine has been shown to cause a decrease in intestinal epithelial TJ permeability via activation of NF-κB (AJP 2004; 286:G367-76). Curcumin, a compound found in turmeric, has been shown to inhibit TNF-α induced activation of NF-κB and subsequent increase in Caco-2 TJ permeability, but the chemical characteristics responsible for these effects are not known. We have synthesized over 20 curcumin analogues and studied their anti-oxidant effects and ability to inhibit NF-κB, but their effects on TNF-α induced increase in Caco-2 TJ permeability are unknown. The aims of this study were to evaluate a select group of curcumin analogues for their ability to inhibit TNF-α induced Caco-2 TJ permeability and their relative ability to inhibit TNF-α induced activation of NF-κB.

**Methods Used:** Caco-2 monolayers were used as an in vitro intestinal epithelial model system to study TJ permeability. NF-κB activation was quantitated by an ELISA-binding assay.

**Summary of Results:** 1) TNF-α treatment resulted in a significant increase in Caco-2 TJ permeability. 2) Over 20 different curcumin analogues were synthesized. 3) Curcumin and selected curcumin analogues significantly inhibited the TNF-α-induced increase in Caco-2 TJ permeability. 4) The TNF-α-induced increase in Caco-2 TJ permeability directly correlated with NF-κB activation and curcumin inhibition of TNF-α-induced increase in Caco-2 TJ permeability correlated with inhibition of NF-κB activation. 5) Curcumin and curcumin analogues were tested for their ability to inhibit NF-κB activation and their anti-oxidant effects.

**Conclusions:** TNF-α caused a significant increase in Caco-2 TJ permeability. Curcumin and curcumin analogues inhibited the TNF-α induced increase in Caco-2 TJ permeability. In studies to be completed, we intend to delineate the relative contribution of anti-oxidant effects and NF-κB inhibitory effects of curcumin analogues in the inhibition of TNF-α induced increased TJ permeability and to identify the chemical characteristics responsible for this inhibition.

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**MECHANISM OF PTEN INTERACTIONS IN COLORECTAL CANCERS AND HAMARTOMATOUS POLyps**

J.K. Lee, S.C. Huang, and J.M. Careathers. UCSD, La Jolla, CA.

**Purpose of Study:** The phosphatase and tumor suppressor gene PTEN is mutated in endometrial and colon cancers with microsatellite instability, and is thought to play a role in their pathogenesis. PTEN encodes two coding hexadecine microsatellites that often change in length within these cancers. Germline PTEN mutations occur in certain hamartomatous polyposis syndromes, some with high colorectal cancer risk in which the polyps may lose PTEN expression indicating somatic inactivation of the wild-type allele. However, the mechanism for silencing the second PTEN allele is unclear. We examine whether familial hamartomatous polyps harbor defects in DNA mismatch repair, with instability in coding microsatellites in PTEN.

**Methods Used:** 99 microsatellite unstable (MSI) sporadic colon cancers and 10 MSI hamartomatous polyps were analyzed for mutations in two hexadecine tracts (exons 7 and 8) of PTEN. PTEN expression was determined by immunohistochemistry using an antibody targeting an epitope beyond the predicted truncated protein.

**Summary of Results:** 5/10 patients with familial hamartomatous polyposis syndrome showed germline PTEN mutations and complete loss of PTEN expression. 11/99 MSI cancers (11%) demonstrated frameshifs in exon 7 and 8 of PTEN while 0/10 MSI hamartomatous polyps showed no frameshifts. Of tumors with mutant PTEN, all demonstrated down regulation or complete loss of PTEN in the epithelium.

**Conclusions:** Microsatellite unstable colon cancers exhibit PTEN exon 7 and 8 frameshift mutations and subsequent loss of PTEN expression. Hamartomatous polyposis syndrome patients exhibit microsatellite instability. Loss of PTEN expression was noted in patients with PTEN germline mutations, but was not associated with microsatellite instability in PTEN. Loss of PTEN expression may be due to other epigenetic phenomena.
233 A NOVEL METHOD OF SEPARATING PROTEINS FOR APPLICATIONS IN BIOMARKER CHARACTERIZATION

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1 Brown University, Providence, RI; 2 University of California, Irvine, Irvine, CA; 3Brown University, Providence, RI and 4University of Florida, Gainesville, FL.

Purpose of Study: The identification and separation of macromolecules with similar mobilities has become important in light of their central role in pathological states of mutations, improper expression or function (e.g. prion diseases, Alzheimer's disease, and cancers). For example, the ratio of two isoforms of alpha-feto-protein is used as a predictor of the severity of liver cancer. Current methods of protein separation require unrealistic amounts of highly concentrated protein and significant processing time; requirements that are difficult to implement in the clinical setting. Additionally, traditional methods attempt to separate protein isoforms in their native or fully denatured state; two biological states in which they have very similar conformations and are thus difficult to separate. A novel methodology integrating microfluidic approaches with protein separation sheds light on the development of a clinically-centered diagnostic device.

Methods Used: Knowing that proteins adopt different conformation pathways as a function of the solvent environment they are equilibrated in, it was hypothesized that a particular buffer in which distinct conformations differences arise between two isoforms would provide for more resolute separation. Two model protein isoforms were generated and analyzed in both solution phase as well as through microfluidic methods. Resolution of separation as a function of the different buffer driven conformation states was monitored.

Summary of Results: Results indicate preferential adsorption of the two model protein isoforms, relative to each other, when in a partially denatured state. This distinct adsorption can allow for resolute separation not traditionally observed when separating proteins in their native or fully denatured state.

Conclusions: The development of a preferential adsorption assay that can be translated to a microfluidic platform is what allows for integration of the proposed methodology in the clinical setting. Having obtained results with laboratory generated model protein isoforms (that mimic potential clinical biomarker properties), specific clinical applications for resolute protein separation can now be investigated.

234 EFFECTS OF PROPARACAINE AND TETRACAINE ON CORNEAL EPITHELIAL CELL MIGRATION IN VITRO

C. Kim, L. Oliveira, and M. Rosenblatt. University of California, Davis, Sacramento, CA.

Purpose of Study: To analyze the effects of the topical anesthetics proparacaine and tetracaine on the motility and morphology of corneal epithelial cells.

Methods Used: Corneal epithelial cells were obtained from rabbit corneas via serial enzymatic digestion. Cells were passaged onto glass bottom dishes at a density of 10^4 cells/cm^2 and 48 well plates at a density of 5 x 10^4 cells/cm^2. Cells plated onto glass bottom dishes were incubated for four hours, whereupon media containing either proparacaine or tetracaine was added, at concentrations ranging from 0.1 to 1.0mM. The cells were then monitored using time-lapse imaging, with images taken every 2 minutes over a 2 hour period. Changes in cellular morphology were observed, while cell motility was assessed by calculating the change in two-dimensional position of individual cells over time. Cells plated onto 48 well plates were incubated overnight and grown to confluency. A single scratch was then created within the monolayer using a 200µL pipette tip. The cells were incubated in media containing varying concentrations of proparacaine or tetracaine, from 0.5mM to 4mM. Wound closure was followed by imaging the scratch area every 6 hours.

Summary of Results: Proparacaine and tetracaine slow cell motility and wound closure in a concentration dependent manner. Compared to control, cells treated with these agents exhibit decreased motility and extension of cellular processes, and assume an altered, spherical conformation. While scratch closure is delayed at all concentrations of proparacaine and tetracaine, concentrations above 1mM cause complete inhibition. Significant levels of cell detachment are also noted at higher concentrations. Furthermore, tetracaine exhibits greater toxicity compared to proparacaine, as it induces a greater degree of morphologic change and detachment at equivalent doses.

Conclusions: We have demonstrated that both proparacaine and tetracaine inhibit cell motility and cause significant morphologic changes in rabbit corneal epithelial cells in a dose dependent fashion. Given the inhibitory effects of these drugs on cell migration, our findings suggest that proparacaine and tetracaine may adversely affect corneal wound healing.

235 OCULAR FINDINGS IN PATIENTS WITH CHRONIC GRAFT-VERSUS-HOST DISEASE

E. Allan1, M. Wu1, P. Martin2, M. Flowers3, and R. Bensinger1. 
1 University of Washington, Seattle, WA; 2 Fred Hutchinson Cancer Research Center, Seattle, WA and 3 Swedish Medical Center, Seattle, WA.

Purpose of Study: Chronic graft-versus-host disease (cGVHD) is a common complication of allogeneic bone marrow transplantation (BMT) with ocular morbidity becoming a greater concern. The goal of this study is to determine prevalence of ocular findings and evaluate their visual acuity in patients with cGVHD following an allogeneic BMT.

Methods Used: A retrospective review was performed on an electronic database containing all patients receiving an allogeneic bone marrow transplantation at Fred Hutchinson Cancer Research Center between 1990 and 2006. Consecutive review was completed on ophthalmic notes of 704 patients, who were referred to an ophthalmologist per protocol because of a Schirmer’s score of <15mm through 2001 or ≤10mm after 2001. Ocular exam findings were extracted from the 494 available records of patients with cGVHD.

Summary of Results: Mean follow up of 494 patients with cGVHD was 979 days (range 58-6089). Chronic GVH was diagnosed in 70.2% of patients. Injection was noted in 10.5% of patients, chemosis in 3.64%, and either pseudomembrane formation or epithelial sloughing in 0.61%. Symbotrophon was not noted among these patients, and subepithelial fibrosis was noted in 0.40% of patients. Fluorescein corneal staining was observed in 30.6% of patients. PSC cataracts were seen in 26.9% of patients. The prevalence of dry eye syndrome (DES) as defined by an average Schirmer’s of ≤5mm was 23.3% at first eye exam. Of those 494 patients, 348 returned for further follow up. Prevalence of DES at the last eye exam was 29.3%. Average visual acuity (VA) at the first visit was 20/27 (range 15-CF) and 20/29 (range 10-LP) at the last visit. Only 1 patient had visual acuity of lower than 20/200 from corneal scarring resulting from BMT related aqueous tear deficiency. Bone marrow transplantation related retinal findings were rare, the most common being hemorrhage due to thrombocytopenia.
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LONG-TERM GLUCOSE CONTROL WITH ORAL AGENTS ONLY IN VETERANS WITH TYPE 2 DIABETES MELLITUS
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Purpose of Study: To evaluate long-term glycemic control in individuals with type 2 diabetes mellitus treated with oral agents only. Our earlier studies showed stability over more than a decade.

Methods Used: We identified in the VANCHCS computer record data the cohort of 191 veterans prescribed hypoglycemic agents every year from 7/92 through 6/07. Hba1c was used to assess glycemic control. Data are expressed as Mean±SD; statistics by t-test and χ². p<0.05 was considered significant.

Summary of Results: Our total hypoglycemic-agent-treated population increased from 2422 to 9529 from 1992-2007; most care was offered in a primary care setting. In the first year, 96 veterans received oral agents only (OAO), 74 insulin only, and 21, combined insulin/oral agents. Fifteen years later, 59 were OAO, 78 insulin only, and 54 both. Six patients receiving insulin in 1992-93 were OAO-treated in 2006-07. Mean Hba1c for the cohort (n=191) overall improved from 8.63±1.50 to 7.65±1.35 (p<0.001). At baseline (1992-93), Hba1c was lower (7.89±1.21) in stable OAO who remained OAO (n=53) than in OAO who received insulin in 2006-07 (8.59±1.19, p<0.01). Duration of diabetes at baseline was similar in both groups (8.9±7.6 yrs; N.S.). In the stable OAO group, Hba1c decreased from 7.89±1.21 to 7.09±1.13 (p<0.001). Veterans who were stable OAO were older at baseline (62.4±6.2) and leaner at 15-yr follow-up (BMI 28.1±4.9) than those who received insulin in 2006-07 (n=43; age=57.9±9.6; BMI=32.3±7.9; p<0.05 and 0.005 respectively). Patients in the stable OAO group (n=53) were 74.0% Caucasian, compared to 51.2% in former-OAO (n=43; p<0.05 (χ²)).

Conclusions: Over half (55%) of patients originally in the OAO group performed well on OAO and were less likely to be leaner at baseline. Some even converted from insulin treatment. Currently used oral agents often maintain, or even improve, glucose control, over 2 decades after diagnosis of diabetes mellitus.

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SNAPSHOTS OF MEDICATION RECONCILIATION: A MEDICAL RECORD REVIEW
C.G. Jette¹, C. Nembeth², M. Nunally³, and R. Cook². ¹University of Washington, Seattle, WA and ²The University of Chicago, Chicago, IL.

Purpose of Study: In order to reconcile medications, clinicians develop a list of a patient’s prescribed, over-the-counter and supplemental medications, then accurately replicate and modify this list over time as a patient’s care is transferred among providers. Long associated with adverse drug events in medicine, the process recently came under increased scrutiny when The Joint Commission named medication reconciliation a 2006 National Patient Safety Goal. Prior improvement efforts include externally imposed protocols, special forms, computer based systems and incident reporting. However, genuine improvement requires understanding of how clinicians reconcile medications based on studies in cognition that reveal how humans process data to make decisions, often subconsciously.

Methods Used: As the initial phase in a multi-year project, we randomly selected, de-identified and reviewed 120 pre-operative anesthesia assessments.

Summary of Results: We analyzed medications within the context of each patient’s medical problem list and developed hypotheses about medication use patterns. Pre-operative anesthesia records can be thought of as a clinician’s “stop point”, where he or she has a good enough understanding of a patient’s medications in order to proceed. Record analysis allowed us to identify five findings which potentially contribute to making medication reconciliation simple or difficult. These findings include drug-drug pairs, drug-disease pairs, webs of drugs and diseases, lifestyle medications and recognizable drug traps.

Conclusions: Further work will observe providers with varying backgrounds performing medication reconciliation on “easy” or “difficult” cases pulled from our dataset. Using research methods aimed at characterizing task complexity and clinician decision-making, we expect to further understand medication reconciliation, discover strategies that experts use to avoid failure, and test tools to improve medication reconciliation safety. This research has direct application to physicians’
work in both hospital and clinic settings, with the goal of reducing adverse drug events.

**239 SOCIODEMOGRAPHIC FACTORS ASSOCIATED WITH HEALTHCARE UTILIZATION IN SIEM REAP CAMBODIA**

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**Purpose of Study:** Cambodia’s public health system faces many challenges as a result of its turbulent political history. Resources are scarce and infrastructure is lacking. Government salaries well below a living wage encourage health workers to neglect their official duties to pursue more lucrative unregulated private practice. At the front lines of the Cambodia’s public health campaign lie its health centers. Historically, these clinics have a reputation for providing poor quality services. These attitudes have led to low utilization levels. The Capacity Building and Health Education Program (CBHEP) is working with the Ministry of Health to develop the capacity of local health centers near Siem Reap. To aid in future interventions, CBHEP would like to know what factors correlate with utilization of different providers. This study seeks to identify these factors and guide future activities of CBHEP and other groups working towards similar goals.

**Methods Used:** The data from this study comes from a cross-sectional survey that was orally administered in Khmer. Subjects verbally responded to survey questions asked by a trained survey administrator. The survey was administered to 618 families representing 4005 individuals in 102 different villages served by the six health centers.

**Summary of Results:** Health centers held the highest percentage of utilization (50.4%), followed by hospitals (14.2%), pharmacies (11.3%) private clinics (4.0%), and traditional medicine (3.9%). A multivariate regression analysis was performed on the survey data. Increasing urbanization of the area served by the health center correlated negatively with utilization (B = −20.95, p<.001, 95%CI = −25.21, −16.99). Higher incomes also correlated with lower health center utilization (B = −5.99, p=.015, 95%CI = −10.82, −1.16). Exposure to negative rumors about private clinics correlated strongly with increased utilization of health centers (B = 21.98, p=.015, 95%CI = 10.82, 33.14).

**Conclusions:** The main factors affecting provider choice in Siem Reap are urbanization, income, and exposure to negative rumors about private clinics. This has implications for future interventions aimed at increasing utilization of area health centers.

**240 THE SPITI MEDICAL PROJECT: A MODEL OF SUSTAINABLE INTERNATIONAL HEALTH DEVELOPMENT**


**Purpose of Study:** The Spiti Valley is a remote region of Northern India that lacks adequate health care. Past international efforts utilized short-term medical aid trips to promote health, thus sustainable efforts were required. The Spiti Medical Project is run by medical and dental students under the supervision of faculty members at the University of British Columbia. It is a collaborative effort between the Trans-Himalayan Aid Society and the UBC Global Health Initiative. The aim of the project is to sustainably promote health in children at a local boarding school: Munsel-ling. In summer 2007, the team traveled to the school to undertake the following: 1) health screening, 2) operation of a primary care centre, 3) health education, and 4) nutritional assessment.

**Methods Used:** To establish a baseline health assessment, the medical and demographic data for 376 students was collected via interview, physical exam and serum haemoglobin measurement. The results were recorded in a personalized health booklet. School-wide empiric treatment of scabies/pediculosis, and anemia were implemented using ivermectin, and iron supplementation, respectively. Education programs of games, crafts, and interactive talks teach children about hand washing, tooth brushing, and nose blowing. Data concerning nutritional intake was collected through staff interviews.

**Summary of Results:** The results of the health screen indicate that scabies/pediculosis, gastroenteritis and anemia were the three most common pathologies present. Skin infections were treated successfully with ivermectin. An improvement in haemoglobin levels was observed three months post initiation of iron supplementation. A primary care center was set up to assess children with health concerns. Effective health education led to improved tooth brushing, nose blowing and hand washing practices. The continuous practice of these routines is reinforced by school staff. An assessment of student nutrition was completed. All data collected will be used for the establishment of future initiatives.

**Conclusions:** We developed a model of health development centred not only on medical aid delivery, but more importantly, on education and community development; both cornerstones to self-sustainability. This program has tremendous potential for ameliorating the standard of health in underprivileged areas of the world.

**241 A TELEMEDICINE MODEL IMPROVES ATTITUDES TO DIABETES IN MEDICAL STUDENTS PARTICIPATING IN A RURAL MEDICINE ROTATION IN NEW MEXICO**

A.M. Sawyer, and K. Collaran. University of New Mexico, Albuquerque, NM.

**Purpose of Study:** Cardiovascular disease, the leading cause of morbidity and mortality in the USA, can be prevented by modification of risk factors including hypertension, dyslipidemia, tobacco use, sedentary lifestyle, poor nutrition, obesity, and diabetes. Dissemination of evidence-based prevention strategies to patients is poor, particularly in underserved rural areas. Unfortunately, programs to improve delivery of specialty level care to these areas are lacking. Project Extension for Community Health Outcomes (ECHO) is a telemedicine and distance-learning program, linking rural health care workers with specialists at the University of New Mexico, in an attempt to improve access to quality health care for rural New Mexicans. We utilized this model in the education and training of medical students on rural rotations, and evaluate whether involvement in this program would change their attitudes to diabetes.

**Methods Used:** Seven second year medical students doing a rural medicine rotation participated in the telemedicine program. On a weekly basis, they were able to present patients with diabetes to the specialists, and together formulate a management plan. Students completed the Michigan Diabetes Attitude Survey, both before and after participation. The valid and reliable overall measure of diabetes-related attitudes, measures attitudes to: (1) The need for health care professionals to have special training to provide diabetes care, (2) the seriousness of type 2 diabetes, (3) the value of tight glucose control, (4) the psychosocial impact of diabetes, and (5) the attitude toward patient autonomy. The 33 questions are graded on a scale of one to five.

**Summary of Results:** The time spent in diabetes training, resulted in a significant improvement in three of the five measured attitudes. (see table below)
Conclusions: The educational experience of participation in project ECHO significantly improved attitudes to diabetes among medical students.

<table>
<thead>
<tr>
<th>Attitude area</th>
<th>Mean Score pre-training</th>
<th>Mean Score Post-training</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Need for specialized training</td>
<td>1.57 (0.43)</td>
<td>1.22 (0.24)</td>
<td>0.045</td>
</tr>
<tr>
<td>Seriousness of diabetes</td>
<td>1.78 (0.14)</td>
<td>1.69 (0.42)</td>
<td>0.685</td>
</tr>
<tr>
<td>Value of sight control</td>
<td>3.51 (0.22)</td>
<td>1.53 (0.24)</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Psychosocial impact</td>
<td>2.07 (0.23)</td>
<td>1.57 (0.41)</td>
<td>0.018</td>
</tr>
<tr>
<td>Patient autonomy</td>
<td>1.84 (0.31)</td>
<td>1.91 (0.43)</td>
<td>0.659</td>
</tr>
</tbody>
</table>

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BACTERIAL VAGINOSIS PREVENTION
EDUCATION OF SEX WORKERS IN CALLAO PERU

H. Sexton. University of Washington, Seattle, WA.

Purpose of Study: Bacterial vaginosis (BV) is the most commonly diagnosed disorder in the female sex worker population at the Posta de Salud, Clinica Barton in Callao, Peru. Education in appropriate hygienic vaginal care practices may reduce the incidence of bacterial vaginosis.

Methods Used: A thorough literature review was conducted to evaluate causes and contributing factors of bacterial vaginosis. Posta de Salud, Clinica Barton health care providers and patients were interviewed to identify vaginal health practices that may contribute to or prevent BV incidence in the female sex worker population.

Summary of Results: Douching, use of synthetic undergarments and inappropriate antibiotic treatments are common practices in the sex worker patient population that likely contribute to changes in vaginal flora precipitating BV. Strategies likely to decrease incidence of bacterial vaginosis include avoidance of douching, use of cotton undergarments and use of antibiotic treatment only under the advice of a health care provider. This information was presented to patients in 3 forms: large posters placed in the clinic waiting room, pamphlets handed out by the clinic counselor and interactive talks given in waiting room.

Conclusions: Bacterial vaginosis the most common diagnosis among female sex workers at the Posta de Salud Clinica Barton in Callao, Peru. Educating health care providers and sex workers in common causes and preventive measures of bacterial vaginosis will improve the health of this community.

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TO GO OR NOT TO GO: DO GLOBAL HEALTH PROJECTS HAVE A POSITIVE IMPACT ON MEDICAL STUDENTS?


Purpose of Study: To assess the impact of the Global Health Initiative’s international health projects on medical students’ awareness and understanding of global health and social responsibility.

Methods Used: The impact of the projects was measured using surveys completed by participants, semi-structured interviews with participants, and the number of interested prospective participants. The surveys assessed, among other things, understanding of key global health themes before and after participating in the international projects and the effect on students both personally and professionally. The interviews provided deeper insight into the impact of the projects on students and their future aspirations. In order to assess the mentorship aspect of the projects, including student presentations and informal meetings, we will compare the number of interested students this year against the number of applicants last year.

Summary of Results: SURVEY

i) Quantitative: There was a 17% increase in students’ perceived abilities of cross-cultural communication, a 23% increase in project development understanding, a 21% increase in project sustainability awareness, a 22% increase in student’s comprehension of community collaboration/local empowerment, and a 23% increase in students’ overall knowledge of global health. Students ranked the importance of mentorship after returning from their project as 9.3/10 and their overall project experience as 8.8/10.

ii) Qualitative: Students identified several important lessons they learned from the international experience. The most common were the importance of being culturally sensitive, cross-cultural communication, organization, and project planning.

INTERVIEW

The interviews confirmed the survey results, as students discussed their increased desire to continue working in the field of global health and the impact the experience had on their global health skills.

MENTORSHIP

Results of the first stage of the mentorship program will be available in November 2007 upon completion of the recruitment process.

Conclusions: The Global Health Initiative’s international health projects were successful in increasing students’ awareness and understanding of key global health issues including cross-cultural communication, sustainability, collaboration and local empowerment.

Immunology and Rheumatology I

Concurrent Session
1:30 PM
Friday, February 1, 2008

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IMMUNE-TO-NEURAL SIGNALING IN RATS
WITH ADJUVANT-INDUCED ARTHRITIS

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1Loma Linda University, Loma Linda, CA and 2Hoover Arthritis Research Institute, Sun City, AZ.

Purpose of Study: In this study, we examined the effect of inducing adjuvant arthritis in male Lewis rats on neural activity and proinflammatory cytokine expression in the hippocampus.

Methods Used: Rats were immunized with complete Freund’s adjuvant (CFA) to induce arthritis, or injected with vehicle, or sterile physiological, endotoxin-free saline. Untreated rats also served as controls. Brains from rats were prepared by immunocytochemistry for localization of c-Fos, a neuronal activation marker, or tumor necrosis factor-α (TNF-α) 4, 14, 21 or 126 days after immunization. Stained tissue was examined morphometrically using an image analysis system. Immunoreactivity in neurons was confirmed by cresyl violet staining.

Summary of Results: c-Fos immunoreactive cells were sparse in hippocampal neurons from nonarthritic control groups. TNF-α was not expressed in control animals. Immunization with CFA induced c-Fos and TNF-α expression in the hippocampal neurons to a similar extent in all regions examined (CA1, CA2, CA3 and dentate gyrus). Similarly, immunoreactivity for TNF-α was induced in all regions of the hippocampus.

Conclusions: Our c-Fos data demonstrates a progressive and chronic increase in neuronal activity in the hippocampus of arthritic rats from 4 to 126 days postimmunization compared with all control groups, suggesting disease specificity. TNF-α expression in the hippocampus of arthritic rats, but not controls, suggests that with the progression of disease, proinflammatory cytokine expression increases in neurons of the hippocampus. The significance of prolonged increase in neuronal...
activity and proinflammatory cytokine expression in the hippocampus is not known and currently under investigation in our laboratory. Widespread and prolonged activation of the hippocampus may be contributory to disease-related cognitive changes, depression, and/or altered pain perception.

245 A COMPARISON OF PSYCHOSOCIAL CHARACTERISTICS IN FIBROMYALGIA AND RHEUMATOID ARTHRITIS PATIENTS INDEPENDENT OF PAIN

E. Katsaros¹ and K. Boyd². 1Loma Linda University Medical Center, Loma Linda, CA and 2Loma Linda University, Loma Linda, CA.

Purpose of Study: The psychosocial aspects of Fibromyalgia (FM) are still poorly understood. We compared FM patients to a group of Rheumatoid Arthritis (RA) patients to determine which psychosocial factors are associated with the diagnosis of FM independently of pain.

Methods Used: We recruited 51 FM and 38 RA patients at a rheumatology outpatient clinic. The patients responded to items that inquired about their demographics, abuse and trauma history, coping, locus of control, anxiety, depression, neuroticism, and strictness in following doctors’ orders. We explored which variables were associated with the diagnosis using binary logistic regression, with pain severity as the first block, and all other psychosocial predictors as a second block.

Forward stepwise regression was used in the second block, with an inclusion criteria p of 0.05.

Summary of Results: The odds ratio of pain severity for FMS diagnosis was 13.20 (95% CI = 3.55 – 49.15). After controlling for pain, the following were the only significant psychosocial predictors of disease: The odds ratio of Child Abuse for FMS diagnosis was 8.17 (CI = 2.16 – 30.91). The odds ratio of ignoring pain for FMS diagnosis was 1.88 (CI = 1.13 – 3.14). The odds ratio of Upper Body Limitations for RA diagnosis was 2.09 (CI = 1.03 – 4.21). Including pain severity, the final model correctly classified 82.0% of the respondents (84.2% of RA, 80.4% of FMS).

Conclusions: Childhood abuse and pain severity most distinguished fibromyalgia from rheumatoid arthritis patients. When controlling for pain, childhood abuse and ignoring pain were the strongest predictor of disease compared to RA. This is in contrast to many studies that demonstrated a correlation of various psychosocial characteristics to FM patients compared to RA patients, but not independently of pain.

246 THE ROLE OF NOD1 AND NOD2 IN THE PRODUCTION OF TNF-α, IL-1β AND IFN-γ IN HUMAN NEONATES

J.E. Caron¹, T.R. La Pine¹, N.H. Augustine¹, T.B. Martins², and H.R. Hill², 1Univ of Utah, SLIC, UT and 2ARUP, SLIC, UT.

Purpose of Study: Human neonates are uniquely susceptible to bacterial infections. The mechanisms for this are poorly understood. Defects in neonatal innate immune responses have been implicated. Nucleotide oligomerization binding domain 1 (NOD1) and NOD2 are novel intracellular pattern-recognition receptors which detect bacterial peptidoglycan (PG) products and initiate pro-inflammatory signaling via NF-kB. The minimal PG breakdown product detected by NOD1 is MurNAc-L-Ala-Glu-meso-diaminopimelic acid (MTP), which is found mostly in gram-negative bacteria. NOD2 detects muramyl dipeptide MurNAc-L-D-isoGln (MDP) found in both gram-negative and gram-positive bacteria. NOD1 and NOD2 may play a role in neonatal innate immune defense. We examine NOD1 and NOD2 production of TNF-α, IL-1β and IFN-γ in mixed mononuclear cells (MMCs) isolated from cord blood and compare these responses to adult MMCs.

Methods Used: Whole blood was collected from healthy adults, umbilical cord blood from healthy term deliveries. MMCs were isolated on Ficoll-Paque. MTP was used for stimulation of NOD1 and MDP for NOD2. Cytokine production was measured using Luminex multi-analyte technology by an in-house developed procedure.

Summary of Results: In response to MTP stimulation through NOD1: 1) TNF-α production was significantly lower for cord blood MMCs [cord 21 pg/mL; adult, 213 pg/mL; (p=0.0028)]; 2) IL-1β production was also significantly lower in cord blood MMCs [cord 4 pg/mL; adult, 5416 pg/mL; (p=0.0090)], and 3) IFN-γ production was lower in cord blood MMCs [cord 3 pg/mL; adult, 13 pg/mL; (p=0.0282)]. MDP stimulation of NOD2 showed: 1) TNF-α production was significantly lower for cord blood MMCs [cord 17 pg/mL; adult, 2089 pg/mL; (p=0.0018)]; 2) IL-1β production was also significantly lower in cord blood MMCs [cord 6 pg/mL; adult, 5858 pg/mL; (p=0.0029)], and 3) IFN-γ production was higher in MMCs from adults compared to cord blood MMCs, though not statistically significant.

Conclusions: Defective cytokine (TNF-α, IL-1β and IFN-γ) production in response to stimulation of NOD1 and NOD2 may play a role in the neonate’s increased susceptibility to bacterial infections. These data are the first to suggest an abnormality of NOD1 and NOD2 signaling in neonatal host defense.

247 ANALYSIS OF LIFESTYLE FACTORS LEADING TO OBESITY IN PSORIASIS PATIENTS

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Purpose of Study: Psoriasis is a non-contagious, life-long, inflammatory skin disease found to be associated with the development of obesity and myocardial infarction. The intent of this study was to identify the factors which contribute to obesity in psoriatic patients.

Methods Used: Subjects were examined to determine a Psoriasis Area and Severity Index (PASI) score and a questionnaire was administered to assess stress level, exercise, nutrition, and the presence of other medical conditions such as arthritis. A total of 100 subjects were recruited from UCLA Dermatology clinics; 51 subjects were psoriasis patients (average age=51.2 years, 22 males, 29 females), while 49 subjects (average age=51.9 years, 25 males, 24 females) served as control non-psoriasis patients.

Summary of Results: Our preliminary results indicate that psoriasis patients (x=27.7, SD=6.41) have a trend toward a higher average body mass index (BMI) than control patients (x=25.98, SD=4.16, p=0.12) with PASI and BMI showing a positive correlation (p=0.03). A statistically significant difference exists between psoriasis individuals and the control group with respect to REAP (Rapid Eating Assessment for Patients) score (p=0.009), total fat (p=0.012), saturated fat (p=0.042), and cholesterol (p=0.032). This difference is maintained in all categories except for saturated fat when controlling for age and BMI, which were found to be significantly correlated with the REAP score and variably associated with individual components of nutrition. There is a trend toward a higher BMI, increased stress level, and less engagement in physical activity in psoriasis patients compared to non-psoriasis controls.

Conclusions: According to our preliminary results, there is a statistically significant correlation between BMI and the severity of disease, as measured by PASI. A statistically significant difference exists between psoriasis individuals and the control group with respect to REAP score, total fat, saturated fat, and cholesterol. The data suggest that patients with psoriasis have less healthful diets than control patients, which may contribute to the increased incidence of obesity in these patients.
248 PAIN AND HEMORRHAGE DURING PHYSICIAN-PERFORMED SYRINGE PROCEDURES IS CAUSED BY POOR NEEDLE CONTROL: A RANDOMIZED CONTROLLED TRIAL


Purpose of Study: During physician-performed syringe and needle procedures including arthrocentesis, physicians refer euphemistically to the presence of blood in the syringe as a “traumatic tap”. We hypothesized that poor needle control during physician-performed syringe procedures traumatizes patient tissues and causes hemorrhage and increased patient pain.

Methods Used: 44 subjects each underwent arthrocentesis of the knee. Pain was measured with the validated Visual Analogue Pain Scale (VAPS). Aspirated fluid was sent for cell count, white blood cell differential, culture, and volume. 22 subjects underwent arthrocentesis with a conventional syringe and 22 subjects with the reciprocating procedure device (RPD). The RPD is a safety device that has been demonstrated to better control the needle and reduce pain compared to the conventional syringe. Red blood cell counts (RBC) were used to measure blood in aspirated fluid, and multivariate logistic regression was used to determine relationships between variables.

Summary of Results: Patient pain during arthrocentesis significantly and independently predicted blood (RBC) in aspirated fluid (r = 0.49, slope = 2.496, CI 95% (862-4130), p = 0.004) and hemorrhage (RBC/WBC) (r = 0.37, slope = 1.52, CI 95% (0.1-2.97), p = 0.04). When the better controlled safety device, the RPD, was compared to the conventional syringe, the RPD reduced blood in aspirated fluid by 40% (RBC: RPD: 10966±12885; Syringe: 16615±16914, p<0.01), reduced hemorrhage by 60% (RBC/WBC: RPD: 4.13±9.54; Syringe: 14.63±14.65, p<0.01), reduced patient pain by 75% (VAPS: RPD: 1.14±2.03; Syringe 4.62±2.87, p<0.01), and improved fluid aspirate yield by 100% (aspirate volume: 15.7±16.7ml; RPD: Syringe: 7.15±3.2ml, p<0.01).

Conclusions: Poor needle control during physician-performed syringe procedures is an important cause of trauma to patient tissues resulting in hemorrhage, increased patient pain, and decreased aspirate yield. Use of the RPD, a safety device that replaces the conventional syringe, reduces needle trauma to patient tissues and improves the outcome and aspirate yield of physician-performed syringe procedures, including arthrocentesis.

249 TRANSITIONAL B CELL STAGES OF DEVELOPMENT IN HUMAN FETAL SPLEEN

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Purpose of Study: Extensive studies of mouse B cell development have used expression of surface markers to define transitional stages of immature B cell development in the mouse spleen. Studies of human B cell development are much more limited and the parallels between B cell production in mouse and human are unclear. To our knowledge there are no reports that examine transitional stages of immature B cell development in human spleen. The aim of this study is to identify human transitional B cell populations in the fetal spleen based on patterns of surface marker expression that have been used to characterize transitional stages of immature B cell development in mouse spleen and in human peripheral blood (PB).

Methods Used: Human fetal spleen cells were stained for 4-color flow cytometry to detect expression of surface markers that have previously been used to identify transitional B cell populations. Co-expression of CD24, CD38, IgD, CD21, and CD23 was examined in gated CD19+ cells. Staining patterns in human fetal spleen were compared to those reported for transitional B cell subsets in mouse spleen and/or human PB.

Summary of Results: Based on patterns of surface marker expression reported for transitional 1 (T1) and transitional 2 (T2) B cell populations in mouse spleen and human PB, we identified phenotypic T1 and T2 B cell populations in human fetal spleen. Size differences in human T1 and T2 cells in fetal spleen are consistent with differences in frequency of cell division that have been reported for T1 and T2 cells in human PB.

Conclusions: Populations of transitional B cells, identified by surface immunophenotypes in the mouse model and in studies of human PB, are present in human spleen at a very early point in human development. Future studies are aimed at comparing and characterizing human B cell development and function in fetal and adult spleen. Further knowledge of B cell development in the spleen is important, given the potential of emerging therapies for B cell driven autoimmune diseases. These include B cell directed therapies such as humanized anti-CD20 and antibodies against BLyS and TACI.

250 MICRORNA EXPRESSION PROFILING OF ARTICULAR CARTILAGE

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Purpose of Study: To determine if there is a differential microRNA expression profile in bovine stifle joint articular cartilage based on histological region and frictional co-efficient properties of the cartilage.

Methods Used: Total RNA from bovine stifle joint articular cartilage was purified and real-time PCR assay performed using a select panel of five microRNAs (miRNA) to determine their expression levels. Cartilage samples representing the superficial, middle, and deep layers were assayed to determine if the miRNA profiles varied based on the histological layer of the articular cartilage. In addition, the miRNA profiles of superficial zone cartilage from anterior and posterior locations along the surface of the medial condyle, known to have varying degrees of friction reduction properties, were compared.

Summary of Results: Based on the panel of five miRNAs selected for expression analysis, the superficial layer of cartilage appeared to express 16 to 32 fold higher levels of miRNAs than the middle and deep layers. There did not appear to be any significant difference in miRNA expression levels between the anterior and posterior region of the medial condyle, which represent the two locations in the knee joint exhibiting the largest difference in frictional coefficient.

Conclusions: The upregulated expression of the examined miRNAs in the superficial layer of knee joint articular cartilage suggest that in the context of the RNA interference pathway, there is a greater degree of post-transcriptional regulation of articular cartilage gene products in the superficial layer as compared to the middle and deep layers. The lack of differential miRNA expression between the anterior and posterior...
articular surfaces of the knee joint suggest no correlation between the five miRNAs in the panel and the gene products responsible for knee joint cartilage articular lubrication.

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PRESSURE-INDUCED URTICARIA SUCCESSFULLY TREATED WITH CYCLOSPORINE


Purpose of Study: Pressure-induced urticaria (PIU) is a mechanical urticaria in which whealing and erythema occur after physical contact. We report on a adolescent girl with severe PIU that was successfully treated with cyclosporine.

Methods Used: A 13 year old girl reported in clinic that she was developing urticarial lesions to areas of her skin to which pressure had been applied. The lesions appeared after minor trauma such as bumping into surfaces or after using her arms to hold items. The lesions usually occur several minutes after contact and were erythematous, pruritic, and painful. They might last several hours and made it difficult to attend school due to the pain. They did not occur unless pressure had been applied. We were able to generate a lesion by scraping the skin with a wooden tongue blade. She had already tried hydroxyzine and cimetidine without relief. She was evaluated for chronic urticaria but thyroid studies were normal and she was negative for thyroid antibodies. Liver function studies were also normal and she was negative for autoantibodies including antinuclear antibody and complement studies were normal. Immunoglobulin levels including IgG, IgA, IgM and IgE were all within normal limits. Chest X-ray did not reveal any abnormalities. She did have a persistently elevated sedimentation rate (up to 35 mm/hr) and C-reactive protein (up to 10.8 mg/L). She was treated with doxipen but this did not alleviate her symptoms. She was also given gabapentin that did not give her relief.

Summary of Results: The patient was started on cyclosporine (100 mg/d) and had great improvement on her disease. She did not develop lesions after minor trauma and was able to discontinue the antihistamines and naproxen for pain. Her CRP did decrease to 3.6 mg/L. She had no adverse effects due to the cyclosporine and her dose was increased to 150 mg/d for further improvement.

Conclusions: This is the first report using cyclosporine to treat pressure-induced urticaria. This case suggests that cyclosporine may be used to treat other patients with physical urticarias. Cyclosporine inhibits T-cell function may explain the mechanism in treating this disorder.

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DEVIC’S NEUROMYELITIS OPTICA (NMO) IN A 20-YEAR-OLD PATIENT WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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Purpose of Study: Systemic lupus erythematosus (SLE) may be associated with multiple neurologic manifestations. NMO; also known as Devic’s syndrome is a combination of bilateral optic neuropathy and transverse myelitis. A serum IgG autoantibody (NMO-IgG) is thought to be a specific marker for NMO. We present a case of neuromyelitis optica in a patient with SLE and antiphospholipid syndrome (APS).

Methods Used: A 20-year-old female with SLE presented with sudden onset of bilateral lower extremity paralysis and paresthesias. She had a temperature of 102.7. Neurologic exam revealed absent reflexes, no motor strength and decreased sensation in bilateral lower extremities. Her ANA, Anti-ds DNA antibodies, lupus anticoagulant and anti-

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ROLE OF EPITHELIAL MEMBRANE PROTEIN (EMP2) IN CHLAMYDIA INFECTIVITY IN VIVO

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Purpose of Study: Chlamydia trachomatis produces significant human disease of the eye and genitourinary tract (GT). Epithelial membrane protein-2 (EMP2) is a candidate host molecule that mediates Chlamydia infection. Our study has two goals: 1) to investigate the effect of EMP2 blockade on GT chlamydial infectivity in vivo; 2) to optimize a murine model of chlamydial conjunctivitis.

Methods Used: In a GT model, BALB/C mice received either negative control or anti-EMP2 diabody (10µg diabody/mouse) preceding infection with C.muridarum (MoPn). Bacterial load and interferon-gamma in GT tissue were respectively quantified by immunofluorescence and ELISA. Short-term and long-term ocular infections involved inoculating C3H/HeN mice with UV-inactivated MoPn or live MoPn (5 x 105 IFU/mouse). Bacterial load in ocular tissue and interferon-gamma secretion were quantified.

Summary of Results: Pretreatment of BALB/C mice with anti-EMP2 diabody significantly reduced bacterial burden and interferon-gamma secretion in the GT model, with greater reduction of ascending infection. In the eye model, transient conjunctivitis was observed only with live organism and there was an associated immunity which appeared protective against repeated ocular infection. Infection-associated immunity is demonstrated by induction of IFN-gamma producing splenic T cells by live MoPn but not by UV-inactivated Chlamydia.

Conclusions: Blockade of EMP2 significantly decreases ascending GT chlamydial infection, supporting a potentially important role for EMP2 as a therapeutic target. The ocular Chlamydia infection model for chlamydial conjunctivitis produces a transient infection followed by an immune response which is protective against repeated infection. Future studies that define specific interactions between Chlamydia and EMP2 may reveal potential targets for disease prevention.
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DETERMINANTS OF VIRAL ASSEMBLY FOR THE NIPAH VIRUS MATRIX, FUSION, AND ATTACHMENT PROTEINS
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Purpose of Study: Nipah (NiV) is an emerging paramyxovirus within the newly defined Henipavirus genus. It is hallmark by microvascular endothelial cell syncytium formation and presents clinically in humans as a respiratory syndrome and often fatal encephalitis. An outbreak in Bangladesh in 2005 had a mortality rate of ~70% in humans. NiV is a negatively stranded RNA enveloped virus with the surface fusion (F) and attachment (G) glycoproteins. The F protein is a Class I fusion protein that contains a tyrosine-based YHQL motif in the cytoplasmic tail which is involved in endocytosis. Although recent studies have elucidated the involvement of the NiV proteins in fusion, assembly and NiV protein association with lipid rafts are still poorly understood.
Methods Used: Methods: NiV-M, -G, and -F proteins and their mutants were codon-optimized and tagged on their C-termini with AU1, FLAG, and HA tags respectively. Viral supernatants from 293T cells expressing these viral proteins were ultracentrifuged to purify viral like particles (VLP’s), and these and their corresponding cell lysates were analyzed by Western blotting. Lipid rafts were collected by sucrose gradient fractionation and precipitated. Cell surface expressions were quantified by flow cytometry.
Summary of Results: The Matrix (M) protein was shown to localize the most in lipid rafts and to bud at the highest levels followed by NiV-F and NiV-G, respectively. Interestingly, the NiV-F glycoprotein was found to bud autonomously, something that has been recorded for very few paramyxoviruses. Additionally, the YHQL motif on NiV-F was shown to be important for NiV-F budding and localization in lipid rafts. Furthermore, when NiV-M was co-expressed with the envelope glycoproteins, it increased NiV-F and NiV-G localization into lipid rafts and their budding efficiencies. NiV-F had a similar effect on NiV-G but did not affect NiV-M.
Conclusions: In toto, these results revealed important determinants for NiV assembly, and implicate lipid rafts in this process. In addition, they comprise an initial understanding of the assembly process for the newly emerging henipavirus genus, and may aid in the elucidation of general paramyxoviral assembly. Further investigation may prove helpful for the development of assembly inhibitors for NiV.

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CCR5-Delta32 MUTATION CORRELATES WITH THE SIZE OF THE SMALLPOX VACCINATION SCAR
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Purpose of Study: The CCR5-delta32 mutation probably arose in Europe between 700 and 1400 years ago and is present in about 10% of Northern Europeans but is virtually unknown in other parts of the world. The mutation attenuates HIV-1 infection and is thought to reduce the transmission of smallpox. We wondered whether there was a possible association of the CCR5 mutation and the size of a smallpox vaccination scar site in previously vaccinated patients.
Methods Used: DNA was isolated from blood using standard procedures and then used as a template to amplify the CCR5 region using two oligonucleotides in the polymerase chain reaction (PCR). The PCR product was loaded on a 12% polyacrylamide gel to analyze the genotype based on size. Individuals with a homozygous mutation show a 200 bp band, and those with no mutation show a 232 bp band. Persons who are heterozygous show both bands on gel. In our analysis, we combined heterozygous and homozygous mutants because only a small number of subjects was studied. Statistical analysis was done using SPSS 10.1 software.
Summary of Results: 28 patients over age 40 were studied. The size of each patient’s smallpox vaccination scar was measured in 2 diameters. 8 of 9 persons with the mutation demonstrated scars that were 1.5 cm or less in diameter, whereas 11 of 19 without the mutation manifested scars that were between 1.5 and 3 cm. These findings are statistically significant when the Pearson Chi Square (P < .001) and Fisher Exact Test (P < .003) are utilized.
Conclusions: We suspect the CCR5-delta32 mutation mitigates smallpox in certain populations. Our small study of 28 patients sought to correlate the diameter of the smallpox scar with the presence or absence of the CCR-5delta32 mutation. The data support the beneficial effect of the mutation and imply it could reduce serious, systemic smallpox. Although smallpox was eradicated from the world 30 years ago, using a patient’s scar size could predict for future vulnerability to the clinical reappearance of smallpox or biological warfare with the virus.

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RAPID MOLECULAR DIAGNOSIS OF THE CLASSIC SEX-LINKED AND VARIANT FORMS OF CHRONIC GRANULOMATOUS DISEASE EMPLOYING HIGH RESOLUTION MELTING ANALYSIS
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Purpose of Study: Chronic granulomatous disease (CGD), which is associated with an absence or severe impairment of the phagocyte respiratory burst, results in severe pyogenic bacterial and fungal infections and is sex-linked in approximately 60% of cases. Characterization of the respiratory burst abnormality utilizing dihydrorhodamine fluorescence may be complicated in sex-linked CGD by patients who have the variant form of the disease with at least some respiratory burst activity. In addition, molecular characterization of the mutations in sex-linked CGD is complicated by the presence of 13 exons necessitating whole gene sequencing, which is both time consuming and expensive.
Methods Used: We have developed a gene scanning technique employing high resolution melting analysis to rapidly (<24 hr) define the involved exon which is then followed by targeted sequence analysis of the amplified product.
Summary of Results: Employing this technique, we have examined 18 classic sex-linked CGD patients, who had typical DHR patterns, and 4 carrier mothers as well as two patients with variant sex-linked disease, whose DHR pattern suggested autosomal recessive disease, but in whom melting analysis and targeted sequencing revealed mutations in the CYBB sex-linked CGD gene. Two carrier females of this variant form of x-linked CGD with unusual DHR patterns, also were found to be heterozygous for the involved mutation.
Conclusions: Gene scanning via high resolution melting analysis followed by targeted sequencing allows rapid molecular diagnosis of sex-linked CGD, which could help to resolve questionable DHR patterns and lead to earlier confirmatory diagnosis and more appropriate treatment of patients.
257 CYTOMEGALOVIRUS-INDUCED INFLAMMATORY RESPONSE IN FETAL ASTROCYTES IS INVERSELY RELATED TO GLIAL DIFFERENTIATION

A. Maheshwary, S. McConaghy, and R. Ohls. University of Alabama at Birmingham, Birmingham, AL. Purpose of Study: Congenital cytomegalovirus (CMV) infection is the most common cause of non-hereditary sensorineural hearing loss (SNHL) in children. Congenital CMV infection affects about 1% of all live births in the US, where 10% are symptomatic at birth and another 10 to 15% develop SNHL or other developmental problems during infancy. For unknown reasons, central nervous system CMV infections are more likely to be symptomatic when the fetus is infected during the first trimester of pregnancy. Because CMV-induced lesions are characterized by inflam- matory responses, we hypothesized that CMV-induced inflammatory changes depend on the stage of fetal development. Methods Used: Human fetal glial cells isolated from 10–22 wk concepti were harvested in the second passage and confirmed to have astrocytic lineage by immunostaining for glial fibrillary acidic protein (>95% purity). Astrocyte cultures were infected with CMV (MOI = 1) for 48 hrs and then stimulated with LPS (1 mcg/mL) and TNF-alpha (10 ng/mL). CMV infection was measured by western blots for immediate-early protein-1. Astrocyte differentiation was assessed by immunocytochemistry for the marker A2B5. Inflammatory responses were measured using a 95-cytokine PCR microarray and ELISA for selected cytokines. NF-kappa B activation was measured as p65 phosphorylation. Summary of Results: CMV-induced inflammatory responses in fetal astrocytes were inversely related to the gestational age. Microarray analysis showed that IL-8 and related CXC chemokines, CXCL10, CXCL11, CCL1, CCL7, CCL11, IL17A, IL23, IL-6, interferon-alpha and gamma were activated in 10–14 wk fetal cells but not in cells from more advanced gestations. Cells from 10–14 wk fetuses produced 3.5–6x more IL-8 and 1.5–2x more CCL7 than at later gestations. These changes correlated with A2B5 immunoreactivity (indicating less differentiated state) and NF-kappa B activation. Conclusions: CMV-induced central nervous system infections may induce local inflammatory changes only in a narrow temporal window related to the stage of glial differentiation. Elucidation of the involved mechanisms will help in the development of anti-inflammatory agents that can be used to reduce tissue damage in conjunction with antiviral drugs.

258 SALVAGE IN TRIPLE CLASS RESISTANT PATIENTS WITH RALTEGRAVIR IN A COMMUNITY BASED PRACTICE

L.S. Newmarch, C.M. Marion, J.A. Dubin, and M. Gottlieb. David Geffen School of Medicine at UCLA, Los Angeles, CA. Purpose of Study: Raltegravir (RAL) is approved for the treatment of HIV-1 integrase inhibitor resistance-associated mutations. The HIV-1 integrase inhibitor raltegravir (RAL), available in expanded access, provides new opportunities for salvage of triple class resistant patients. Methods Used: Twenty subjects received RAL. The mean baseline viral load (VL) by bDNA was 97,535 copies and CD-4 count was 151. Previous protease inhibitor usage was 5.3 drugs. Mean GSS and PSS scores were 2.8 and 2.5 respectively, where those naive to enfuvirtide (ENF) earned a score of 1. 17 were naive to darunavir (DRV), while 13 received DRV in the OBR. 13 patients were naive to ENF, however only 1 received ENF in OBR. 18/20 (90%) had VL < 400 at 4 weeks; 12/20 (60%) had VL < 75 and 11 of those 12 were DRV naive at baseline. At 10 weeks, 15/17 (88%) had VL < 400 and 13/17 (77%) had VL < 75. The log10 change in VL overall at 4 weeks was −2.4. There was no significant decline in VL after 4 weeks. CD4 counts increased by 81 cells in the first 4 weeks overall, and by an additional 29 cells (110 cells total) at 10 weeks for 16/20 evaluable patients. Multivariate regression analysis examining the change in VL and CD-4 from baseline, controlling for ARV regimen prior to RAL as well as OBR was performed. We did not find significant correlates for the observed VL reduction in the OBR, including DRV and ENF. Patients with higher baseline VLs had a larger increase in CD4 at 4 weeks. All results significant at 95% level. RAL was well tolerated. Conclusions: RAL was a potent foundation for salvage regimens in triple class resistant patients. It produced rapid declines in VL and more gradual recovery of CD4 cell counts. Outcome at 10 weeks was independent of DRV and ENF in OBR. Effects on VL suppression to <400 and <75 and on CD-4 count confirmed those reported in Benchmark studies.

259 CHARACTERIZATION OF A NOVEL RECOMBINANT ENTEROVIRUS

R. Hammon, N. Halnon, and P. Krogstad. David Geffen School of Medicine at UCLA, Los Angeles, CA. Purpose of Study: Coxsackievirus B (CVB) is the most commonly identified cause of viral myocarditis. Determinants of cardiotoxicity in CVB strains have been localized to the stemloop II (SLII) structural domain in the 5' untranslated region (5' UTR). A virus isolated in 2005 from a neonatal myocarditis patient at Mattel Children’s Hospital in Los Angeles (strain CVB3-MCH1) was initially typed as a coxsackievirus B3. The purpose of this study was to investigate the possible reasons for the increased virulence seen in this particular isolate. Methods Used: The isolate was sequenced using random and non-random PCR primers. Phylogenetic analysis was done using SimPlot to analyze sequence similarity and ClustalW to generate alignments for creation of phylogenetic trees. Summary of Results: Phylogenetic analysis and nucleotide sequence similarity plots confirm that the P1 region is most similar to CVB3 while P2 and P3 are most similar to echovirus 30 and the recently typed enterovirus strain EV86. Conclusions: Sequencing of the entire genome reveals divergent sequence homologies in the capsid and nonstructural regions, indicating that a recombination event has occurred. We then compared the translated virus sequence to that of similar myocarditic coxsackievirus strains and identified mutations in the coding region of CVB3-MCH1 leading to amino acid substitutions in antibody binding sites (previously identified in mice). Also, folding of the 5' UTR of CVB3-MCH1 differs from the prototype strains. In particular, SLII of CVB3-MCH1 contains nucleotide substitutions that create an extra stemloop fold in the middle of SLII that is not present in other CVB3 strains. This may lead to altered interactions with viral and host proteins, resulting in a more virulent phenotype. Finally, we attempted to stably clone this virus to create a characterized myocarditic virus for use in experimental models.
THE PREVALENCE AND ANTIBIOTIC SUSCEPTIBILITY OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS BACTERIA IN PEDIATRIC PATIENTS IN LAS VEGAS

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Purpose of Study: Colonization and infection by community-associated resistant strains of Staphylococcus aureus are being reported in epidemic proportion globally. Community-associated methicillin-resistant Staphylococcus aureus (CA-MRSA) has been implicated in invasive infections in children, with wide geographical diversity in rates of colonization and infection. The purpose of this study was to determine the local prevalence of MRSA colonization in children and to characterize the MRSA isolates in the laboratory with regard to antibiotic susceptibility patterns, the presence of the mecA gene, and the presence of a specific virulence factor, the Panton-Valentine leukocidin (PVL) gene.

Methods Used: Nasal swabs were collected at two pediatric clinics for a total of 497 children between 2 weeks and 21 years of age. A questionnaire was administered by the pediatric staff to collect demographic data, medical, family and social history. Samples were cultured onto 2 selective media for Staphylococcus aureus and MRSA. Potential MRSA isolates were further evaluated by real-time polymerase chain reaction (PCR) assays specific for the mecA and PVL genes, and for susceptibility to eight antibiotics by Kirby-Bauer disk diffusion on Mueller-Hinton medium.

Summary of Results: Culture results showed that MRSA was present in 15 of the 497 samples (3.0%). Four different antibiotic susceptibility profiles were observed among the MRSA isolates. All 15 MRSA isolates were positive for the presence of the mecA gene, and 10 MRSA isolates contained the PVL gene. During the same study period, a countywide hospital survey revealed that MRSA accounted for 34% of species isolated from infected patients. The most commonly identified risk factor among participants was having been hospitalized (18%), followed by antibiotic usage in the preceding 1 month (10.6%) and having a family member who works in a hospital/clinic (8.2%).

Conclusions: Discordance exists in the prevalence of MRSA colonization versus MRSA infection. Understanding the role that colonization plays in infection is needed to develop effective interventions to reduce the growing epidemic of MRSA infections.

SAFETY OF INTRAVENOUS ANTIBIOTIC THERAPY IN CHRONIC LYME DISEASE

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Purpose of Study: Treatment of Lyme disease is controversial. Although intravenous antibiotic therapy has been used to treat neurologic Lyme disease for up to 30 days, treatment beyond this length of time in patients with persistent neurologic symptoms is considered dangerous. The goal of our study was to evaluate the safety of intravenous antibiotic therapy in a cohort of patients with chronic neurologic Lyme disease.

Methods Used: We enrolled 199 consecutive patients in the study. All patients had significant neurologic symptoms and positive testing for Borrelia burgdorferi consistent with chronic neurologic Lyme disease. Patients were treated with intravenous antibiotics via a central venous catheter (11 patients), a peripherally inserted central catheter (PICC line, 138 patients), a midline catheter (5 patients), a subcutaneous port (20 patients) or a peripheral catheter (25 patients). Standard intravascular device (IVD) care was administered to all patients, and monitoring for medication reactions and IVD infection, clotting or infiltration was performed on a weekly basis.

Summary of Results: For the 199 patients, the mean length of intravenous antibiotic treatment was 118 days (range, 7–750 days) representing 23,654 IVD-days. Seven patients (3.5%) experienced allergic reactions to the antibiotic medication, and two patients (1.0%) had gallbladder toxicity during the study. IVD complications occurred in 15 patients (7.5%) representing an incidence of 0.63 per 1000 IVD-days. Thirteen complications involved PICC lines and two involved ports. The IVD problems occurred an average of 81 days after initiation of treatment (range, 7–240 days). There were three suspected line infections for an incidence of 0.13 per 1000 IVD-days. Only one of the IVD infections was confirmed, and no resistant organisms were cultured from any patient. None of the IVD complications were fatal.

Conclusions: Prolonged intravenous antibiotic therapy is associated with low morbidity and no IVD-related mortality in patients with chronic neurologic Lyme disease. With proper IVD care, the risk of extended antibiotic therapy in these patients appears to be low.
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Methods Used: to develop obesity.

The objective is to determine the desaturation index (ratio of oleate to stearate) as a measure of SCD1 activity in rats nutritionally programmed central appetite suppression that is impaired in maternal food restriction. The hypothesis is that SCD1 will upregulate in the pups from maternal environment of maternal food restriction. This difference was not observed at 1 day of age. Programming effects on the desaturation index may not be manifest reflect postnatal nutritional exposure and time-dependent events of growth and development. This observation suggests a potential role of stearoyl-CoA desaturase in prenatal nutritional programming of adult obesity in rats

Summary of Results: Plasma samples from offspring of a model of rat dams 50% food restricted during pregnancy were used as a control group. All offspring were fed ad libitum after birth. Plasma samples were obtained from 1 day, 3 weeks, and 9 months of age. Plasma was saponified with heptadecanoic acid added as an internal standard, fatty acids were extracted, and GC/MS performed. Desaturation indices were calculated.

Conclusions: At 1 day and at 3 weeks of age, there was no difference in the desaturation index. At 9 months of age, the desaturation index was increased in the rats programmed to develop obesity in comparison to the control group. The desaturation index trended toward a decrease at 9 months of age in the control group.

Methods Used: Plasma samples from offspring of a model of rat dams 50% food restricted during pregnancy were assessed for fatty acid composition. Samples from offspring of ad libitum fed dams were used as a control group. All offspring were fed ad libitum after birth. Plasma samples were obtained from 1 day, 3 weeks, and 9 months of age. Plasma was saponified with heptadecanoic acid added as an internal standard, fatty acids were extracted, and GC/MS performed. Desaturation indices were calculated.

Summary of Results: Results are expressed as percentage of control +/- SEM. IUGR increased expression of GR and 11βHSD1 in the retroperitoneal adipose depot of the male rat. (GR 200.9% +/- 14.3% (p < 0.03), 11βHSD1 233.9% +/- 44.9% (p < 0.01)) Conversely, there were no significant differences in expression of either GR or 11βHSD1 in subcutaneous adipose tissue.

Conclusions: We conclude that IUGR increases GR and 11βHSD1 mRNA in retroperitoneal adipose of day 21 male rats, without affecting mRNA levels of these genes in subcutaneous adipose. These results reveal that the response to IUGR is dependent of the location of the adipose depot. Interestingly, in both the human and the rat, GR mRNA levels are characterized by multiple exon variants. We speculate that the changes observed here will due to specific epigenetic modifications of the GR genes and subsequent increases of specific GR exon variants. (supported by HD41075).

ROLE OF STEAROYL-COA DESATURASE IN PRENATAL NUTRITIONAL PROGRAMMING OF ADULT OBESITY IN RATS

J.K. Yee1,2, S. Lim1, M. Desai1,3, M. Rossi1,3, and W.P. Lee1,2, 1Los Angeles BioMedical Research Institute at Harbor-UCLA, Torrance, CA; 2David Geffen School of Medicine at UCLA, Los Angeles, CA and 3David Geffen School of Medicine at UCLA, Los Angeles, CA.

Purpose of Study: In utero exposure to undernutrition increases risk of development of obesity. Targets for prevention and treatment of obesity include stearoyl-CoA desaturase enzyme 1 (SCD1) which converts stearate to oleate (C18:0 to C18:1). Oleate may normally have a role in central appetite suppression that is impaired in maternal food restriction. The hypothesis is that SCD1 will upregulate in the pups from maternal food restriction, leading to an increase in the ratio of oleate to stearate. The objective is to determine the desaturation index (ratio of oleate to stearate) as a measure of SCD1 activity in rats nutritionally programmed to develop obesity.

Methods Used: Plasma samples from offspring of a model of rat dams 50% food restricted during pregnancy were used as a control group. All offspring were fed ad libitum after birth. Plasma samples were obtained from 1 day, 3 weeks, and 9 months of age. Plasma was saponified with heptadecanoic acid added as an internal standard, fatty acids were extracted, and GC/MS performed. Desaturation indices were calculated.

Summary of Results: At 1 day and at 3 weeks of age, there was no difference in the desaturation index. At 9 months of age, the desaturation index was increased in the rats programmed to develop obesity in comparison to the control group. The desaturation index trended towards a decrease at 9 months of age in the control group.

Conclusions: The desaturation index was increased compared to controls in 9 month old obese rats who had been exposed to an in utero environment of maternal food restriction. This difference was not observed at 1 day of age and 3 weeks of age. At 9 months of age, the desaturation index in control animals trended toward a decrease compared to 1 day of age. Programming effects on the desaturation index may not be manifest in the fatty acid composition until adulthood. The programming effects reflect postnatal nutritional exposure and time-dependent events of growth and development. This observation suggests a potential window of time for obesity prevention in early postnatal life.

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ECTOPIC FAT ACCOMPANIES VISCERAL ADIPOSEITY AND TOGETHER EXPLAIN THE MAJORITY OF VARIATION IN INSULIN RESISTANCE

W.C. ChapinC. KriskyU. SzumowskiS. Bacon, and J.Q. Purnell. OHSU, Portland, OR.

Purpose of Study: Intra-abdominal fat (IAF), intra-hepatic lipid (IHL), and intra-myocellular lipid (IMCL) have all been separately associated with insulin resistance. Few studies have, however included measurements of all these variables and examined their relative contributions to insulin sensitivity and other components of the metabolic syndrome.

Methods Used: 21 subjects (10 women, 11 men) with an average (range) age of 43 (23 to 62) years and BMI of 27 (19 to 38) kg/m2 underwent studies of insulin sensitivity (Si) by the FSIVGTT, IAF by MRI, IMCL and extra-cellular lipid (EMCL) by MRS, and fasting lipids and apolipoprotein measurements. Four subjects with type 2 diabetes were also studied but were not on lipid lowering therapy and had their diabetes medications held 24-hrs before study.

Summary of Results: Si correlated with IAF (r = 0.72, p = 0.004), IHL (r = 0.75, p = 0.03), and IMCL (r = 0.70, p = 0.04), but not EMCL (r = 0.16, p = 0.68). On multiple linear regression, inclusion of IAF, IHL, and IMCL as independent variables explained 81% of the variability in Si (r^2 = 0.81), with IAF having the highest standardized coefficient: −0.41, −0.31, and −0.30 for IAF, IHL, and IMCL, respectively. IAF, but not IHL or IMCL, also correlated with fasting glucose.

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insulin ($r = 0.75$, $p = 0.002$), triglyceride levels ($r = 0.59$, $p = 0.02$) and VLDL-C ($r = 0.69$, $p = 0.005$); and reached borderline significant correlation with total HDL ($r = -0.48$, $p = 0.07$) and HDL3 ($r = -0.50$, $p = 0.06$) levels.

**Conclusions:** Accumulation of ectopic fat (IMCL and IHL) and IAF together explain ~80% of the variation in insulin sensitivity in these subjects. Although IAF accumulation best correlated with various glucose and lipid components of the metabolic syndrome, ectopic fat accumulation is a common feature in viscerally obese subjects with each depot likely contributes to the pathogenesis of the metabolic syndrome.

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**266 EVIDENCE OF APOPTOSIS IN HUMAN ADIPOSE TISSUE FROM HEALTHY INDIVIDUALS**


**Purpose of Study:** Apoptosis in human adipose tissue has been recently characterized in disease states such as Human Immunodeficiency Virus (HIV). However, there is a paucity of data on the role of apoptosis in adipose tissue from healthy subjects. This study was undertaken to determine if the downstream signaling protein of apoptosis, cleaved poly (ADP-ribose) polymerase (cPARP), is differentially expressed over time in healthy subjects and in subjects undergoing suction lipectomy.

**Methods Used:** Adipose tissue biopsies were procured from 9 subjects [Age: 42 ± 2.5 (means ± SE), body mass index: 25.6 ± 0.9, males (M) = 2, females (F) = 7] from 2 distinct anatomical regions at 3 different time points. Following the initial biopsy, subjects were randomized to suction lipectomy surgery (n = 5, M = 1) or a control group (n = 4, M = 1). Biopsy sites were determined prior to randomization so that adipose tissue was obtained from a potential surgical site (PSS), which would only be suctioned in individuals undergoing surgery, and from a non-potential surgical site (NPSS), which remained unsuctioned in all individuals at 6-wk and 6-mo visits. Protein expression of cPARP was quantified by western immunoblot analyses.

**Summary of Results:** cPARP expression did not differ between groups or biopsy site at baseline [NPSS: 1.8 ± 10^4 densitometric units (DU) ± 3.4 ± 10^3, PSS: 1.9 ± 10^4 ± 2.0 ± 10^3 DU]. There was no difference in cPARP in control subjects from baseline up to 6-mo in either PSS (baseline: 2.1 ± 10^4 ± 4.8 ± 10^3 DU, 6wk: 2.3 ± 10^4 ± 4.4 ± 10^3 DU, 6mo: 1.8 ± 10^4 ± 4.9 ± 10^3 DU, P = 0.15) or NPSS (baseline: 2.1 ± 10^4 ± 2.2 ± 10^3 DU, 6wk: 2.2 ± 10^4 ± 4.4 ± 10^3 DU, 6mo: 2.5 ± 10^4 ± 1.1 ± 10^3 DU, P = 0.175). However, there was a trend in cPARP expression over time as seen in the surgery group in both PSS (baseline: 1.5 ± 10^4 ± 4.7 ± 10^3 DU, 6wk: 1.6 ± 10^4 ± 2.8 ± 10^3 DU, 6mo: 2.4 ± 10^4 ± 3.5 ± 10^3 DU, P = 0.082) and NPSS (baseline: 1.8 ± 10^4 ± 3.2 ± 10^3 DU, 6wk: 1.2 ± 10^4 ± 3.6 ± 10^3 DU, 6mo: 1.9 ± 10^4 ± 2.5 ± 10^3 DU, P = 0.068). Additional subjects are being analyzed.

**Conclusions:** These preliminary data provide evidence that apoptosis may occur in healthy individuals and may be differentially regulated in subcutaneous adipose tissue by suction lipectomy.

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**268 PIOGLITAZONE STIMULATES ADIPONECTIN SECRETION FROM 3T3-L1 ADIPOCYTES VIA ACTIVATION OF THE PHOSPHATIDYLINOSITOL 3’-KINASE**

R.I. Pereira, J. Leiner, and B. Draznin. Denver Veterans Affairs Medical Center, Denver, CO; UCCHC, Aurora, CO; and Denver Health, Denver, CO.

**Purpose of Study:** Thiazolidinediones (TZDs) have been observed to increase circulating adiponectin levels, but the mechanism of this effect remains unclear. We investigated the acute effects of the TZD pioglitazone on adiponectin secretion, and the involvement of the phosphatidylinositol 3-kinase (PI3K) signaling pathway in this action.

**Methods Used:** We treated 3T3-L1 adipocytes with 1-10μM pioglitazone for 2–24 hours, +/− PI3K inhibition by Wortmannin. Adiponectin secreted into growth media was measured by Western blot. PI3K activity was measured by thin layer chromatography. Pioglitazone effect on adiponectin synthesis and on secretion of newly synthesized adiponectin was studied using a pulse-chase technique.

**Summary of Results:** In 3T3-L1 adipocytes, pioglitazone significantly increased both adiponectin secretion and PI3K activity in a dose-dependent manner. At two hours, pioglitazone increased adiponectin secretion by 61 +/− 16% (P < 0.004), and this stimulatory effect was maintained after 24 hours (64 +/− 23%, P < 0.05). Wortmannin completely blocked the pioglitazone effect and decreased adiponectin secretion to below control levels at 2 (28% +/− 3% of control, P < 0.0001) and at 24 hours (52 +/− 5% of control, P < 0.03). Pioglitazone treatment increased PI3K activity in a dose-dependent manner (1.8 vs. 2.7-fold increase over control at 2 μM vs. 10 μM dose, P = 0.02).

**Conclusions:** Our data show that pioglitazone acutely stimulates adiponectin secretion from 3T3-L1 adipocytes and for the first time demonstrate that this action is dependent on PI3K activation.
269 THE EFFECT OF COMBINATION ROSIGLITAZONE/METFORMIN THERAPY ON ADIPOCYTE AND SERUM ADIPOnectin CONCENTRATION

J. Kung1, C. Cho1, T.P. Ciaraldi2, and R.R. Henry2. 1University of California, San Diego, San Diego, CA and 2VA, San Diego, CA.

Purpose of Study: Adiponectin (Ad), a secretory product of adipose tissue, has previously been shown to be decreased in individuals with type 2 diabetes (T2D), insulin resistance, and obesity, and increased in response to thiazolidinedione (TZD) therapy. We investigated the dose-response effect of TZD alone and in combination with metformin on subcutaneous adipocyte protein content and serum levels of Ad.

Methods Used: T2D subjects were randomized to receive either high-dose rosiglitazone (R-8 mg/day), high-dose metformin (M-2000 mg/day), or low-dose rosiglitazone/metformin combination therapy (r+m-4 mg + 1000 mg/day) for a period of 4 months. All subjects were then switched to high-dose rosiglitazone/metformin combination therapy (R+M-8 mg + 2000 mg/day) for another 4 months. Adipose tissue biopsies as well as clinical data were obtained at each step of the trial.

Summary of Results: M had no effect on Ad, either adipocyte content or circulating levels. R significantly increased Ad content in adipocytes (p = 0.0386), while r+m combination therapy had no effect on cellular Ad, suggesting that rosiglitazone’s ability to increase cellular Ad is dose-dependent. Serum adiponectin levels increased with R treatment (p = 0.059) and low-dose r+m combination therapy (p = 0.062). Adding R to M increased serum Ad levels the same as R alone. When patients were then switched to high-dose R+M combination therapy, they experienced a significant rise in serum Ad levels as compared to baseline (p = 0.0012). Changes in cellular adiponectin levels correlated positively with changes in HDL (p = 0.014, r = 0.43).

Conclusions: In summary: 1) M has no effect on adipocyte adiponectin content or presence in the circulation, 2) R and R+M increase Ad levels, 3) Increases in Ad correlated positively with clinical markers such as HDL. We conclude that in combination therapy, cellular and circulating adiponectin levels rise in a dose-dependent manner in response to R, independent of the M component.

270 COMPARISON OF RT3 TRIAXIAL ACCELEROMETER AND 7-DAY PHYSICAL ACTIVITY RECALL IN ADOLESCENTS

A.S. Kong1, A. Harris1, and B. Skipper2. 1University of New Mexico, Albuquerque, NM and 2University of New Mexico, Albuquerque, NM.

Purpose of Study: Obesity and type 2 diabetes are increasing among adolescents, and physical activity is a key factor for preventing and treating these problems. This study was designed to compare the RT3 accelerometer and the 7-day Physical Activity Recall (7dPAR) in estimating energy expenditure in adolescents of mostly Hispanic and American Indian descent for an obesity intervention.

Methods Used: Eighteen participants (age = 16–18 years, BMI = 29 ± 8 kg/m2, 61% females, 44% Hispanic, 44% American Indian) were studied. Height and weight were obtained at the job training center’s health clinic along with programming the accelerometer. Participants were instructed to wear the accelerometer on the right hip for 7 days, except for during showers, swimming, and sleeping. On the seventh day, accelerometers were retrieved, data downloaded and the 7dPAR was administered to participants to recall the previous week of physical activity. Participants with at least 4 complete days of accelerometer data and who completed the 7dPAR interviews were analyzed. Relationships of the two instruments with regards to average daily energy expenditure, minutes of moderate physical activity (4–6 METs) and minutes of vigorous physical activity (≥6 METs) were determined using Spearman rank correlation.

Summary of Results: The RT3 and 7dPAR yielded comparable mean daily energy expenditure (2,731.5 vs. 2,971.9 kcal/day, p = 0.15). Spearman correlation was high and statistically significant for daily energy expenditure between the RT3 and 7dPAR, r = 0.88 (p < 0.001). Minutes of moderate physical activity collected by the 7dPAR was 99 ± 103 vs. 45 ± 21 min by the RT3; r = 0.56 (p = 0.02), while minutes of vigorous physical activity collected by the 7dPAR was 38 ± 55 vs. 56 ± 45 min by the RT3; r = -0.20 (p = 0.44).

Conclusions: Although, these data show that energy expenditure is highly correlated between the RT3 accelerometer and 7dPAR, designation of moderate and vigorous physical activity were incongruent between the RT3 and 7dPAR. Use of both instruments may be necessary to accurately designate physical activity levels in obesity interventions.

Morphpogenesis and Malformations
Concurrent Session
1:30 PM
Friday, February 1, 2008

271 KLIPPEL-FEIL ANOMALY: ASSOCIATED FEATURES AND UNDERLYING DYSDMORPHOGENESIS

S. Dugan, and L. Hudgins. Stanford University, Stanford, CA.

Purpose of Study: Klippel-Feil anomaly (KFA) is a malformation sequence involving segmentation failure of at least two cervical vertebrae and classically leading to low posterior hairline and short neck with limited range of motion. The purpose of this report is to review the wide range of associated findings and to classify possible subgroups based on proposed mechanisms.

Methods Used: Using a patient database, we retrospectively reviewed the charts of patients seen in Medical Genetics consultation for vertebral anomalies. The database included all inpatients and outpatients seen at a single tertiary-care center over the past three years. A given patient was considered to have KFA if he or she had fusion of at least two cervical vertebrae and classically leading to low posterior hairline and short neck with limited range of motion. The purpose of this report is to review the wide range of associated findings and to classify possible subgroups based on proposed mechanisms.

Summary of Results: Malformations and Malformations

ACTIVITY RECALL IN ADOLESCENTS

ACCELEROMETER AND 7-DAY PHYSICAL COMPARISON OF RT3 TRIAXIAL

CONCENTRATION

Rosiglitazone/Metformin Therapy

On Adipocyte and Serum Adiponectin Concentration

J. Kung1, C. Cho1, T.P. Ciaraldi2, and R.R. Henry2. 1University of California, San Diego, San Diego, CA and 2VA, San Diego, CA.

Purpose of Study: Adiponectin (Ad), a secretory product of adipose tissue, has previously been shown to be decreased in individuals with type 2 diabetes (T2D), insulin resistance, and obesity, and increased in response to thiazolidinedione (TZD) therapy. We investigated the dose-response effect of TZD alone and in combination with metformin on subcutaneous adipocyte protein content and serum levels of Ad.

Methods Used: T2D subjects were randomized to receive either high-dose rosiglitazone (R-8 mg/day), high-dose metformin (M-2000 mg/day), or low-dose rosiglitazone/metformin combination therapy (r+m-4 mg + 1000 mg/day) for a period of 4 months. All subjects were then switched to high-dose rosiglitazone/metformin combination therapy (R+M-8 mg + 2000 mg/day) for another 4 months. Adipose tissue biopsies as well as clinical data were obtained at each step of the trial.

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Conclusions: In summary: 1) M has no effect on adipocyte adiponectin content or presence in the circulation, 2) R and R+M increase Ad levels, 3) Increases in Ad correlated positively with clinical markers such as HDL. We conclude that in combination therapy, cellular and circulating adiponectin levels rise in a dose-dependent manner in response to R, independent of the M component.

270 COMPARISON OF RT3 TRIAXIAL ACCELEROMETER AND 7-DAY PHYSICAL ACTIVITY RECALL IN ADOLESCENTS

A.S. Kong1, A. Harris1, and B. Skipper2. 1University of New Mexico, Albuquerque, NM and 2University of New Mexico, Albuquerque, NM.

Purpose of Study: Obesity and type 2 diabetes are increasing among adolescents, and physical activity is a key factor for preventing and treating these problems. This study was designed to compare the RT3 accelerometer and the 7-day Physical Activity Recall (7dPAR) in estimating energy expenditure in adolescents of mostly Hispanic and American Indian descent for an obesity intervention.

Methods Used: Eighteen participants (age = 16–18 years, BMI = 29 ± 8 kg/m2, 61% females, 44% Hispanic, 44% American Indian) were studied. Height and weight were obtained at the job training center’s health clinic along with programming the accelerometer. Participants were instructed to wear the accelerometer on the right hip for 7 days, except for during showers, swimming, and sleeping. On the seventh day, accelerometers were retrieved, data downloaded and the 7dPAR was administered to participants to recall the previous week of physical activity. Participants with at least 4 complete days of accelerometer data and who completed the 7dPAR interviews were analyzed. Relationships of the two instruments with regards to average daily energy expenditure, minutes of moderate physical activity (4–6 METs) and minutes of vigorous physical activity (≥6 METs) were determined using Spearman rank correlation.

Summary of Results: The RT3 and 7dPAR yielded comparable mean daily energy expenditure (2,731.5 vs. 2,971.9 kcal/day, p = 0.15). Spearman correlation was high and statistically significant for daily energy expenditure between the RT3 and 7dPAR, r = 0.88 (p < 0.001). Minutes of moderate physical activity collected by the 7dPAR was 99 ± 103 vs. 45 ± 21 min by the RT3; r = 0.56 (p = 0.02), while minutes of vigorous physical activity collected by the 7dPAR was 38 ± 55 vs. 56 ± 45 min by the RT3; r = -0.20 (p = 0.44).

Conclusions: Although, these data show that energy expenditure is highly correlated between the RT3 accelerometer and 7dPAR, designation of moderate and vigorous physical activity were incongruent between the RT3 and 7dPAR. Use of both instruments may be necessary to accurately designate physical activity levels in obesity interventions.
observed developmental disability, encephalocele, tethered cord, and situs ambiguous.

Conclusions: The findings in our study and those in the literature suggest etiologic heterogeneity in Klippel-Feil anomaly. The sequence usually occurs in association with other anomalies, particularly of the ear, kidneys, and radial rays. The spectrum of associated findings has led to much speculation on the mechanism or mechanisms behind KFA. Proposed mechanisms include disruptions in blood supply and altered molecular signaling at the midline during embryogenesis. The anomaly’s regional distribution, as well as its frequency in the OHV spectrum, suggest a vascular etiology; associated arterial anomalies are well-described in the literature. However, sporadic vascular disruption is difficult to implicate in patients with anatomically distant malformations such as renal agenesis or radial ray defects. More plausible explanations in this subset of patients would include systemic tendency toward vascular disruption or pleiotropic effects of a genetic abnormality.

FEMORAL FACIAL SYNDROME AND HYDROCEPHALY: A NOVEL PRESENTATION
A. Ho1, N. LeFloch1, M.L. Levy1,2, and L.M. Bird3,4, 1University of California, San Diego, La Jolla, CA; 2Rady Children’s Hospital of San Diego, San Diego, CA; 3University of California, San Diego, La Jolla, CA and 4Rady Children’s Hospital of San Diego, San Diego, CA.

Purpose of Study: The purpose of this study is to present a case that may reveal a novel feature of femoral facial syndrome.

Methods Used: Case Report

Summary of Results: A Mexican male infant was born at 37 weeks gestation to a 31 year-old woman with type 2 diabetes mellitus that was in poor control during the first trimester of pregnancy. At birth, he presented with hydrocephalus due to aqueductal stenosis, with a head circumference of 53 cm and widely split cranial sutures. He had a long, featureless philtrum and Robin sequence (micrognathia, U-shaped cleft palate, glossoptosis creating airway obstruction). He had short immobile legs and bilaterally clubbed feet. Radiographs demonstrated absence of the femur on the right and severe femoral hypoplasia on the left. The right fibula was hypoplastic, and there were segmentation anomalies of the thoracic spine.

Conclusions: This infant of a diabetic mother displays a pattern of malformation consistent with femoral facial syndrome (FFS) as well as hydrocephaly, presumably due to aqueductal stenosis. There are three previous reports of central nervous system (CNS) defects in FFS, but none included hydrocephalus. Two of these individuals were born to women without diabetes, allowing us to conclude that CNS defects are part of FFS and not a coexistent expression of diabetic embryopathy. In the case presented herein, either the hydrocephaly is a novel feature of FFS, or the hydrocephaly is a separate diabetes-related malformation. The information we have currently does not allow us to determine which of these interpretations is correct.

FAMILIAL PREDISPOSITION TO DEVELOPMENTAL DYSPLASIA OF THE HIP
D. Stevenson1,2, G. Mineau3, R. Kerber4, D. Viskochil1,2, A. Schiffem4,2, and J. Roach3,4, 1University of Utah, SLC, UT; 2Shriners Hospital Intermountain, SLC, UT; 3University of Utah, SLC, UT and 4University of Utah, SLC, UT.

Purpose of Study: Developmental dysplasia of the hip (DDH) is a common birth defect affecting 1.5% of neonates and thought to have genetic contributions to the phenotype. The purpose of this study is to assess the degree of relationship between individuals with DDH.

Methods Used: The Utah Population Database (UPDB) is a computerized integration of pedigrees, vital statistics, and medical records representing over six million individuals. Data sets were created from UPDB state-wide birth certificates and from the University of Utah Health Sciences Center enterprise data warehouse using records for DDH and linked to UPDB. Controls for the dataset were selected that matched cases on birth year and sex and 10 controls were randomly selected per case. Statistics computed for each family were the number of descendants, the observed and expected number of affected, P-value, familial standardize incidence ratio (FSIR), relative risks and standard error. A kinship analysis tool was used to find pedigrees with excess DDH.

Summary of Results: Combined data resulted in 1649 individuals with DDH. Relative risk (RR) was significantly increased in first degree relatives (RR = 12.1; p < 0.000001), sibs (RR = 11.9; p < 0.000001), first cousins (RR = 1.7; p = 0.04), and second cousins (RR = 0.6; p = 0.03). A total of 468 families were identified with at least 5 affected individuals in a family. These results were then filtered to only contain families that had a p-value < 0.01. This resulted in 141 founders with anywhere between 4 to 30 affected living descendants with a p-value < 0.01 with family sizes ranging from 594 to 44819 descendants. A total of 28 founders had an FSIR of ≥5.0.

Conclusions: Our data suggest a genetic contribution to DDH with a 12-fold increase in risk for first degree relatives. Still, DDH appears to be multifactorial, as there is an apparent relative protective effect for second cousins, which does not follow a simple genetic inheritance pattern. This may be the result of the sample size, multiple genes interacting, and/or environmental effects. Several large families were identified, which will allow for future genetic linkage or association studies.

POPULATION STUDY OF LIMB-BODY WALL COMPLEX IN UTAH:
PREVALENCE, DESCRIPTIVE ANALYSIS AND CLUES TO PATHOGENESIS
J.C. Carey1,2, J. Byrne1,2, K. Lecheminant2, and M. Feldkamp1,2, 1University of Utah, Salt Lake City, UT and 2Utah Department of Health, Salt Lake City, UT.

Purpose of Study: In 1987 Van Allen et al. established the limb-body wall complex (LBW) as a distinct and recognizable entity. Despite many subsequent reports, the etiology and the pathogenesis of LBW remain elusive. The relationship of LBW to the amniotic band sequence (ABS) has been recognized for years but not clarified. Two recent papers in 2007 suggest that LBW is on the same pathogenic continuum as cloacal exstrophy (CE) and the OEIS complex. Here we report on a population-based series of 27 cases of LBW with the purpose of attempting to elucidate pathogenesis and relationships to ABS and CE/OEIS. Preliminary elements of these data were included in a prior presentation on CE; this study provides the details.

Methods Used: We analyzed all cases of LBW reported in the Utah Birth Defects Network (UBDN) 1997–2005. Records, including prenatal diagnosis and autopsy examinations were available in the UBDN, which is an active and comprehensive statewide birth defects surveillance program.

Summary of Results: There were 27 well-documented infants indicating a prevalence in Utah of 1 in 16,033 total births, a figure that is higher than all previous prevalence studies. All infants died in perinatal period, and all were prenatally diagnosed by sonogram. Seven cases had LBW without any limb defects and 7 had limb deficiencies of ABS; 6 of these had orofacial clefts or acrania, while 13 were pure LBW without craniofacial involvement or bands. Three of the cases were 1 of MZ twins.

Conclusions: Our series, one of the largest number reported, documents the highest prevalence among the epidemiologic studies where other figures are about 1 in 40,000 (Spain, Europe, Japan). We confirm that
there are two groups of infants with LBW, those with primarily upper limb and craniofacial involvement and those with lower limb and caudal defects. Two cases had some overlap with CE/OEIS and support some relationship between the two patterns.

275 FAMILIAL OCCURRENCE OF SCHWANNOMATOSIS AND MALIGNANT RHABDOID TUMOR ASSOCIATED WITH A Duplication IN Snf5/Ini1/Smarcb1 M. Williams1, J. Keyser2, and J. Swensen3. 1Intermountain Healthcare, Salt Lake City, UT; 2Intermountain Healthcare, Logan, UT and 3University of Utah, Salt Lake City, UT.

Purpose of Study: Evaluate a family with autosomal dominantly inherited schwannomatosis in which several individuals have died of malignant rhabdoid tumor. Autosomal dominant inheritance of both schwannomatosis and malignant rhabdoid tumor has been reported in the literature, but never in the same family. Germline mutations in the hSNF5/INI1/SMARCB1 gene have been identified in families with hereditary predispositions to each tumor type.

Methods Used: Review of pathology from both schwannomas and malignant rhabdoid tumors including immunohistochemistry. Molecular analysis of the hSNF5/INI1/SMARCB1 gene in surviving family members with schwannomatosis and in tissue from both schwannomas and malignant rhabdoid tumors excised from family members.

Summary of Results: Review of the histology and immunohistochemistry of the pathologic specimens confirmed the diagnosis of schwannoma and malignant rhabdoid tumors. One of the schwannomas had some features of a malignant peripheral nerve sheath tumor. Malignant transformation of schwannomas has not previously been reported in individuals with schwannomatosis. Analysis of the hSNF5/INI1/SMARCB1 gene in 3 affected family members revealed the same 2,631 base-pair duplication containing exon 6. Duplication of exon 6 in the mRNA transcript results in frameshift and protein truncation. Molecular analysis of the tumors is pending at the time of abstract submission.

Conclusions: This is the first reported instance of a family manifesting both schwannomatosis and malignant rhabdoid tumor due to a germline mutation in the hSNF5/INI1/SMARCB1 gene. Possible mechanisms as well as additional findings from the tumor analysis will be discussed.

276 FETUS-IN-FETU: TWINNING OR HIGHLY-DIFFERENTIATED TERATOMA? J. Kaplan1, A. Kwan1, A. Cherry2, D. Perry2, and J. Shieh1. 1Stanford University, Stanford, CA and 2Stanford University, Stanford, CA.

Purpose of Study: Fetus-in-Fetu is an uncommon condition in which a mass comprised of fetiform structures is found within a developing fetus or living individual. Given the controversy over whether these masses represent a form of twinning or differentiated teratomas, we present a case of a female with multiple intra-abdominal fetiform masses and an ovarian teratoma.

Methods Used: Case presentation and literature review. We performed chromosome analysis on the infant, four of the eleven fetiform masses, and the ovarian mass.

Summary of Results: A 30-week gestation female fetus was found to have intra-abdominal fetal structures on prenatal ultrasound. Following delivery, the child demonstrated a grossly distended abdomen and, upon laparotomy, eleven fetiform masses were found within the peritoneum/retroperitoneum. Of these masses, four had vertebral columns, eight had recognizable limbs or appendages, and three had neither but did have complex tissue architecture. In addition to these fetiform masses, a mature ovarian teratoma was observed. Karyotype revealed 46, XX on each of the analyzed specimens. Interestingly, the family history was significant for twinning.

Conclusions: Since this case represents one of the largest number of fetiform masses described to date, we critically examine the definition of fetus-in-fetu and propose potential mechanisms behind this condition.


Purpose of Study: The purpose of this study was to determine if multiple echogenic foci within the fetal heart were associated with an increased risk of fetal chromosomal aneuploidy as compared to a single focus in our patient population.

Methods Used: During a span of 30 months, all women referred to our institution for obstetrical ultrasound were evaluated prospectively for the presence of echogenic cardiac foci in the fetal heart. Each patient was also evaluated for the presence of other risk factors for aneuploidy including other ultrasound findings, biochemical screening and maternal age. A population of patients with two or more fetal echogenic cardiac foci and a comparable population of patients with single fetal echogenic cardiac foci were then identified and their neonatal outcomes were followed.

Summary of Results: 53 patients with multiple (more than one) fetal echogenic foci were identified. During the time period that these patients were identified, 55 patients with single fetal echogenic foci were also identified for comparison and a control group with normal fetal cardiac findings on ultrasound was also identified to use as a control. Of the 53 patients with multiple fetal echogenic foci there were 5 cases of Trisomy 21, 3 of these cases without other ultrasound abnormalities and in women under 35 years of age. Of the 55 patients with the finding of single fetal echogenic foci, there was one case of Trisomy 21, the patient in this case was 40 years old but without other abnormalities on ultrasound. In the control group there were no cases of Trisomy 21.

Conclusions: Though the significance of echogenic cardiac foci in fetal ultrasound remains controversial, this data suggests that the identification of more than one echogenic cardiac focus may be of greater significance in terms of association with aneuploidy when compared to having a single echogenic cardiac focus which in turn carries greater risk than having no echogenic cardiac focus. This was found to be the case even in the absence of other risk factors such as other sonographic anomalies and advanced maternal age.

278 RACIAL DIFFERENCES IN SOUTHERN NEVADA’S DOWN SYNDROME INCIDENCE AND THE OCCURRENCE OF ASSOCIATED CONGENITAL CARDIAC MALFORMATIONS W.N. Evans1,2, R.I. Acherman1,2, K.T. Kim1,2, and H. Restrepo1,2. 1Children’s Heart Center, Las Vegas, NV and 2University of Nevada, School of Medicine, Las Vegas, NV.

Purpose of Study: To review the incidence of DS and related heart disease in Southern Nevada between July of 2002 and July of 2007 with respect to race

Methods Used: We searched our electronic database for patients with DS with age = 5 years of age. We assigned mother’s race by self report. We tabulated patients in four racial categories: Hispanic, White, Black, and Asian. Mother’s age was calculated from her date of birth and child’s date of birth. We used vital statistics for Clark County, Nevada,
for calculating live births (LB) and racial percentages during the study period. Our program is the sole provider of pediatric cardiology services in Southern Nevada. Primary care providers refer DS patients with and without suspected heart disease for cardiac evaluation. We used chi square for statistical analysis.

**Summary of Results:** There were 147 children with DS, 76 males and 71 females. The live birth incidence of DS varied by race with a significant difference between the highest and lowest incidence (p = 0.03). Maternal mean age was similar between groups. The percentage of congenital cardiac abnormalities was significantly lower in Asians compared to other races (p = 0.003). We lacked data on pregnancy terminations.

**Conclusions:** In Southern Nevada, the live birth occurrence of DS and associated congenital heart disease varied by race. The incidence of DS was higher in Hispanics and Asians when compared with whites and blacks. Heart disease occurrence was similar in Hispanics, Whites, and Blacks but less in Asians, but mean maternal age was similar between races.

<table>
<thead>
<tr>
<th>Item</th>
<th>Hispanic</th>
<th>White</th>
<th>Asian</th>
<th>Black</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mother’s age in years (mean ± s.d.)</td>
<td>31.8 ± 7.0</td>
<td>32.2 ± 8.0</td>
<td>34.6 ± 7.2</td>
<td>35.6 ± 9.5</td>
</tr>
<tr>
<td>Prevalence of DS per 1,000 LB</td>
<td>1.3</td>
<td>0.8</td>
<td>1.1</td>
<td>0.6</td>
</tr>
<tr>
<td>Percentage of LB by race</td>
<td>39%</td>
<td>43%</td>
<td>8%</td>
<td>10%</td>
</tr>
<tr>
<td>Percentage of DS by race</td>
<td>50%</td>
<td>35%</td>
<td>8%</td>
<td>6%</td>
</tr>
<tr>
<td>Percentage of DS with cardiac disease</td>
<td>62%</td>
<td>73%</td>
<td>17%</td>
<td>67%</td>
</tr>
</tbody>
</table>

### 279

**INBORN ERROR OF METABOLISM PRESENTING AS MALFORMATION SYNDROME: HYDROCEPHALUS AS A MANIFESTATION OF COBALAMIN D DISEASE**

J.A. Bernstein1, C. Lee-Messer2, K. Cusmano3, D.S. Rosenblatt1, T. Cowan4, and G.M. Enns1. 1Stanford University, Stanford, CA; 2Stanford University, Stanford, CA and 3McGill University, Montreal, QC, Canada.

**Purpose of Study:** Hydrocephalus occurs in approximately 1 in 2,000 live births. Recognized causes include infection, vascular events, and central nervous system malformations. We recently diagnosed cobalamin D (cblD) disease in a 4-month-old girl with hydrocephalus, developmental delay and seizures. CblD disease is a rare, potentially treatable disorder of vitamin B12 metabolism associated with developmental delay and neurologic signs. Hydrocephalus has not previously been reported in cblD disease, although it has been observed in the related conditions of cblC disease and methylene tetrahydrofolate reductase (MTHFR) deficiency. Through this case report we demonstrate that cblD disease in addition to related metabolic disorders can result in hydrocephalus.

**Methods Used:** This study is based on review of case records and related literature.

**Summary of Results:** A 4-month-old girl presented with hydrocephalus, developmental delay, seizures and anteriorly displaced anus. Laboratory evaluation demonstrated moderately elevated plasma homocysteine and low methionine. Methylmalonic acid (MMA) was not detected in urine. These findings suggested the diagnoses of cblE/bialk disease or MTHFR deficiency. Hospital course was complicated by inferior vena cava thrombosis. After initiation of cofactor therapy a decrease in plasma homocysteine and normalization of methionine levels was observed. Complementation studies of cultured fibroblasts revealed a definitive diagnosis of cblD variant-1 disease.

**Conclusions:** We report hydrocephalus as a novel manifestation of cblD disease. Hydrocephalus has also been observed in related disorders of B12 and folate metabolism. Thus, we recommend measurement of plasma homocysteine to screen for these conditions in patients with unexplained tetraventricular hydrocephalus, especially if accompanied by developmental delay. Diagnosis of cblD disease or a related recessive disorder allows for accurate counseling of recurrence risk. Additionally, treatment may improve clinical outcome.

### 280

**MYCOPHENOLATE MOFETIL AS A TERATOGEN**

P.Y. Jackson1,2, L. Paquette1,2, R. Ramanathan1,2, and I. Seri1,2. 1LAC-USC, Los Angeles, CA and 2Children’s Hospital, Los Angeles, CA.

**Purpose of Study:** We report a term infant with multiple congenital anomalies possibly due to mycophenolate mofetil alone or in combination with other immunosuppressive medications.

**Methods Used:** Detailed review of the patient’s chart, maternal history, and a literature search for other similar patients.

**Summary of Results:** The teratogenic potential of new generation immunomodulators, such as mycophenolate mofetil, is not well described. Rare cases of congenital anomalies in infants born to mothers following solid organ transplant and exposed to mycophenolate mofetil have been reported, including patterns of microtia, cleft lip/palate, and digit abnormalities. This patient was born at 35 weeks gestation to a 20-year-old mother 18 months after liver transplant. All prenatal labs were normal. Throughout the pregnancy, mother was treated with mycophenolate mofetil, tacrolimus, and prednisone, in addition to prophylactic antibiotics, prenatal vitamins, and folic acid. Once the pregnancy was discovered, the dose of mycophenolate mofetil was decreased from 1000mg to 500mg BID. At 18 weeks, the fetus was noted to have bilateral cleft lip/palate. At 23 weeks, double-outlet right ventricle with mitral stenosis and moderate hypoplasia of the left ventricle were discovered. Amniocentesis revealed normal chromosomes, 46 XX, and a negative FISH for 22q11.2. Both the complex congenital heart disease and cleft anomalies were confirmed at delivery, in addition to cataracts, microphthalmia, abnormal fingers, fused ribs, microtia, and intestinal malrotation. After birth, high resolution chromosomes, and microarray to rule out hereditary and syndromic causes for the multiple congenital anomalies were noncontributory.

**Conclusions:** This case is consistent with previous reports supporting a mycophenolate mofetil embryopathy. It is impossible to know if the additional anomalies not previously reported for mycophenolate mofetil resulted from embryotoxic effect of other maternal medications or a specific combination or dosing regimen. Counseling about the risks and close surveillance of the fetus during the prenatal period for ophthalmic and auricular anomalies, cleft lip/palate, and cardiac anomalies are necessary in fetuses exposed to mycophenolate mofetil.

### 281

**NEONATAL PRESENTATION OF GLUTARIC ACIDURIA TYPE 1**

K.P. Cusmano-Ozog1, J.R. Waterson2, G.M. Enns1, and T.M. Cowan2. 1Stanford University, Stanford, CA; 2Children’s Hospital & Research Center at Oakland, Oakland, CA and 3Stanford University, Stanford, CA.

**Purpose of Study:** Glutaric aciduria type 1 (GA1) is an autosomal recessive inborn error of metabolism caused by glutaryl-CoA dehydrogenase deficiency. Individuals have normal early growth and development and typically present between 3 and 36 months of age with acute focal striatal necrosis during an intercurrent illness. Unlike other organic acidemias, significant metabolic acidosis does not occur. We describe an unusual presentation of GA1.

**Methods Used:** Retrospective chart review.
Summary of Results: Following a normal birth, a four-day-old male presented to the Emergency Department (ED) with lethargy, respiratory distress and poor feeding. Neonatal exam was normal except for macrocephaly. Physical exam findings in the ED included hypothermia, pallor, severe dehydration and Kussmaul respirations. Laboratory studies revealed a severe metabolic acidosis with an increased anion gap and ketonuria. Ammonia, lactate and glucose were normal. Plasma free and total carnitine were low with elevated glutaryl carnitine on acylcarnitine profile. Urine organic acids showed an abnormal excretion of glutaric, glutaconic, and 3-hydroxyglutaric acids with massive ketonuria. The newborn screen results were then called out as being positive for GA1.

Additional studies included serum glutaric and 3-hydroxyglutaric acids and urine glutaryl carnitine, all of which were elevated. MRI of the brain revealed classic findings of GA1 including open opercula, fronto-temporal hypoplasia, diffuse white matter changes with hyperintensity of the putamen, and subdural hemorrhage. The patient was started on carnitine supplementation and Glutarex-1 formula. He recovered and was discharged home; however, long term prognosis is uncertain.

Conclusions: Patients with GA1 can present in the neonatal period, even before the results of the newborn screen are known. Macrocephaly may be the only presenting sign in an otherwise healthy neonate. Sialal necrosis can occur in the neonatal period and without an obvious trigger. During an acute episode, severe metabolic acidosis and ketonuria may be seen. GA1 should be considered in any individual presenting with acute metabolic decompensation.

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CROUZON SYNDROME WITH ACANThOSIS NIGRICANS IN TWO GENERATIONS: CASE REPORT OF MOTHER AND PERNATALLY DIAGNOSED SON

K. Zakarian. UCI, Glendale, CA.

Purpose of Study: We offer a review of the current literature on the genetics of Crouzon-acanthosis and detail a case report involving a mother and son with Crouzon-acanthosis. Second semester prenatal ultrasound diagnosed the condition of the fetus in a mother we tested positive for the known Ala 391Glu mutation.

Methods Used: Medline was used to perform a review of current literature on Crouzon syndrome with acanthosis nigricans and two cases of this rare condition are reviewed, in a mother and son, including positive gene mutation testing, prenatal ultrasound positive for Kleeblattschadel, or “clover leaf” craniosynostosis in son as well as imaging with 3-D head CT of skull in son.

Summary of Results: A 20 year-old female with Crouzon-acanthosis phenotype features including cloital astasia, curved nose, high-arched palate, irregular dentition and periorbital and perioral acanthosis was referred to prenatal clinic for genetic counseling after ultrasound at 24/37 weeks gestation showed abnormal head shape. Her medical history included coronal craniosynostosis and cloital astasia requiring VP shunting and foreificial advance surgery as well as permanent tracheostomy respectively.

Mother’s sequence analysis of FGFR3 (exon 10) which is 99% sensitive for detecting mutations in Crouzon-acanthosis, confirmed the A391E mutation. Her son was born vaginally at 37 4/7 wk gestation. He was intubated secondary to severe cloital astasia and had early tracheostomy placement. He had bilateral ptosis, hypertelorism and a Kleeblattschadel, or clover-leaf skull associated with widely patent metopic sutures anterior sagittal and Mendozal sutures. CT scan demonstrated a non-communicating hydrocephalus for which he had shunt placement and a tracheostomy for severe cloital astasia.

Conclusions: Crouzon-acanthosis is seen in 5% of patients with Crouzonoid phenotype. This subtype also has acanthosis nigricans changes as well cloital astasia, jaw cementomas and hydrocephalus. Often it is associated with pansynostosis leading to Kleeblattschadel. Prenatally, Kleeblattschadel can be seen in 2nd trimester. This craniosynostosis is associated with Chiari malformation in 100% of cases, mainly secondary to jugular foramen stenosis. Nearly half of patients will also have cloital astasia and require tracheostomy placement.

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Concurrent Session
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Friday, February 1, 2008

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INTRAUTERINE GROWTH RESTRICTION ALTERS TISSUE NECROSIS FACTOR ALPHA LIGAND AND RECEPTOR EXPRESSION IN A GENDER DEPENDENT MANNER


Purpose of Study: Intrauterine growth restriction (IUGR) predisposes towards neurological morbidities in both humans and rats, with males experiencing worse outcomes. IUGR and neurological morbidities have been correlated with perinatal inflammatory status and elevated serum Tissue Necrosis Factor Alpha (TNFα). Despite the extent of neurological morbidity, little is known about the effect of IUGR on expression of TNFα and its receptors in the brain. We hypothesize that IUGR will significantly increase mRNA levels of TNFα and its key receptors in the male IUGR rat brain.

Methods Used: IUGR conditions were achieved by bilateral uterine artery ligation in pregnant rats on day e19, with brain collection from IUGR and sham pups on day 0 of life. Whole organ mRNA was extracted and assessed for levels of genes TNFα, TNF Receptor1 (R1) and TNF Receptor2 (R2) via real time polymerase chain reaction.

Summary of Results: We first compared sham females to sham males and found that sham females had lower TNFα and R1 mRNA levels and equal R2 levels. While IUGR decreased mRNA levels of TNFα, R1 and R2 in IUGR female compared to sham female, it increased mRNA levels of TNFα and R2 in the IUGR male compared to sham.

Conclusions: We conclude that IUGR increases mRNA of TNFα ligand and Receptor 2 in the brain of male rats, and a decreases them in the female. Our findings are consistent with previously published data on systemic TNFα system and correspond to known gender biased neurologic morbidities in both rat and human IUGRs. The surprising finding is the down-regulation of mRNA in the IUGR female, despite the already lowered baseline in female shams. We speculate that factors specific to the female brain regulate the TNFα mRNA levels in a gender specific manner, which thereby dampen the consequences of IUGR.

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SHH IS NECESSARY FOR LIMB REGENERATION

L.N. Bautista1, N. Mishima1, and K.C. Oberg1,2. 1Loma Linda University, Loma Linda, CA and 2Loma Linda University, Loma Linda, CA.

Purpose of Study: The Shh-Fgf feedback loop is important for patterned limb outgrowth. Shh emanating from the ZPA in the posterior limb bud up-regulates Fgfs in the apical ectodermal ridge (AER). Fgfs secreted from the posterior AER maintain the expression of Shh in the posterior distal aspect of the limb bud. However, it is not clear how this feedback loop works during limb regeneration. Amputation of a chick
825 INTRAUTERINE GROWTH RESTRICTION AFFECTS NEUREGULIN-1 MEDITATED NEURONAL DIFFERENTIATION IN THE HIPPOCAMPUS


Purpose of Study: The overall objective is to understand neural stem cell (NSC) biology in face of the prenatal insult of uteroplacental insufficiency (UPI). UPI with resultant intrauterine growth restriction (IUGR) predisposes human and rat brains towards hippocampal injury. IUGR male rats perform poorly on hippocampal memory tasks compared to controls. The mechanisms of injury however are unknown. Neuregulins (NRGs) are regulatory signaling molecules essential for NSC differentiation and migration. We have previously demonstrated that IUGR rat females showed increased whole brain NRG-1 mRNA expression and increased mature neuron numbers in the hippocampus at term. In contrast, IUGR males showed decreased NRG-1 mRNA expression and decreased mature neuron numbers. We hypothesize that whole brain NRG-1 mRNA expression reflects hippocampal NRG-1 localization. We further hypothesize that IUGR males cease differentiating into mature neurons due to decreased NRG-1.

Methods Used: IUGR was produced through UPI by bilateral uterine artery ligation in pregnant Sprague-Dawley rats at E19 (term=21.5 days). Controls were produced by sham surgery. Brains were perfused and fixed at term. Immunofluorescent (IF) quantification of NRG-1, α-III-tubulin (neuronal progenitors) was done in CA1, CA3, and dentate gyrus (DG) of the hippocampus of both sexes (n=2/group) using confocal microscopy.

Summary of Results: IUGR females showed increased NRG-1 mRNA expression in all hippocampal regions consistent with mRNA expression. They also showed increased α-III-tubulin staining in similar regions denoting increased neuroblast migration. IUGR males however exhibited no change in hippocampal NRG-1 IF in all hippocampal regions consistent with mRNA expression. They also showed increased doubledtalin staining in similar regions denoting increased neuronal progenitor migration. IUGR males however exhibited no change in hippocampal NRG-1 IF despite a decrease in mRNA expression but showed increased neuronal progenitors as evidenced by increased βIII-tubulin staining.

Conclusions: IUGR females respond to UPI by upregulating NRG-1 leading to enhanced neuronal migration to the hippocampus, possibly explaining our previous finding of increased neuron numbers. By comparison, IUGR males retain more immature neurons in the absence of NRG-1, possibly resulting in decreased neuron numbers. We speculate that the difference in hippocampal neuronal phenotypes in the two sexes after UPI may depend on NRG-1’s role in neuronal differentiation and migration.

826 EFFICACY OF CHROMIUM MESOPORPHYRIN IN INHIBITING HEME OXYGENASE ACTIVITY IN HEME-LOADED NEWBORN MICE


Purpose of Study: Heme oxygenase (HO) catalyzes the degradation of heme to produce bilirubin. Because excess bilirubin production due to increased heme loads (hemolysis) can lead to neonatal jaundice, use of metalloporphyrins (Mps), HO-inhibitors, may be an ideal treatment for its prevention. We have shown that the Mp, tin mesoporphyrin (SnMP), is a potent HO inhibitor, however, it is photoactive and can induce HO-1 transcriptional activity, thereby negating its clinical utility. Our objective was to investigate the efficacy of an alternative Mp, chromium mesoporphyrin (CrMP), in inhibiting in vivo HO enzyme activity after heme loading in newborn mice, a model analogous to that of a hemolytic infant.

Methods Used: 7-d-old mice were given 30-μmol heme (H)/kg or vehicle (V) by subcutaneous (SQ) injections on Day 1. On Day 2, mice were given 30, 15, or 7.5 μmol CrMP/kg (CrMP30, CrMP15, or CrMP7.5) or V orally. On Day 3, V or a 2nd H load was given SQ. On Day 4, mice were sacrificed. Liver and brain were harvested for HO activity measurements.

Summary of Results: %HO activity left (mean ± SD) in liver and brain tissues (n=3 per tissue) compared to control (VVV) tissues is shown in the Table below. After the 2nd heme load (HVH), liver HO activity was induced significantly (54%). In the HCr 30H group, heme-induced liver HO activity was abolished and significantly inhibited to 46% of VVV levels. In the HCr 15H group, heme-induced liver HO activity was also eliminated, but only to baseline levels. In the HCr 7H group, heme-induced liver HO activity was only partially eliminated (57%) and significantly higher than VVV levels. In contrast, brain HO activity was not induced by successive heme loading nor affected by CrMP.

Conclusions: These findings show that after the 2nd heme load, all doses of CrMP were effective in inhibiting heme-induced increases in liver HO activity, but not in the brain. We conclude that low doses of CrMP exerts long-term inhibition of liver HO activity in the context of repeated heme loads. Thus, CrMP may be ideal alternative compounds for use in the treatment of neonatal jaundice caused by hemolytic disease.

<table>
<thead>
<tr>
<th>Group</th>
<th>VVV</th>
<th>HVH</th>
<th>HCr30H</th>
<th>HCr15H</th>
<th>HCr7H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>100±0%</td>
<td>154±25%*</td>
<td>54±22%*</td>
<td>105±12%*</td>
<td>123±19%*</td>
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<tr>
<td>Brain</td>
<td>100±0%</td>
<td>99±12%</td>
<td>97±11%</td>
<td>103±22%</td>
<td>92±9%</td>
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</table>

827 MECHANISM OF NICOTINE-INDUCED UP-REGULATION OF WINGLESS/INT (Wnt) SIGNALING IN HUMAN ALVEOLAR INTERSTITIAL FIBROBLASTS (AIFs)

L.M. Cerny, R. Sakurai, Y. Wang, P. Guo, J.S. Torday, and V.K. Rehan. Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, CA.
Purpose of Study: Nicotine exposure alters the normal homeostatic pulmonary epithelial-mesenchymal paracrine signaling pathways, resulting in ALI-to-myofibroblast (MYF) transdifferentiation. Since the ALI is a frequent cause of adult respiratory distress syndrome (ARDS), a better understanding of the pathobiology of pulmonary mesenchymal-epithelial signaling is important.

Methods Used: Adult male rats were exposed to 250 ppiottometrically identified mesenchymal-epithelial (ME) clusters were mechanically isolated and cultured in vitro. The effect of nicotine on ALI signaling was assessed using qRT-PCR, Western blotting, and functional assays.

Summary of Results: Nicotine exposure significantly decreased ME cluster proliferation and increased ME cluster apoptosis. These changes were associated with alterations in the expression of ME cluster markers, including the ME cluster transcription factor Sia1rl1.

Conclusions: Nicotine exposure alters the normal homeostatic pulmonary epithelial-mesenchymal paracrine signaling pathways, resulting in ALI-to-myofibroblast (MYF) transdifferentiation. This up-regulation of certain ALI signaling is accompanied by increased ME cluster apoptosis, which may play a role in the development of ARDS.

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LATE PRETERM INFANTS PROCESS AUDITORY STIMULI DIFFERENTLY THAN FULL-TERM INFANTS

J.E. Barthelet1,2, C.T. Worwa2, J.M. Therien1,2, and R.O. deRegnier3.

1 Univ. of MN, Mpls, MN; 2Children’s Hosp. & Clinics of MN, St. Paul, MN and 3Northwestern Univ., Chicago, IL.

Purpose of Study: Despite being the largest subgroup of preterm births, very limited data exists documenting the neurodevelopmental course of late preterm infants who are medically healthy, yet still incur the risks of being born prematurely. Our purpose was to evaluate the effects of late prematurity on the development of auditory recognition memory in healthy newborn infants using event-related potentials (ERPs), a technique commonly used in cognitive neuroscience.

Methods Used: Subjects (n=9) who were late preterm newborns born at 35–37 weeks gestational age tested at <6 days of age compared with 28 healthy, full-term newborns born at 39–41 weeks. ERPs were recorded from 16 standard scalp leads during active sleep while listening to recordings of the word “baby” in the mother’s voice alternating with a female speaker’s voice.

Summary of Results: Compared with full-term (FT) newborns, late preterm (PT) infant ERPs demonstrated a significant difference in auditory processing of mother’s and stranger’s voices over the left hemisphere leads (central [C3], frontal [F3], and parietal [P3]). At 1200–2000 msec after the stimulus presentation, the mean ERP amplitudes for PT infants were significantly larger than those elicited by FT infants (Table).

Conclusions: Our findings demonstrate that the last few weeks of pregnancy are associated with significant neural development in the auditory processing pathways, particularly over the left hemisphere. It is not known whether premature exposure to postnatal experiences may have an impact on the development of auditory processing. Further research investigating the effects of postconceptional age and postnatal experience will be useful in understanding interactions between experience and brain development in premature infants.

<table>
<thead>
<tr>
<th>Lead</th>
<th>Mean Amplitude (microvolts)</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3</td>
<td>PT: 5.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>FT: 1.389</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F3</td>
<td>PT: 2.778</td>
<td>1.66</td>
<td>0.0045</td>
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<tr>
<td></td>
<td>FT: 2.696</td>
<td>0.915</td>
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<td>P5</td>
<td>PT: 7.111</td>
<td>2.249</td>
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<tr>
<td></td>
<td>FT: 0.179</td>
<td>0.090</td>
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290 MODIFIABLE SIDS RISK FACTORS AND SIDS IN TORAJA, INDONESIA

T.T. Hoppenbrouwers1, S. Sandarupa2, J. Hodgman1, and T.G. Keens1.
1Childrens Hospital Los Angeles; Women's and Childrens Hospital LAC+USC Medical Center, Los Angeles, CA and 2Hasanuddin University, Makassar, Indonesia.

Purpose of Study: Two assumptions tend to explain the absence of SIDS research in developing countries: SIDS would be unrecognized by medical personnel. SIDS would be overwhelmed by other causes of post-neonatal deaths. Objectives: (a) quantify modifiable SIDS risk factors in a province of a developing country. (b) Explore the incidence of SIDS.

Methods Used: A survey of 250 mothers with an infant between 1 week and 6 months of age was performed in their homes in Toraja, a rural region of Indonesia. Mothers were drawn from diverse geographic locations and were representative of the local population in educational level. Public health records were scrutinized for incidence of neonatal and post-neonatal deaths.

Summary of Results: With the exception of the controversial bed-sharing, modifiable risk factors for SIDS recognized in the West are favorable for Torajan babies. Preterm infants <2500 gms are not viable and even those >2250 gms may not survive. Postneonatal mortality from prematurity dominated. Some deaths from prematurity could have in fact been SIDS.

Conclusions: In light of a comparable post-neonatal mortality, the inevitable danger of co-sleeping should be questioned. Whereas this rural area in a developing country exhibited only a very low post-neonatal mortality, the completeness of these births /deaths records should be examined further. Autopsies are not routinely performed but SIDS could well be rare. If confirmed, Toraja could provide a unique opportunity to study the role of modifiable risk factors.

291 DEVELOPMENT OF SMAD3 (−/−) ALVEOLAR TYPE II CELL LINES

C.G. Ramos, C. Tiozzo, and P. Minoo. University of Southern California, Keck School of Medicine, Los Angeles, CA.

Purpose of Study: TGF-β is a multifunctional signaling molecule with established roles in normal development and pathogenesis of disease. We have shown that bioactive TGF-β in the lungs of preterm neonates predicts bronchopulmonary dysplasia. Intracellular TGF-β signaling is mediated via the activation of second-messenger Smad proteins. We have shown that Smad3 mediates the inhibitory effect of TGF-β on surfactant protein B, SP-B gene transcription in lung epithelial cells. There are currently no epithelial cell lines that could be used for analysis of Smad3-dependent TGF-β signaling.

Methods Used: In this study, we developed SV40 immortalized Smad 3(−/−) alveolar type II cell lines and characterized their phenotype. The cells were generated by selective mating between Smad3(−/−) and SpC-SV40 transgenic mice. Subsequently tumors were isolated from the lungs of double transgenic mice from which viable immortalized type II cell clones were derived. The cell clones were characterized by RT-PCR and Western Blots.

Summary of Results: Smad3(−/−) type II cells express all three SP genes and proteins plus AQPS. TGF-β responsiveness in the absence of Smad3 is not eliminated, but blunted as evidenced by decreased PAI-response.

Conclusions: The Smad3-deficient immortalized mouse alveolar type II cell lines may provide a novel experimental model system in which to study Smad3-dependent TGF-β-responsive gene regulation. For example, gene array analysis may distinguish specific differences between Smad2 vs. Smad3 target genes in the lung. Further characterization of potential interactions between the TGF-β pathway and other intracellular pathways, such as the Wnt signaling are also currently ongoing. Supported by NHLBI and the Hastings Foundation.

292 A POSITIVE FAMILY HISTORY OF DYSLIPIDEMIA NEGATIVELY IMPACTS THE SUCCESS OF LIFE-STYLE INTERVENTION

K.T. Kip1,2, W.N. Evans1,2, G.A. Mayman1,2, R.J. Acherman1,2, and H. Restrepo1,2. 1Children’s Heart Center, Las Vegas, NV and 2University of Nevada, School of Medicine, Las Vegas, NV.

Purpose of Study: To explore the relationship between positive and negative family histories of hypercholesterolemia (+FHH and −FHH) and lipid profiles in obese children referred to a lifestyle-intervention program.

Methods Used: We analyzed data from 397 children referred to our program between March 2003 and April 2007. All patients had BMIs = 95th percentile. We drew initial fasting triglycerides, total cholesterol, LDL, and HDL levels. We considered a +FHH when one or more first or second degree relative was reported in the family questionnaire. We analyzed data with the t and the chi-square tests.

Summary of Results: We recorded 207 patients with a +FHH and 190 patients with a −FHH that completed our 12-week program. There were no racial differences between the groups. The +FHH group had 102 females and 105 males with a mean age of 11.3 years (range 6–17 years). The −FHH group had 85 females and 105 males with mean age of 11.4 years (range 6–18 years). Initial total cholesterol and LDL levels in the +FHH group were significantly higher than in the −FHH group. After 12 weeks, both groups showed significant decreases in BMIs. There was no significant change in the lipid panel in the +FHH group, but the −FHH group showed a significant decrease in total cholesterol and triglycerides (table summarizes results). The chance to have hypercholesterolemia was higher when there was a +FHH (relative risk: 1.4, p = 0.001).

Conclusions: Lipid profiles in obese children with +FHH were more abnormal than those with −FHH. Following lifestyle modifications, those with −FHH showed a greater increase in their cholesterol values than those with +FHH, suggesting that adjunct pharmacologic therapy may be required in +FHH obese children to more effectively reduce dyslipidemia and secondarily the risk of atherosclerosis.

<table>
<thead>
<tr>
<th></th>
<th>Positive Family History</th>
<th>Negative Family History</th>
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<tr>
<td>BMI Z score</td>
<td>Week 1</td>
<td>Week 12</td>
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<td></td>
<td>p</td>
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<td>2.28 ± 0.29</td>
<td>2.23 ± 0.32</td>
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<tr>
<td></td>
<td>&lt; 0.01</td>
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<tr>
<td>Cholesterol mg/dL</td>
<td>170 ± 10</td>
<td>160 ± 12</td>
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<tr>
<td></td>
<td>0.02</td>
<td>0.02</td>
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<tr>
<td>Triglycerides mg/dL</td>
<td>158 ± 9.68</td>
<td>139 ± 8.66</td>
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<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
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<tr>
<td>HDL mg/dL</td>
<td>45 ± 10</td>
<td>45 ± 13</td>
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<td>N.S</td>
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<tr>
<td>LDL mg/dL</td>
<td>106 ± 34</td>
<td>107 ± 29</td>
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<td></td>
<td>0.14</td>
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Supported by NHLBI and the Hastings Foundation.
Conclusions: 
Years ago by retrotransposition of a copy of chromosome 22qter. 
indicates that the RPL23A7 pseudogene arose approximately 3.5 million 
cancers. Preliminary phylogenetic analysis with related sequences 
The RPL23A7 pseudogene is conspicuous by its expression in proliferat-
tional elements which may also drive an adjacent Ras-related oncogene.

Methods Used: 
Comparisons were made using the human and chimpanzee assemblies (March 2006) at the UCSC Genome Browser. BLAST analysis was carried out at the NCBI website. Motif and Pfam analyses were performed at the SwissProt website.

Summary of Results: 
The peritelomeric region of the chimpanzee chromosome encodes a phospholipase A2 (PtChr2a-PLA2) paralogous to that of human PLA2G4 on human chromosome 15, however no ortholog of PtChr2a-PLA2 was detected in the syntenic region of human chromosome 2. In addition, human chromosome 2 has a nucleotide sequence in the vicinity of the telomere fusion site that encodes a 60S ribosomal protein (RPL23) pseudogene (RPL23A7) that is absent in the syntenic regions of both chimpanzee and rhesus chromosomes. A region 5' to the RPL23A7 pseudogene comprises potential GC-rich transcriptional elements which may also drive an adjacent Ras-related oncogene. The RPL23A7 pseudogene is conspicuous by its expression in proliferating cells and tissue, including human fetal brain and a range of human cancers. Preliminary phylogenetic analysis with related sequences indicates that the RPL23A7 pseudogene arose approximately 3.5 million years ago by retrotransposition of a copy of chromosome 22qter.

Conclusions: 
Phospholipase A2 expression regulates growth and differentiation via synthesis of eicosanoids. The deletion of a phospholipase A2 and absence of its expression in early hominids during embryonic development may have delayed cessation of neural crest cell migration and proliferation resulting in a more cranial tissue in the fetus. Elevated levels of transcript for ribosomal protein L23 (RPL23) in hippocampus and proliferation resulting in a more cranial tissue in the fetus. Elevated levels of transcript for ribosomal protein L23 (RPL23) in hippocampus from post-coital day 18 (E18) rat have been observed (Kaser (2000) J. Invest. Med., 48(1): 282A). A novel mechanism of action by pseudogenes that mediates gene expression is proposed than may, in turn, have propelled neural crest cell proliferation and migration with similar consequences.

Nephrology and Hypertension 
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THE PREVALENCE OF DIABETES 20 YEARS FOLLOWING EXTRACORPOREAL SHOCKWAVE LITHOTRIPSY

Purpose of Study: SWL has become a mainstay in the treatment of kidney stones. It has been proposed that patients undergoing SWL have a higher risk of developing diabetes. We determined the prevalence of diabetes in patients who underwent SWL 20 years ago. The study rate was compared to background provincial rates.

Methods Used: 357 patients who had undergone SWL between 1985 and 1987 were identified from our database at Vancouver General Hospital. A telephone survey was conducted and patients were questioned on whether they had developed diabetes since SWL. Information was gathered regarding BMI, hypertension, smoking, recurrent stone disease, and family history of diabetes. It was difficult to identify patients diagnosed with kidney stones who did not have SWL during the study period and who have never had subsequent SWL; therefore, the prevalence of diabetes in our study group was compared to the provincial prevalence as reported by the BC Ministry of Health Services in 2002.

Summary of Results: Of the 357 contact letters mailed, 247 were ineligible due to: deceased (18), incorrect address (104), unable to reach (82), refused/unable to consent (11), other (12). 130 patients completed the telephone questionnaire (36.4% response rate). 4 were excluded for diagnosis of diabetes prior to SWL. The median age was 67.4 year, and the median BMI was 26.7 kg/m². Background BMI and smoking rates for this age group in BC are comparable with the study group. The overall prevalence of diabetes in the study group was 25.4%. Males had a prevalence of diabetes of 28.7% (25/87) and females 17.9% (7/39). The provincial prevalence of diabetes for this age group is 12–18% for men and 9–15% for women.

Conclusions: There is an elevated prevalence of diabetes in male patients who had undergone SWL 20 years previously. The shortcomings of this study are that proper controls were difficult to obtain, and it would be premature to conclude that SWL alone causes this increased prevalence. Part may be attributable to suggestions that people with stone disease have a higher risk of diabetes due to a common precipitating metabolic derangement. The observation that patients have a higher prevalence of diabetes 20 years following SWL remains to be completely explained, but this study supports that an association exists.
13C-alanine or its incorporation into protein in comparison above those levels in spermine only group.

**Conclusions:** The premix-F1 and F2 (glutathione precursors with selenium) are promising anti-oxidants in preventing uremic-toxin-induced apoptosis. Restoration of protein metabolism may be one of the mechanisms by which F1 formulation protects spermine-induced smooth muscle from cell death, and supports an enhanced efficacy of synthesizing and restoring glutathione, also surpassing a cysteine compound (NAC), with the more effective cystine, a physiologically stable cysteine carrier. We speculate that F2 possibly prevents apoptosis through a different metabolic pathway.

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**RHO GTPASES, ACTIN DEPOLYMERIZING PROTEIN COFILIN AND P38 MITOGEN ACTIVATED PROTEIN KINASE IN DIABETIC NEPHROPATHY (DN)**

M. Chamberlin\(^1\), T. Dai\(^1\), I. Jayaratne\(^1\), J. LaPage\(^3\), R. Natarajan\(^2\), C. Nast\(^1\), and S. Adler\(^1\). \(^1\)Los Angeles Biomedical Research Institute, Torrance, CA; \(^2\)Beckman Research Institute/City of Hope, Duarte, CA and \(^3\)Cedars Sinai Medical Center, Los Angeles, CA.

**Purpose of Study:** Albuminuria is associated with podocyte (pod) effacement and actin cytoskeleton (ACSKN) disruption. We assessed relationships between RhoA, downstream cofilin, p38 MAPK and phospho-p38 MAPK (pp38),and GAPDH were measured by Western blot. Active RhoA was measured using a Rhotekin-Rho-GTP binding/GTP-RhoA complex pull-down assay; and monomeric (G) and total actin by the DNase1 inhibition assay.

**Summary of Results:** RhoA activity increased 3-fold at 1 wk (p = 0.01) in DM vs C, decreased to C at 4 mos (NS). In pod activated RhoA increased 4-fold in HG (p = 0.03); this was suppressed in the presence of C3 exo (p = 0.03). RhoA activation in HG was associated with increased p-cofilin (the inactive form) compared to LG (p = 0.01) and was inhibited by C3 exo (p = 0.004). pp38 MAPK increased 1.5 fold in HG compared to LG. The activity reverted to C value in the presence of C3 exo (p < 0.05). Glom F/G actin was stable at 1 wk (NS) and disrupted at 4 mos (p < 0.05). In vitro, F/G actin was increased in HG conditions and the increment abrogated in the presence of C3 exo (p < 0.05).

**Conclusions:** 1)RhoA is activated in the presence of HG; 2)Cofilin is inactivated by phosphorylation in HG; 3) p38 MAPK is phosphorylated in HG conditions and is blocked by RhoA inhibitor 4)ACSKN shows preservation early in vivo and in vitro in HG in association with Rho activation and downstream phosphorylation of cofilin. RhoA inactivation, downstream cofilin activation, and disruption of ACSKN, occurs late. The results suggest for the first time, a role for pod RhoA/cofilin signaling in mediating HG-induced podocyte ACSKN regulation. pp38 MAPK is activated by RhoA, at least in part.

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**MAMMALLIAN TARGET OF RAPAMYCIN (mTOR) SIGNALING AND APOPTOSIS IN POLYCYSTIC KIDNEY DISEASE (PKD)**

C.L. Edelstein\(^1\), Y. Tao\(^2\), I. Zafar\(^1\), and R.W. Schrier\(^1\). \(^1\)UCHSC, Denver, CO and \(^2\)Texas Tech University HSC, Amarillo, TX.

**Purpose of Study:** The aim of the present study was to investigate mTOR signaling and apoptosis in PKD.

**Methods Used:** An ELISA kit for free IGF-1 was used. Akt was immunoprecipitated using a specific antibody and then an in vitro kinase assay was performed using GSK-3 fusion protein as a substrate. TUNEL-positive apoptotic cells with condensed pyknotic nuclei or apoptotic bodies were quantitated.

**Summary of Results:** IGF-1 is known to activate the mTOR pathway via stimulation of Akt kinase. Free IGF-1, the biologically active form of IGF-1, was measured in cyst fluid from 14 ADPKD patients. Free IGF-1 levels were high in cyst fluid: 1710 ± 119 pg/mL. Experiments were next performed in kidneys from 8 w old heterozygous Han:SPRD rats with PKD (Cy/+ ) treated with rapamycin 0.2 mg/kg IP or vehicle vs. normal littermate controls (+/+). On immunoblot analysis of whole kidney there was a 50% increase in IGF-1Rα in Cy/+ vs.+/+ (n = 3).

**Conclusions:** End-stage renal disease (ESRD) is associated with severe alterations of T and B lymphocyte compartments and increased susceptibility to infections. We recently reported that naive and central memory, but not effector memory CD4+ and CD8+ T cells, were depleted in ESRD patients (Kidney Int 70: 371–376, 2006). Earlier studies have documented significant reduction in peripheral blood B cell count in ESRD. However, the effect of ESRD on B cell subpopulations has not been studied previously.
Conclusions: decreases p70S6 kinase activity, caspase-3 and apoptosis. In Cy/+ and 0.1 in Cy/+ treated with rapamycin (P = 0.06 vs. Cy/+). In summary, in PKD kidneys there are high levels of IGF-1 in cyst fluid, increased Akt kinase activity, P70S6K activity and protein. Rapamycin decreases P70S6K activity, caspase-3 and apoptosis.

Conclusions: In conclusion, the signaling pathways that connect mTOR signaling with apoptosis in PKD merit further study.

299 ANTIOXIDANT THERAPY IMPROVES ANTIOXIDANT ACTIVITY OF HDL WITHOUT AFFECTING OXIDATIVE STRESS AND INFLAMMATION IN PATIENTS WITH END STAGE RENAL DISEASE (ESRD)

H. Moradi, R. Elahimehr, M. Kamgar, M. Pahl, and N. Vaziri. University of California, Irvine, Orange, CA.

Purpose of Study: ESRD is associated with oxidative stress, inflammation, reduced HDL concentration/activity and high risk of atherosclerotic cardiovascular disease (CVD). By promoting reverse lipid transport and its potent antioxidant/anti-inflammatory actions, HDL protects against CVD. Oxidative stress and inflammation convert HDL to a pro-oxidant/pro-inflammatory agent. We therefore, explored the effects of antioxidant therapy on markers of oxidative stress and inflammation and redox activity of HDL in ESRD patients.

Methods Used: 37 ESRD patients participated in the study. After a 4-week washout period during which all antioxidant supplements were withheld, patients were randomized to the antioxidant-treated (vitamin E, 800 IU; vitamin C, 250 mg; B6, 100 mg; B12, 250 mcg; and folic acid, 10 mg/day for 8 weeks) or placebo-treated groups. At the conclusion of the double blind phase, placebo-treated patients were switched to active treatment for 8 weeks. Plasma F2-isoprostane, C-reactive protein (CRP), interleukin-6 (IL-6) and Apo-AI concentrations, paraoxonase and glutathione peroxidase activities and HDL redox properties were measured.

Summary of Results: Blood hemoglobin, total leukocyte count, and plasma transferrin saturation, ferritin, calcium, phosphorus, bicarbonate and albumin levels were comparable in the two groups at baseline and remained unaffected by either active or placebo treatment. Similarly, erythropoietin requirement, pre- and post-dialysis blood pressure, inter-dialytic weight gain, and frequency of intra-dialytic hypotension were similar among the study groups at all points. Likewise, F2-isoprostane, CRP, IL-6, Apo-AI as well as paraoxonase and glutathione peroxidase activities were similar in the two groups and were not altered by antioxidant therapy. However, antioxidant activity of HDL, which was poor in ESRD patients, improved significantly in antioxidant-treated group compared to the placebo-treated patients.

Conclusions: While antioxidant therapy does not reverse ESRD-associated oxidative stress and inflammation, it does increase antioxidant properties of HDL. Consequently, the clinical significance of this phenomenon which is likely due to tocopherol enrichment of HDL is uncertain.

301 DOES TACROLIMUS CAUSE SUBCLINICAL NEPHROTOXICITY IN CHILDREN WITH STEROID RESISTANT NEPHROTIC SYNDROME

L. Butani, R. Ramsamoorthy. 1University of California Davis Medical Center, Sacramento, CA and 2University Of California Davis Medical Center, Sacramento, CA.

Purpose of Study: To evaluate the occurrence and risk factors for development of interstitial fibrosis in children receiving tacrolimus (T) for steroid resistant nephrotic syndrome (SRNS).

Methods Used: From 7/95–10/03 11 children with SRNS received T, 10 of whom achieved remission. Follow-up renal biopsies were obtained in 7 of the 10 children who had received T for at least 1 year (median duration 24 months). Biopsies were analyzed in a blinded manner by a single pathologist and scored for tubular atrophy (TA) and interstitial fibrosis (FIB); tissue was stained for transforming growth factor (TGF) β 1, 2 and 3 using a polyclonal antibody by an immunoperoxidase technique. Paired biopsies (pre and post T) were compared for increases in tubular TGF β immunoreactivity and TA/FIB.

Summary of Results: At follow-up 2 patients had increases in both their TA and FIB scores. Four children (57%) had a significant increase in extent of tubular TGF β expression, including the 2 with changes on light microscopy. There was a correlation (r = 0.94, p = 0.002) between change in FIB and TA scores. There was no correlation between weight
adjusted T dose or duration of therapy with change in TA/FIB scores or TGF β staining. The 2 children with increased histologic damage were exposed to a higher mean T dose (0.36 vs. 0.15 mg/kg/day, p = 0.02).

Conclusions: The prolonged use of T, while effective in maintaining remission in children with SRNS, is associated with chronic tubulointerstitial injury, especially at higher doses. Serial renal biopsies are recommended in patients who continue to receive T for an extended period of time. Alternative medications/strategies need to be studied to prevent renal injury in children who are T dependent.

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ATYPICAL HEMOLYTIC UREMIC SYNDROME AND ACUTE LYMPHOBLASTIC LEUKEMIA CAN PRESENT SIMULTANEOUSLY


Purpose of Study: Malignancy related thrombotic microangiopathy (TM) is an uncommon but well documented complication. There are very few case reports describing the association of acute leukemia with TM manifesting as hemolytic uremic syndrome (HUS).

Methods Used: We report a case who presented with both entities simultaneously.

Summary of Results: A 3.5 year old girl presented with fever, hepatosplenomegaly and significant cervical lymphadenopathy. She was found to have anemia, thrombocytopenia, neutropenia, and elevated liver enzymes. The peripheral blood smear was consistent with microangiopathic hemolytic anemia and interestingly also revealed some blast cells. Urinalysis showed microscopic haematuria and proteinuria Bone marrow biopsy showed leukemic infiltration. She was treated initially with dexamethasone only. Over the next two weeks, she developed hyper- tension, nephrotic range proteinuria, and doubling of her serum creatinine. The renal biopsy was consistent with TM. Factor H and Factor I levels were measured and were within normal range. The leukemia responded well to chemotherapy. At one year post diagnosis she is in remission and has a normal urinalysis and blood pressure.

Conclusions: >The present case is quite unique as it had features of both microangiopathic hemolytic anemia (MAHA) and acute leukemia at presentation. Only six cases are reported in the literature of which both microangiopathic hemolytic anemia and interestingly also revealed some blast cells. Urinalysis showed microscopic haematuria and proteinuria Bone marrow biopsy showed leukemic infiltration. She was treated initially with dexamethasone only. Over the next two weeks, she developed hyper-tension, nephrotic range proteinuria, and doubling of her serum creatinine. The renal biopsy was consistent with TM. Factor H and Factor I levels were measured and were within normal range. The leukemia responded well to chemotherapy. At one year post diagnosis she is in remission and has a normal urinalysis and blood pressure.

Conclusions: The prolonged use of T, while effective in maintaining remission in children with SRNS, is associated with chronic tubulointerstitial injury, especially at higher doses. Serial renal biopsies are recommended in patients who continue to receive T for an extended period of time. Alternative medications/strategies need to be studied to prevent renal injury in children who are T dependent.

304
SUPPRESSION OF HOMOQUINOLINIC ACID ENHANCED JUNCTIONAL POTENTIALS BY THE D3 STEREOISOMER OF A CARBOXY DERIVATIVE OF BUCKMINSTERFULLERENE

D.W. Stockwell1, T.D. Vogel1, N. Jahe1, R.N. Friedman1, and L. Dugan2. 1Indiana U School of Med, Indianapolis, IN and 2UC San Diego, San Diego, CA.

Purpose of Study: In Spinal cord injury (SCI), glutamate excitotoxicity can contribute to secondary damage. One way to reduce this excitotoxicity is to block NMDA glutamate receptors. These are present at the dactylopodite opener muscles of crayfish walking limb excitatory neuromuscular junctions (NMJs). Homoquinolinic acid (HQA), an NMDA glutamate receptor agonist, enhances excitatory junctional potentials (EJPs) at these NMJs. The D3 stereosomer of the carboxyderivative of buckminsterfullerene suppresses EJPs in this model. This study examines the effect of D3 on HQA-enhanced EJPs.

Methods Used: The 1st or 2nd walking limb was removed from a crayfish. The merpopodite portion of the limb was dissected to isolate the excitatory axon for the dactylopodite opener muscle. The preparation was bathed in Van Harreveld’s (VH) solution, pH of 7.2 ± 0.1, during the dissection and during the pre- and posttreatment control EJP recordings. A microelectrode was placed in a muscle fiber in a dactylopodite opener

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and the excitatory axon was electrically stimulated to evoke EJPs. We utilized short term facilitation evoked by 10 sec of suprathreshold stimulus at 30 Hz prior to each data collection. A digital oscilloscope averaged 8 traces per second for 10 sec for each data record. After the pretreatment control we replaced the bath (VH) with 50 μM HQA in VH. EJPs were recorded at 10 s, 1 m, 2 m, and 3 m and then the bath was exchanged with 50 μM HQA and 50 μM D3 in VH. EJPs were recorded at 10 s, 1 m, 2 m, and 3 m. Posttreatment control data were then obtained following 3 complete exchanges of the bath with VH.

**Summary of Results:** Data are reported as percent change in EJP amplitude from control. 50 μM HQA enhanced the amplitude of EJPs 40% and the 50 μM HQA, 50 μM D3 combination depressed the amplitude of EJPs 20%. The EJP depression was not completely reversed with post treatment washes. Qualitatively similar results were obtained in 5 additional preps.

**Conclusions:** 50 μM D3 can suppress 50 μM HQA-induced EJP enhancement. This study suggests that D3 may be useful in suppressing excitotoxicity secondary to NMDA receptor activation and confirms NMDA glutamate receptor presence at the crayfish opener muscle NMJ preparation.

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**305 CLINICAL AND PATHOLOGICAL FEATURES IN PEDIATRIC BRAIN DEATH**

J.E. Jocelyn and M. Mathur. Loma Linda University School of Medicine, Loma Linda, CA.

**Purpose of Study:** Guidelines for determining brain death in children are complex and differ with patient age. Current U.S. criteria for determination of brain death are thought to reflect death of the entire brain. However, certain functions such as temperature regulation/neurohormonal control may be preserved in patients even when “brain death” has been determined clinically and confirmed by laboratory studies. We investigated the correlation between the clinical and pathological findings for determining whole brain death.

**Methods Used:** Medical records of children (0–18 years of age in whom brain death was determined) admitted to our Pediatric ICU from 2000–2005 were reviewed for performance of 14 specific examination elements derived from the current guidelines and confirmatory testing. On patients in whom an autopsy was performed, pathological findings were reviewed in order to determine the extent of neuronal cell death and to search for correlations with the clinical findings.

**Summary of Results:** 16 children had clinical and pathological data available for review. Fifteen had 2 brain death exams as recommended in the guidelines. One patient did not have a second exam, apnea test or lab test recorded. Only 25% of the children less than one year and 55% of the older children had age appropriate intervals between exams. Of 14 specific exam elements, 5–10 were completed during exam 1 and 6–12 during exam 2. 3 of 4 patients <1 year had a recommended confirmatory laboratory test. Gross pathology of 4 patients showed “respirator brain”, 15 had edema, 12 had visible hemorrhage and 9 showed signs of herniation. Histological appearance revealed non-uniform areas of edema, necrosis, engorgement, ischemia or hemorrhage in various areas of the brain and brainstem. Absence of cerebral blood flow on radionuclide examination and no spontaneous breaths on apnea testing were reported in 13 patients, however only 7 showed evidence of ischemia or necrosis in the medulla.

**Conclusions:** Despite clinical determination of brain death, brainstem herniation was not found in all patients. The correlation between the clinical basis for determination of brain death and pathological findings appears to be poorly consistent. This correlation should be further studied to determine whether the current clinical whole brain death definition in the United States remains applicable.

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**306 EFFECTS OF NICOTINE ON AUDITORY GATING IN MANIC ILLNESSES**

T. Parker, L. Martin, J. Lins, R. Freedman, and A. Olin. University of Colorado Health Sciences Center School of Medicine, Denver, CO.

**Purpose of Study:** Deficits in the ability to inhibit evoked EEG responses to repetitive auditory stimuli are noted in a variety of mental illnesses. This gating deficit is most markedly noticeable in schizophrenia, however those with a history of mania are also commonly affected. This study is being undertaken to provide further data concerning the effects of bipolar disorder to explore theories of potential underlying mechanisms.

**Methods Used:** Men and women between the ages of 18 and 60, both smokers and nonsmokers, were recruited from the Denver metro area. Diagnosis of either Bipolar Disorder, Type I, with or without psychosis, or Schizoaffective Disorder, Bipolar Type, was confirmed through the Structured Clinical Interview for DSM-IV Diagnoses (SCID) and a detailed life history. Three standardized surveys assessed current symptoms: Beck Depression Inventory, Young Mania Rating Scale, and the Brief Psychiatric Rating Scale. Baseline EEG recordings of the P50 auditory evoked potential was collected. Subjects then chewed either nicotine or placebo gum, and immediately thereafter P50 recordings post drug administration were obtained. Subjects returned at a later date to be tested with the alternate gum, in a session otherwise identical to the first.

**Summary of Results:** It is anticipated that subjects who show a reduced ability to gate P50 responses at baseline will demonstrate a marked improvement in these responses after nicotine administration, over any gain from placebo. Other studies have indicated that increased adrenergic activity causes P50 gating deficits as well. We therefore also predict that nicotine-induced gating improvements will be more significant for those with a greater degree of psychosis history. Specifically, bipolar subjects with psychosis are expected to show greater improvements than those without, and schizoaffective subjects are expected to demonstrate the most significant normalization.

**Conclusions:** Nicotine exposure leading to improvements of deficient P50 gating ability would lend evidence to the theory of a shared cholinergic mechanism. Degree of improvement correlating to degree of mania psychosis would support the involvement of noradrenergic inhibition of interneurons as a secondary etiological pathway in P50 gating deficiencies.

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**307 MILD COGNITIVE IMPAIRMENT AND PET: AN EVIDENCE-BASED APPROACH TO A CASE STUDY**

L.V. Onofrei1, R. Wetherington2, and P.C. Heyn1. 1University of Colorado Health Sciences School of Medicine, Denver, CO and 2University of Colorado at Colorado Springs, Colorado Springs, CO.

**Purpose of Study:** Alzheimer’s disease (AD) is the most common form of dementia and has been associated with diabetes and metabolic syndrome (MS). It is important to detect AD at early stages to allow prompt initiation of therapy and counseling of the patients and family. The aim of the current study is twofold: 1) to systematically analyze the literature to investigate the clinical significance of FDG-PET in the early diagnosis of mild cognitive impairment (MCI), and 2) to examine by a case study design the patterns of hypometabolism found in two older female subjects with metabolic syndrome and subjective memory complaints.

**Methods Used:** Systematic Review Methods: An extensive review of literature has been conducted, using PubMed as the primary search engine. A set of predefined inclusion criteria was used to determine inclusion and analysis of the papers. Case Study Methods: Two older female patients (P1 & P2) were evaluated by a comprehensive battery of neurological, memory, and laboratory tests.
308 INTEGRATION OF AUTOMATION AND DATA MANAGEMENT TO AID IN FMRI ANALYSIS
D. Buxton and J. Brinkley. University of Washington, Seattle, WA.

Purpose of Study: The primary purpose of this project is to create a database of information regarding analyzed functional MRI (fMRI) data. Once created, this database could become part of brain data integration projects such as DXBrain (http://sig.biostr.washington.edu/projects/dxbrain/index.html); thus increasing the utility of the data. The secondary purpose of this project is to improve productivity of fMRI researchers with respect to data analysis. This helps further our primary objective by giving them an incentive to use our software (called fMRI Book Keeper, or fMBK), thus insuring the creation of the database.

Methods Used: Information is recorded at initiation of fMRI image analysis and at each step until completion. The initial information consists of information such as subject demographics and the protocol followed in obtaining the brain scan. It is extracted primarily from the DICOM source images that are normally discarded. Recording each step is done with software wrappers around the analysis software (FSL) that are completely transparent to the researcher.

We created tools to help automate some of the more tedious analysis steps. These tools increase researcher productivity and ensure database creation by encouraging use of fMBK.

Summary of Results: Initial feedback indicates fMBK is useful in increasing researcher productivity. Formal evaluation of efficacy, however, is out of scope for this project at this time. With respect to our primary objective, an XML file is available after fMRI data analysis is completed that can easily be transformed into a database, thus making the data exploitable in larger scale projects.

Conclusions: This project provides a means of increasing researcher productivity while producing a database that records the details of their analysis. This database increases the usefulness of their work by making their data available in other, possibly multi-modal, projects with no extra work required by them.

309 SELF-REPORTED WRITTEN JAPANESE LANGUAGE ABILITY AND RISK FOR ANY DEMENTIA, ALZHEIMER’S DISEASE (AD) AND VASCULAR DEMENTIA (VD): THE HONOLULU ASIA AGING STUDY (HAAS)
K.N. Arani1, L. Gibbons2, L. White3, and P.K. Crane2. 1School of Medicine, Seattle, WA; 2University of Washington School of Medicine, Seattle, WA and 3Pacific Health Research Institute, Honolulu, HI.

Purpose of Study: To evaluate the association between self-reported ability to read and write Japanese and risk of developing any dementia, AD, and VD.

Methods Used: We analyzed HAAS data from 3,734 Japanese-American men born between 1900 and 1919. Self-reported ability to speak and read and write Japanese was measured in midlife. We categorized subjects into those who reported no spoken or written Japanese skills (n = 570), those who reported ability to speak Japanese but no written Japanese ability (n = 1898, reference category for all analyses), and those who reported both spoken and written Japanese ability (n = 1266). We examined three outcomes at their initial evaluation in 1991-1993: any dementia, AD, and VD.

Statistical Analysis: We used separate logistic regression models to determine adjusted relationships between exposure and outcomes.

Summary of Results: After adjusting for such characteristics as APOE, income, and years of education, we did not find differences in risk between those who reported both spoken and written Japanese ability and our reference group for any dementia (OR 1.95, 95% CI 0.72–5.34), AD (Odds Ratio, OR 2.84; 95% CI 0.79–10.22), or VD/OR 0.70, 95% CI 0.35–1.39). We also found no differences in risk between those who reported neither spoken nor written Japanese ability and our reference group for any dementia (OR 0.67, 95% CI 0.28–1.58), AD (OR 0.65, 95% CI 0.17–2.40), or VD (OR 0.65, 95% CI 0.23–1.86).

Conclusions: Our findings are not consistent with the hypothesis that Japanese written language proficiency is associated with prevalent dementia, AD, or VD. Further work is needed to identify specific factors associated with protection from dementia.

310 ONCOGENE ADDICTED MELANOMAS
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Purpose of Study: MEK (mitogen-activated protein kinase) is thought to be a therapeutic target for B-raf mutated melanomas. B-raf mutated melanomas often have an accumulation of oncogenic mutations. This accumulation would normally be dealt with by apoptotic activity. The B-raf mutation, however, produces MEK dependent anti-apoptotic signals. Assays for oncogenic mutations in clinical specimens will help match patients with the most beneficial targeting agent for the individual.

Melanoma can particularly be studied with these assays by targeting tumor specific signaling molecules as most of these cancers carry somatic mutations in the B-raf kinase and preliminary data indicates that most B-raf mutated melanomas exhibit “oncogene addiction”. It has been shown that MEK inhibitor causes regression of human melanoma lung metastases in a xenograft mouse model. Preliminary studies show that MEK inhibition therapy targets cells with ERK activation and does not affect the viability of normal melanocytes or melanoma cells that lack elevated pERK levels. Thus, by discovering the oncogene mutations specific to MEK more efficient treatment modalities may be considered for melanoma cases.

Methods Used: A375 cell lines were cultured and grown to be used for experimentation. The cells were incubated in a DMSO media. Reverse transfection experiments were necessary to label the cells with specific oncogenes. Reverse transfection involves the printing of specific DNA on a printing slide. It was determined, after practicing with blue food dye mixtures, that a 10% gelatin-DNA mixture would give the best results for the experiment as this mixture would provide even printing of DNA. Slides were printed with a 10% food dye-water mixture, 10% food dye-SDS solution mixture,10% food dye-gelatin mixture, and 10% food dye-PBS mixture, respectively.
Summary of Results: The SDS-food dye mixture displayed the best results, however SDS would destroy the DNA in the actual experimentation. Thus, a gelatin-food dye mixture would be the best combination as it provided fairly even printing results and it would not harm DNA. The PBS-food dye mixture did not print as evenly as the gelatin mixture. The water-food dye mixture yielded uneven printing.

Conclusions: More work needs to be performed in order to conclusively identify oncogene addicted melanomas.

311 USE OF AMNIOTIC MEMBRANE TRANSPLANTATION IN TRABECULECTOMY AND AHMED VALVE IMPLANTATION

M. Saedian1 and A.J. Khodabakhsh2, 1The David Geffen School of Medicine at UCLA, Beverly Hills, CA and 2The Beverly Hills Vision Center, Beverly Hills, CA.

Purpose of Study: To describe the use and evaluate the effect of amniotic membrane transplantation for the repair of the ocular surface during trabeculectomy or ahmed valve implantation.

Methods Used: A comprehensive literature review of cases demonstrating the utilization of amniotic membrane transplantation in trabeculectomy and ahmed valve implantation.

Summary of Results: The successful management of glaucoma entails a delicate balance between sufficient anti-inflammatory therapy and proper lowering of intraocular pressure (IOP) to prevent loss of vision. While medical management may suppress the inflammatory process, the intraocular pressure (IOP) may not subsequently become lowered in some cases and surgical intervention becomes necessary. The current surgical options most effective for uncontrolled IOP despite medical therapy are trabeculectomy or a glaucoma drainage device. However, the condition of the conjunctiva in those with ocular surface disease, such as patients with uveitis or burns, not only has been indicated to decrease the efficacy of trabeculectomy, but also deter glaucoma drainage devices from achieving a higher rate of long term results. Amniotic membrane can be utilized as a graft to reconstruct ocular surface. This has been shown to effectively reduce neovascularization, fibrosis and inflammation and lead to higher rates of success through decreased IOP when used during trabeculectomy and ahmed valve implantation.

Conclusions: Amniotic membrane grafting is an effective surgical technique in surgical glaucoma cases with ocular surface disease. The use of amniotic membrane transplantation in trabeculectomy and ahmed valve implantation can significantly lower the intraocular pressure as pathological tissues are removed and replaced with amnion. This technique therefore has the potential to contribute toward decreasing the future detrimental effects of glaucoma.

312 ENSURING SUSTAINABILITY FOR THE BRIGHTER SMILES AFRICA PROGRAM IN UGANDA


Purpose of Study: The purpose of this study is to determine the success of various measures set in place to ensure the sustainability of the “Brighter Smiles Africa Program”.

Methods Used: The implementation of a successful international project requires measures to be set in place in order to ensure continuation and expansion of the project. During the summer of 2007, an interdisciplinary team of seven students from the University of British Columbia (UBC) and several third year dental students from Makerere University collaborated to promote oral health care education for children in rural Uganda. Community involvement via meetings with principals, teachers, parents and elders were discussed and dates and times were suggested. During the pilot project in the summer of 2006, it was established that third year Makerere University dental students would visit the rural communities once a month for 2–3 days to provide supplies and evaluate the progress in each community. UBC students also sought to establish local partners to support the program for example Colgate and The Rotary Club to provide toothbrushes, toothpaste and dental examination kits. Teachers from the four communities, Makerere University dental students and the UBC teams of 2006 and 2007 initiated the establishment of a daily brushing schedule for the children at each school. Data surveys were conducted at each location to provide scientific means of evaluating the progress of each community.

Summary of Results: The dental student-teacher meeting provided positive feedback from the teachers and a community meeting is scheduled for October 2007. Furthermore, the school children brush everyday before lunchtime in the 4 schools as a mandatory activity. Survey data collected showed an improvement in oral health of children that received fluoride the previous year in 2006.

Conclusions: Measures set in place during the 2006 UBC Brighter Smiles Africa Program ensure progress and sustainability. However, further involvement and commitment is required from local Ugandan partners and conduction of workshops demonstrating proper data collection must continue to improve the accuracy of results.

313 SUSTAINABLE INTERNATIONAL HEALTH: A STUDENT’S PERSPECTIVE


Purpose of Study: In today’s shrinking world, the health of those across the world can deeply affect the health of us here in the third world. This past summer, 10 medical and dental students traveled to the Himalayan region of Northern India. This was a student-directed and led project with faculty advisors in Canada. There, they created a partnership with the community and the school in order to provide health services to the community. Students were exposed to challenging travel, living and nutritional conditions, as well as challenges working across a language barrier. Looking at the students’ perspective of this project will help improve the project for future years and create a template for future student-led global health projects.

Methods Used: Upon return, the students filled in feedback forms, looking at the project itself, as well as feedback forms on their travel experiences. This, combined with interviews detailing the experiences of the students, allowed an assessment of the benefits to the students of the project. As well, it detailed their views on the sustainability of the project. Travel safety feedback was also collected and analyzed.

Summary of Results: Overall, the students felt that their skills in cross-cultural caring, their abilities to create and generate a sustainable health care project had increased. As well, they feel they are more able to create community partnerships and engage community members in various health projects. The students felt safe with the travel as they had been prepared for this beforehand and had anticipated these challenges.

Conclusions: Students from the India Spiti Valley Health Project felt that they were able to achieve a successful, sustainable Global Health project that met the goals they had set out with regards to community partnership. As well, they created a community partnership that can be encouraged throughout years to come.
314 INTERNATIONAL COLLABORATION BETWEEN CANADIAN AND UGANDAN UNIVERSITY STUDENTS WITHIN A GLOBAL HEALTH INITIATIVE


Purpose of Study: Global health projects involving university students abound with varying degrees of meaningful international collaboration resulting from the interaction and exchange. International partnerships and project planning should include community-led objectives and goals from the local overseas partners. During the summer of 2007 Canadian students from the University of British Columbia (UBC) embarked on the program’s second phase of the Brighter Smiles Africa Program in Uganda to implement the pediatric oral health program piloted in 2006. The purpose of this study was to document and assess the value of international collaboration between Canadian UBC students and the Ugandan Makerere university students.

Methods Used: Surveys distributed to the Ugandan and Canadian university students to assess the value of international student collaboration within the program were reviewed. Interviews with students from both countries were videotaped and summarized.

Summary of Results: All Ugandan students surveyed rated the inter-university collaboration as excellent. They valued highly learning about research design, accurate data collection and evaluation within a workable and practical health initiative. The opportunity to work alongside the Canadian students in community-based project involving school children in rural areas of Uganda was especially important to the Ugandan students. All UBC university students rated the experience of collaboration with the Ugandan Makerere University students as excellent and stated that the enthusiasm generated from their partnership would promote sustainability for the program in Uganda. Video material from student interviews will encourage the Canadian UBC students to join the global initiative.

Conclusions: International university student collaboration involving educational and cultural exchange during global health projects lays the groundwork for meaningful and lasting partnerships between universities. Team building, cooperation and working together with university students from other countries builds mutual trust and understanding provide steps toward addressing millennium goals for improving child health worldwide.

Western Student Medical Research Forum
Student Scientific Session VI
1:30 PM
Friday, February 1, 2008

315 EFFECT OF VOCAL CORD HEIGHT ASYMMETRY ON THE AERODYNAMIC PROPERTIES OF PHONATION

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Purpose of Study: Vocal cord height asymmetry represents one manifestation of superior laryngeal nerve paralysis (SLNp).

Methods Used: An ex vivo human laryngeal model was employed to study the effect of vocal fold height asymmetry on the aerodynamics of phonation, such as threshold flow and subglottic pressure. Supraglottic structures were excised, vocal cord adduction sutures were applied bilaterally, and a single suture was administered directly into the posterior aspect of the left vocal cord to induce height asymmetry. The prepared larynx was furnished with a flow supply that was controlled manually. A pulley mechanism with a variable amount of mass (0–50 g) was administered on the left posterior vocal cord suture to manipulate vocal cord height asymmetry. A hi-fidelity microphone and a pressure transducer were utilized to determine phonatory onset and subglottic pressure, respectively. A high-speed camera was used to assess vibratory pattern.

Summary of Results: With increasing height asymmetry, volume flow rate increased to achieve an equivalent amount of subglottic pressure. A mathematical model of Q (flow) = (A × Pressure^1.5) + (B × Pressure^1.5) was developed to describe the pressure-flow relationship. Terms “A” and “B” were proposed as pre-phonatory area and vocal fold stiffness, respectively. With increasing height asymmetry threshold flow increased, threshold subglottic pressure is unchanged, and threshold frequency increased. With increasing vocal cord asymmetry, high-speed photography revealed increased pre-phonatory area, reduced effective vibratory mass, and an increasingly anterior vibratory pattern.

Conclusions: The vocal breathiness and vocal fatigue linked to SLNp may be attributed to the increasing pre-phonatory area and threshold flow observed in this investigation.

316 SELECTIVE USE OF INTRAOPERATIVE CELL SAVER MACHINES IN PEDIATRIC SPINAL SURGERY

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Purpose of Study: This study aims to determine if routine cell saver (CS) machine use decreases allogeneic blood transfusion in pediatric spinal deformity surgery.

Methods Used: This study examined 191 patients undergoing spinal fusion of >5 segments. There were 42 surgeries performed in the pre-cell saver era (BCS) and 149 in the cell saver era (ACS). The Haemonetics Cell Saver 5 system with a 125 cc bowl was used in the ACS group (Haemonetics, Braintree, MA). Perioperative data collected included diagnosis, curve magnitude, extent of fusion, preoperative hematocrit, estimated blood loss (EBL), surgical approach, intraoperative and postoperative transfusion and postoperative hematocrit.

Summary of Results: The overall transfusion rate was 114/191 (60%). The rate of allogeneic transfusion was 72.1% vs. 22.3% in BCS and ACS, respectively. Using a Pearson Chi-square analysis, the difference was statistically significant. There were 31 patients receiving allogeneic blood only (A), 82 patients receiving CS blood only and 13 patients receiving both allogeneic and CS blood (B). “A” patients received an average of 20.23 cc/kg and “B” patients received 1.42 cc/kg of allogeneic blood. There was no difference in postoperative hematocrit or intraoperative EBL in ACS and BCS. No CS blood was transfused in patients <20 kg, undergoing primary dual growing rod placement or initial VEPtr placement. 89% of patients (16/18) with >10 thoracic pedicle screws and 80% (8/10) of patients fused to the pelvis received CS blood. Using a multivariate linear regression analysis, these two factors were statistically significant. There was no diagnosis associated with increased CS transfusion, nor were there threshold values of preoperative hematocrit, curve magnitude, or number of fused levels associated with increased CS transfusion.
Conclusions: This study demonstrates that the selective use of an intraoperative CS machine decreases intraoperative allogeneic blood transfusions in pediatric spinal fusion surgery. Because there is a blood loss volume threshold before the CS machine can be used, its use was not beneficial in patients <20 kg. CS blood transfusion was particularly high in surgeries involving fusion to the pelvis or a large number of thoracic pedicle screws.

317 HIGH-TENSION DOUBLE-ROW FOOTPRINT REPAIR VERSUS REDUCED-TENSION SINGLE-ROW REPAIR IN MASSIVE ROTATOR CUFF TEARS
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Purpose of Study: In spite of the added strength of double-row repairs, some authors have advocated a medicated single-row repair under reduced tension for massive retracted rotator cuff tears. The purpose of the current study was to perform a biomechanical comparison of the two repair constructs using accordingly different loading conditions.
Methods Used: A tension differential of 50 N was used for the biomechanical testing based on the in vivo measurement of the tension differential between the two repair sites. 20 paired cadaveric shoulders were dissected and standardized massive cuff tears were created. Each pair was randomized to receive a double-row footprint repair in one specimen and a single-row repair at the articular margin in the other. All repairs were then loaded to failure. Data collection included strain and stiffness for the first and final cycles between the two repair sites. All repairs were then loaded to failure. Data collection included strain and stiffness for the first and final cycles, gap formation between the first and final cycles, yield strength, failure. Data collection included strain and stiffness for the first and final cycles, gap formation between the two repair sites. 20 paired cadaveric shoulders were dissected and standardized massive cuff tears were created. Each pair was randomized to receive a double-row footprint repair in one specimen and a single-row repair at the articular margin in the other. All repairs were then loaded to failure. Data collection included strain and stiffness for the first and final cycles, gap formation between the first and final cycles, yield strength, failure.
Summary of Results: For the double-row and single-row repairs respectively, first cycle strain was 2.16 mm and 4.51 mm; final cycle strain was 0.66 mm and 1.31 mm; first cycle stiffness was 66.2 N/mm and 54.1 N/mm; final cycle stiffness was 197.8 N/mm and 131.1 N/mm; gap formation was 6.05 mm and 8.16 mm; yield strength was 498.4 N and 437.1 N; and ultimate failure strength was 594.6 N and 443.5 N. Using paired t-test analysis, the double-row construct had significantly lower strain in the first and final cycles, higher stiffness in the final cycle, lower gap formation, and higher yield and ultimate failure strength (P < 0.05).
Conclusions: In spite of higher-load cycling conditions, the double row construct demonstrated superior biomechanical properties.

318 COMPARING THE EFFECTS OF INTERFERENCE TONES ON DISTORTION PRODUCT OTOACOUSTIC EMISSIONS IN RABBITS AND HUMANS USING LEVEL/PHASE MAPS
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Purpose of Study: Distortion Product Otoacoustic Emissions (DPOAEs) first discovered by Kemp are sounds which come back out of ears in response to two pure tone stimuli and have proved useful in objectively assessing hearing in both animals and humans. The level and phase of these emissions can be plotted versus the ratio of the two pure tone eliciting stimuli to make level/phase maps. Typically these maps show horizontal “wave-fixed” phase bands and vertical “place-fixed” phase bands. Shera and Guinan (1999) suggested that these phase banding patterns originate from two sources, the ”wave-fixed” from the traveling wave of the stimuli as well as the “place-fixed” from a reflection component originating from the DPOAE place. The commonly accepted belief is that rabbits and humans both have these two mechanisms of DPOAE generation. However, contrary to expectation, a careful comparison of DPOAE level/phase maps show that DPOAEs in both rabbits and humans behave differently.
Methods Used: The level/phase maps were collected from 4 normal-hearing rabbits and 2 normal-hearing humans. First, control data was collected and plotted on a level/phase map. A second map was collected with an interference tone (IT) placed 44 Hz below the DP place in order to suppress the vertical place-fixed bands. The vector difference or “residual” was then taken to quantify the effects of the ITs on the DPOAE.
Summary of Results: In humans, the IT removes “fine structure” variations in DPOAE level and the residual is substantial with fine vertical phase banding. In rabbits, vertical banding is not eliminated and the residual is small and suggests that IT has little effect on the DPOAE place fixed emissions.
Conclusions: Thus, one source of the DPOAEs in rabbits may not be a place fixed emission as it is in humans. The results suggest that rabbits and human cochleae may not behave as similarly as was once thought and must be elucidated through further experimentation.
three-prong grasper resulting in increased extraction force. Basket-type graspers were able to stones of all sizes with significantly less force. The novel single-step percutaneous access sheath was thinner and more pliable resulting in decreased force for removal compared to two conventional percutaneous access sheaths.

320 TREATMENT OF THE DÉCOLLÉTÉ AREA IN FEMALES: PHYSICIAN PRESCRIBED SKIN CARE SYSTEMS AND FRACTIONAL LASER

M.J. Matus, A. Gabriel, S. Gupta, and T. Wharton. Loma Linda University, Loma Linda, CA.

Purpose of Study: Many methods have been proposed to reduce mottled pigmentation and improve skin tautness. Décolleté wrinkles are transient in females around the ages of 35-40, but often become permanent by the age of 50. Fractional laser therapy and physician prescribed skin care systems have both been successfully used separately as a means to improve skin quality notably improving skin tautness and fighting unwanted melanocytic pigmentation in various regions of the body. Therefore the purpose of our study was to investigate the collective effects of a skin care system with fractional laser therapy of the décolleté.

Methods Used: Six consecutive female patients, ages 44–66, were prospectively followed over a 6-month period. Each patient began a physician prescribed skin care system (Obagi NuDerm® System) in the décolleté area as a pre-treatment protocol and continued throughout the duration of the entire study. At the 6-week mark each patient underwent 3 consecutive sessions of fractional laser therapy (Palomar Lux1540™) 4 weeks apart. Pinch tests were performed at each visit and each patient was photographed. The data was analyzed with a student t-test and p < 0.05 was considered significant.

Summary of Results: All 6 patients achieved a significant exponential decrease in the skin recoil time with the pinch test at the end of the study. In addition, subjective analysis of photographs shows a reduction in the amount of dark skin spots, discolorations and hyperpigmentation.

Conclusions: This trial illustrates that dual treatment with a physician prescribed skin care system followed by fractional laser therapy is an effective and safe method of management of aged and sun damaged skin in the décolleté. It should therefore be considered as a treatment option in the rejuvenation of this area.

321 TECHNIQUES TO IMPROVE EARLY RECIPIENT GRAFT FUNCTION FOLLOWING HAND-ASSISTED LAPAROSCOPIC DONOR NEPHRECTOMY

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Purpose of Study: In recent years, laparoscopic methods of kidney procurement have become more prevalent due to the reduced morbidity for the donor. At the same time, an increase was seen in delayed graft function in recipients of laparoscopically procured kidneys. The objective of this study was to evaluate a novel protocol designed to reduce the incidence of delayed graft function in recipients of laparoscopically procured kidneys.

Methods Used: A retrospective chart review was performed of the first 50 consecutive hand-assisted laparoscopic donor nephrectomy (HALDN) patients operated on under a new protocol and was compared to the 50 consecutive prior open donor nephrectomy (ODN) patients. The new protocol involved the use of a two person surgical team including an endourologist and a transplant surgeon and advanced 4-phase CT angiogram screening to delineate the anatomy of the kidney and its blood supply. Short and long term recipient graft function was compared between the two groups. Variables compared included demographic variables, surgical parameters, complications and outcome in donors and recipients.

Summary of Results: When comparing HALDN patients to ODN patients ODN patients had shorter operating time (179 vs. 267 min; p < 0.001), while HALDN patients had decreased blood loss (134 vs. 401 cc; p < 0.001) and length of stay (3.2 vs. 3.8 days; p = 0.001). There was no difference in donor post-operative peak creatinine. When comparing the recipients, those receiving laparoscopically procured kidneys had a decreased operative time (185 vs. 216 min; p = 0.019) and lower creatinine at one week (1.2 vs. 1.9; p = 0.038). Delayed graft function was 2% (1 of 50 patients) in the HALDN recipient group compared to 14% (7 of 50) in the ODN recipient group (p = 0.027).

Conclusions: In contrast to previously published series, the recipients of HALDN kidneys procured using this novel protocol had a significantly lower creatinine at one week along with a lower incidence of delayed graft function. This suggests that implementing this new protocol for HALDN procedures could remove one of the last remaining disincentives for laparoscopic kidney procurement.

322 LONG-TERM MORBIDITY, PAIN AND DISABILITY FOLLOWING REPAIR OF SEVERE CHEST WALL INJURY

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Purpose of Study: To determine the long-term outcomes, including complications and pain and disability measurements, of patients with acute chest wall injury repair.

Methods Used: Retrospective medical record review supplemented with McGill Pain Questionnaire (MPQ), RAND-36 Health Survey, & level of daily activity (LDA, 1 = vigorous up to 4 = sedentary) outcomes of patients responding to postal contact.

Summary of Results: From 1996–2006, 48 patients with flail chest (n = 17), intractable pain associated with displaced rib fractures (n = 17), chest wall defects (n = 7), or thoracotomy for other indications (n = 7) underwent chest wall repair. There were 6 (12.5 %) long-term complications: fixation failure (4 patients with posterior absorbable plates alone), osteomyelitis (1 patient), chest wall rigidity necessitating plate removal (1 patient). 15 patients were contacted 48 (range 19–96) months post-injury. Rib-only Pain Rating Index (PRI) was relatively low and RAND-36 indices indicated high levels of function as seen in the accompanying tables.

Conclusions: Surgical repair of severe rib fractures, including flail chest and severe displacements, is associated with low long-term morbidity, pain, and disability. Absorbable plates provide unreliable fixation when placed alone posteriorly.

<table>
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<tr>
<th></th>
<th>Current Age (range)</th>
<th>60.6 (30.91)</th>
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<tbody>
<tr>
<td># Rib Fractures (SEM)</td>
<td>9.1 (±9)</td>
<td></td>
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<tr>
<td>ISS</td>
<td>30.1 (±2.9)</td>
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<tr>
<td>Chest AIS</td>
<td>4.0 (±0.1)</td>
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<tr>
<td>Rib PRI (0-50 scale)</td>
<td>6.7 (±2.1)</td>
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<tr>
<td>Pre-injury LDA</td>
<td>1.5 (±0.16)</td>
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<tr>
<td>Current LDA</td>
<td>2.0 (±0.24)</td>
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323 THE LIGAMENT OF WRISBERG, LATERAL DISCOID MENISCI, AND ASSOCIATED FINDINGS: A STUDY UTILIZING IMAGING AND ARTHROSCOPY

R. Parikh1,2, D. Patel1, G. Rebello1, and S. Kattapuram1. 1Massachusetts General Hospital, Boston, MA and 2SUNY Brooklyn, Brooklyn, NY.

Purpose of Study: Lateral discoid meniscus is characterized by the meniscus appearing as a full biconvex disc, and its cause is unclear. Complete lateral discoid meniscus is a type of discoid meniscus that is characterized by filling entirely the lateral compartment, and an incomplete discoid is smaller and does not fill the entire lateral compartment. Associations of the ligament of Wrisberg, complete/incomplete discoid meniscus, and insufficiency of the meniscotibial ligament are considered some of the factors associated with lateral discoid meniscus. This study attempts to gain a further understanding of these relationships.

Methods Used: 15 random patients (range 6.8 to 62.7 years, mean of 41.2 years of age, 12 female, 3 male) and 16 knees suspected of lateral discoid meniscus were confirmed via MRI and arthroscopy treatment to have a lateral discoid meniscus. From arthroscopy and MRI findings we summarized the types of discoid meniscus (complete and incomplete), and their relationship to the ligament of Wrisberg. Associated findings such as lateral/medial meniscal tears and chondral injury or ligament damage were looked into as well.

Summary of Results: 13 of our discoid menisci were complete and 3 were incomplete. We did not observe any deficiencies posterior to the tunnel. All but 1 patient (1 knee) had ligaments of Wrisberg with an average width of 28.22 mm. Comparing the widths Wrisberg ligaments of complete and incomplete discoid meniscus we found t value was .5447, and the p-value was found to be .6406, including ligaments of complete discoid is smaller and does not fill the entire lateral compartment. All but 1 patient (1 knee) had ligaments of Wrisberg with an average width of 28.22 mm. Comparing the widths Wrisberg were found to have T-BILI = 4 mg/dl on hospital day 2 is a good predictor for the presence of retained common bile duct (CBD) stones, and ultimately for the need for preoperative endoscopic retrograde cholangiopancreatography (ERCP) in patients with gallstone pancreatitis (GP). Our previous study indicated that day 2 T-BILI > 4 mg/dl is the best predictor of a retained CBD stone. However, the optimal T-BILI level at which ERCP should be performed is unclear.

Methods Used: Consecutive patients with GP who were admitted to an urban teaching hospital over a 54 month period were evaluated from January 2003 through July 2007. GP was diagnosed based on the following criteria: 1) upper abdominal pain and tenderness, 2) hyperamylasemia, 3) documented cholelitiasis, 4) absence of alcohol use, hypercalcemia, hypertriglyceridermia, or medications known to cause pancreatitis. A p-value < 0.05 using a Fischer’s test was considered to be statistically significant.

Summary of Results: 200 patients with documented GP were divided into two groups: those with hospital day 2 T-BILI > 4 mg/dl and <4 mg/dl (FIG 1). 20 out of 200 patients were found to have T-BILI = 4 mg/dl. 11 of these 20 were found to have CBD stones. 180 of the total 200 patients were found to have T-BILI < 4 mg/dl. 22 of these 180 were found to have CBD stones. A Fischer’s Exact Test showed that day 2 T-BILI level was statistically significant for predicting a CBD stone (p < 0.0001, odds ratio = 8.78, 95% confidence interval [3.27–23.6], sensitivity = 34%, specificity = 95%, PPV = 0.55, NPV = 0.88, likelihood ratio = 6.19).

Conclusions: In patients with GP, hospital day 2 serum T-BILI = 4 mg/dl significantly predicts the presence of CBD stones. This confirms our previous finding. Given the 95% specificity, this predictor can potentially be used to determine whether a preoperative ERCP should

324 RESULTS OF PTERYGIUM EXCISION USING AMNIOTIC MEMBRANE BENEATH THE HEALTHY CONJUNCTIVA SURROUNDING A CONJUNCTIVAL AUTOGRRAFT


Purpose of Study: Pterygium is characterized by fibrovascular proliferation and overgrowth of the cornea, which causes ocular irritation, disfiguration, and potentially affects visual acuity. Today, two methods of pterygium surgery are common. The first is surgical excision with a conjunctival autograft harvested from the same eye. A second technique is to fill the void of the removed pterygium with amniotic membrane. Recurrence with each technique occurs in up to 10%. To further decrease the rate of recurrence we studied the use of both conjunctival autograft and amniotic membrane where the amniotic membrane graft is placed beneath the healthy conjunctiva surrounding the conjunctival autograft.

Methods Used: After removal of the pterygium, an amniotic membrane transplant graft of similar size was placed into the potential space beneath the conjunctiva surrounding the defect where the pterygium was removed. A conjunctival autograft was harvested from the superior limbal conjunctiva. The graft was held into place using Tisseel VH fibrin sealant. We reviewed outcomes of all patients undergoing this procedure over a 14 month period.

Summary of Results: Forty eyes of 35 patients underwent surgery. Mean follow-up was 10 months (range 4–17 months). Postoperatively, all conjunctival and amniotic membrane grafts remained viable and in place. One eye developed a pyogenic granuloma at the nasal edge of its autograft. Recurrences did not occur in any eyes in this series.

Conclusions: Pterygium excision using combined conjunctival autograft and adjacent subconjunctival amniotic membrane transplantation is a viable technique for treatment of pterygium. Results compare favorably with established techniques.

325 Gallstone Pancreatitis: Predictive Value of Serum Total Bilirubin for Common Bile Duct Stones

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Purpose of Study: The purpose of this study is to confirm whether a serum total bilirubin (T-BILI) > 4 mg/dl on hospital day 2 is a good predictor for the presence of retained common bile duct (CBD) stones, and ultimately for the need for preoperative endoscopic retrograde cholangiopancreatography (ERCP) in patients with gallstone pancreatitis (GP). Our previous study indicated that day 2 T-BILI > 4 mg/dl is the best predictor of a retained CBD stone. However, the optimal T-BILI level at which ERCP should be performed is unclear.

Methods Used: Consecutive patients with GP who were admitted to an urban teaching hospital over a 54 month period were evaluated from January 2003 through July 2007. GP was diagnosed based on the following criteria: 1) upper abdominal pain and tenderness, 2) hyperamylasemia, 3) documented cholelithiasis, 4) absence of alcohol use, hypercalcemia, hypertriglyceridermia, or medications known to cause pancreatitis. A p-value < 0.05 using a Fischer’s test was considered to be statistically significant.

Summary of Results: 200 patients with documented GP were divided into two groups: those with hospital day 2 T-BILI = 4 mg/dl and <4 mg/dl (FIG 1). 20 out of 200 patients were found to have T-BILI = 4 mg/dl. 11 of these 20 were found to have CBD stones. 180 of the total 200 patients were found to have T-BILI < 4 mg/dl. 22 of these 180 were found to have CBD stones. A Fischer’s Exact Test showed that day 2 T-BILI level was statistically significant for predicting a CBD stone (p < 0.0001, odds ratio = 8.78, 95% confidence interval [3.27–23.6], sensitivity = 34%, specificity = 95%, PPV = 0.55, NPV = 0.88, likelihood ratio = 6.19).

Conclusions: In patients with GP, hospital day 2 serum T-BILI = 4 mg/dl significantly predicts the presence of CBD stones. This confirms our previous finding. Given the 95% specificity, this predictor can potentially be used to determine whether a preoperative ERCP should
be performed. This will reduce the number of unnecessary procedures and minimize risk for patients.

<table>
<thead>
<tr>
<th>CBD Stone</th>
<th>T-Bili &gt;4</th>
<th>T-Bili &lt;4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>11</td>
<td>22</td>
</tr>
<tr>
<td>No CBD Stone</td>
<td>9</td>
<td>158</td>
</tr>
</tbody>
</table>

326 (Withdrawn)

327
SCANNING, PRODUCTION AND CHARACTERIZATION OF HUMAN SCAFFOLDS FOR CARTILAGE TISSUE ENGINEERING

P. Manson, J. Szivek, and N. Webber. University of Arizona, Tucson, AZ.

Purpose of Study: Joint pain and loss of mobility represents the most common cause of impairment in the US (Buckwalter and Mankin, 1998). Nearly 50% of Americans between 25 and 74 years of age report having had knee pain (National Centers for Health Statistics, 1992). More patients undergo surgery to place Orthopaedic implants than any other type of implant. Although numerous procedures have been developed for the treatment of damaged articular cartilage, currently none consistently restore the long-term function of articular cartilage.

Methods Used: We took core samples of femoral condyles from cadavers and then scanned them using a micro CT scanner. The 3D data from the scans was ported to a rapid prototyping machine and several scaffolds were made. These scaffolds are being implanted into cadaver femoral condyles for testing. Scaffold loading is carried out at 49 N/s and/or 3923 N/s to simulate loading conditions of slow or rapid gait. In all tests, load and displacement are monitored simultaneously and results will be compared to previous measurements collected from similar sized osteochondral human specimens. Strain gauges on the scaffold surfaces provide measurements of stiffness characteristics of the scaffold.

Summary of Results: The micro CT signs were completed successfully and translated into 3D files that were used to make the scaffolds. The scaffolds looked visually similar to the trabecular bone of the original core sample.

Conclusions: We are in the implanting and testing phase. In a previous studies with a canine model loading of scaffolds in joints during gait and exercise activities were measured. That model validated the belief that measurements could be used to monitor healing. The concept of using a scaffold with cells or tissue is not without merit but clearly requires a good understanding of the ideal properties for a scaffold and tissue engineered surface. Use of in vivo sensors attached to the scaffold provides an opportunity to measure scaffold properties and to monitor healing. This will allow a better understand of the next steps in the scaffold development process.

Concurrent Session
8:30 AM
Saturday, February 2, 2008

328
ANAPHYLACTOID REACTION AND ITS TREATMENT AS A CAUSE OF AN ATYPICAL GLOBAL TAKO-TSUBO CARDIOMYOPATHY

M. Fraley1, 2, and D. Ratliff1, 2. 1University of New Mexico, Albuquerque, NM and 2Veteran’s Administration Medical Center, Albuquerque, NM.

Purpose of Study: To illustrate a unique cause and cardiac manifestations of Tako-tsubo cardiomyopathy.

Methods Used: Prospective, direct history and physical examination, laboratory data, initial cardiac catheterization with coronary angiography and left ventriculography, echocardiogram as well as clinical and echocardiographic follow-up.

Summary of Results: A 42-year-old diabetic female experienced an anaphylactoid reaction after eating almonds characterized by lip tingling, throat tightening, and shortness of breath. She received diphenhydramine, oxygen, and two doses of 0.3 mg subcutaneous epinephrine. Her original symptoms resolved. However, she developed chest tightness and diaphoresis after epinephrine administration, which required one sublingual nitroglycerin for relief. The initial electrocardiogram showed sinus tachycardia with diffuse non-specific ST-segment abnormalities. Cardiac biomarkers revealed only mild elevation of CKP as well as a nonspecific rise of troponin I. Though she had no further symptoms, due to her history of diabetes and mildly abnormal troponin, she underwent a coronary angiogram and left ventriculography. These studies revealed angiographically normal coronary arteries with global hypokinesis, worse in the anterolateral, diaphragmatic and apical segments, with an ejection fraction of 25%. These findings were confirmed on an echocardiogram performed immediately following the cardiac catheterization. There was no infectious prodrome, previous cardiac history, or evidence of infiltrative causes for her cardiomyopathy. The diagnosis of Tako-tsubo cardiomyopathy was made based upon the constellation of findings in this setting of significant catecholamine exposure due to the anaphylactoid reaction and subsequent subcutaneous epinephrine administration. Follow-up echocardiogram obtained four weeks after her original presentation showed dramatic improvement of her ejection fraction (50%) and wall motion further supporting the diagnosis.

Conclusions: To the best of our knowledge, this is the first case of an anaphylactoid reaction and its treatment causing global Tako-tsubo cardiomyopathy.

329
LONG TERM RISKS OF STEROID WEANING AFTER HEART TRANSPLANTATION

A. Vaidya, M. Kawano, M. Hamilton, and J. Kobashigawa. David Geffen School of Medicine at UCLA, Los Angeles, CA.

Purpose of Study: Corticosteroids (CS) after heart transplant have significant adverse effects. In many programs, corticosteroid weaning (CSW) is successfully performed with good outcome. Yet there are risks to CSW in terms of late rejection, which may result in graft dysfunction. The purpose of this study is to characterize the risks involved in CSW and analyze the long term follow up of these patients.

Methods Used: Between 1994 and 2006, 305 heart transplant patients underwent CSW at a mean of 11.1 months after heart transplantation. CSW was performed by reducing prednisone from a baseline of 5 mg daily and reducing the dose by 1 mg monthly with monthly endomyocardial biopsies until off. Patients were followed for a mean of 5 years after cessation of CS with outcome recorded.

Summary of Results: Of those patients initiated on CSW, 272 (89.2%) were successfully weaned off CS. Those that failed included 27 (8.9%) due to rejection during CSW and 6 (1.9%) due to intolerable withdrawal side effects. For patients who were successfully weaned completely off CS and followed for 5 years, 52/272 (19%) required reinitiation of CS (mean period of 16 months from time of wean), mainly for rejection. Successfully weaned patients had a significantly better 5 year survival (conditional to being alive at 1 year) compared to the non-weaned group (93% vs. 83%, p = 0.001). Freedom from cardiac allograft vasculopathy...
(CAV) at 5 years was comparable in the successfully weaned and non-weaned groups (83% vs. 79%, p = 0.21). The 52 patients who required re-institution of CS had comparable 5 year survival to the non-weaned group (90% vs. 83%, p = 0.15). When re-institution of CS was required, this led to a significantly lower freedom from CAV when compared with the non-weaned group (60% vs. 79%, p = 0.003).

Conclusions: Corticosteroid weaning appears safe in most patients and successful, is associated with improved survival. However, there is a long-term risk of increased CAV in those patients who require re-institution of corticosteroids. CAV in these patients may not have occurred had they maintained corticosteroid therapy.

**330 THE USE OF ALISKIREN, A NEW BLOOD PRESSURE MEDICATION, IN HEART TRANSPLANT PATIENTS**

A. Ankrom, M. Hamilton, and J. Kobashigawa. David Geffen School of Medicine at UCLA, Los Angeles, CA.

**Purpose of Study:** Hypertension after heart transplant is a common complication due in part to the use of calcineurin inhibitors (CNI) used as standard immunosuppressive therapy. This CNI hypertension is associated with a hyper-renin state. Anti-hypertensives are limited by adverse effects (edema from calcium channel blockers and hyperkalemia from ACE inhibitors). Refractory hypertension after heart transplant may require the use of 2 or more anti-hypertensives. Aliskiren, a direct-renin inhibitor, represents a new class of anti-hypertensives which may be effective in this patient population.

**Methods Used:** Between April 2007 and September 2007 we reviewed 10 patients treated with aliskiren 150 mg (4 patients were on 300 mg). Patients were followed for a mean of 8.4 weeks. All patients were on an average of 2.2 anti-hypertensive medications prior to aliskiren and no other anti-hypertensives were changed during this period.

**Summary of Results:** The mean systolic blood pressure drop was 25 mmHg (all patients decreased). Diastolic pressure decreased a mean of 16 mmHg (8 of 10 patients decreased). Heart rate was unchanged. Serum creatinine, potassium, and immunosuppression blood levels were unchanged. 3 of the 10 stopped medication after 8 weeks due to back pain, hypotension, nausea/vomiting, and diarrhea which all resolved after cessation of medication.

**Conclusions:** Aliskiren appears to be safe and effective in heart transplant patients and all side effects were reversible upon cessation of therapy. A larger study is warranted to establish the role of direct-renin inhibitors in CNI-associated hypertension.

**331 THE OUTCOME OF LOWER SOCIAL ECONOMIC STATUS AFTER HEART TRANSPLANTATION**

D. Patel, K. Kiyosaki, M. Kawano, M. Hamilton, and J. Kobashigawa. David Geffen School of Medicine at UCLA, Los Angeles, CA.

**Purpose of Study:** Medical insurance can reflect socioeconomic status (SES). Patients with private health insurance pay insurance premiums and thus may be of higher SES. Patients on Medicare are often older or chronically ill, and mainly of lower SES. After heart transplant, health care costs can cause financial stress and lowering of one’s SES. We followed transplant patients over time to determine the effect to changing insurance (and indirectly, changing SES) on outcomes.

**Methods Used:** Between 1994 and 2006 we reviewed 649 heart transplant patients, divided based on medical insurance at transplant: Medicaid (group 1, n = 78), private insurance (group 2, n = 474), and Medicare (group 3, n = 70). HMO (Kaiser, n = 20) patients were excluded because they were not followed at our institution.

**Summary of Results:** Over 10 years, group 1 had significantly lower survival vs. group 2 (61.5% vs. 75.9%, p = 0.04; see graph) and a trend to lower survival vs. group 3 (61.5% vs. 71.4%, p = 0.26). During the 10-year follow up, no patient in group 1 changed insurance, while 22 patients (4.6%) in group 2 and 17 patients (24.3%) in group 3 switched to Medicaid. Of those patients who switched from private insurance to Medicaid, survival was less than those patients who did not switch (68.2% vs. 76.3%, p = 0.32).

**Conclusions:** Patients starting on Medicaid at transplant (in a lower SES) have lower survival 10 years after heart transplant. Switching to Medicaid from private insurance or Medicare is also associated with poorer outcome. This warrants further assessment.

**332 REDUCTION IN MALIGNANCY WITH USE OF NEWER IMMUNOSUPPRESSIVE REGIMENS IN HEART TRANSPLANT PATIENTS**

A. Brown, J. Kawano, M. Kawano, M. Hamilton, and J. Kobashigawa. David Geffen School of Medicine at UCLA, Los Angeles, CA.

**Purpose of Study:** Malignancy is now gaining as the major factor limiting long-term survival in heart transplant patients. Recent reports have shown that newer anti-proliferative agents may cause less cancer. However, the true risk of malignancy lies not only in the individual immunosuppressive agent, but rather the immunosuppressive regimen which leads to the global immunosuppressed state. It is not yet known to which immunosuppressive regimen may harbor the greatest malignancy risk among patients on triple drug immunosuppressive therapy without induction in heart transplant patients.

**Methods Used:** Between 1996 and 2006 we have reviewed 320 heart transplant patients divided into 6 groups based on immunosuppressive regimen and anti-proliferative drug. Patients were divided into 6 groups: azathioprine (AZA)/cyclosporine (CSA), AZA/tacrolimus (TAC); mycophenolate mofetil (MMF)/CSA, MMF/TAC; sirolimus (SRL)/CSA, SRL/TAC. Based upon conditional 1 year survival, patients were followed for 4 years to assess for differences in malignancy rates.

**Summary of Results:** For patients on AZA-based immunosuppressive regimen, freedom from malignancy was numerically lower compared to MMF and SRL based regimens (AZA/CSA = 82.5%, AZA/TAC = 78.9%; MMF/CSA = 88.9%, MMF/TAC = 89.8%; SRL/CSA = 100%, SRL/TAC = 100%, all p = NS) (see table). The SRL based regimens had 100% freedom from malignancy, but the numbers are small.
333 THE OUTCOME OF SUCCESSFULLY TREATED PRE-TRANSPLANT SENSITIZED PATIENTS AFTER HEART TRANSPLANTATION

E. Sue, M. Kawano, M. Hamilton, and J. Kobashigawa. David Geffen School of Medicine at UCLA, Los Angeles, CA.

Purpose of Study: Pre-heart transplant patients with elevated panel reactive antibody (PRA) levels are reported to have poor outcome post transplant. Usually, patients with prior transplant, multiparous females, and patients with previous blood transfusions have markedly elevated PRAs. Some patients may need therapies such as plasmapheresis or intravenous immunoglobulin (IVIG) to reduce these elevated PRAs to allow for safe heart transplantation. The outcome of those patients who successfully reduce pre-transplant PRAs is not known.

Methods Used: Between 1994 and 2006, 516 patients awaiting heart transplant were reviewed. 23 of these patients had elevated PRAs and had at least two positive prospective donor specific crossmatches (DSXM), which necessitated treatment (with plasmapheresis and/or IVIG and/or rituximab) to reduce these elevated PRAs. The remaining 493 patients who did not have elevated PRAs and subsequently underwent heart transplant acted as controls. Immunosuppression for these patients included tacrolimus, mycophenolate, and corticosteroids.

Summary of Results: 21 of 23 patients with significantly elevated PRAs (range 28–100%) were successfully treated with a mean PRA reduction of 40%. These 21 patients underwent heart transplant with a negative prospective DSXM. The 2 patients who were unsuccessfully treated died from advanced heart failure. The treated patients compared to controls had significantly lower freedom from any treated first year rejection (57.1% vs. 88.8%, p < 0.001). The treated group compared to the control group had comparable 5 year survival (66.7% vs. 74.6%, p = 0.373) and 5 year freedom from cardiac allograft vasculopathy (77.3% vs. 79%, p = 0.823).

Conclusions: Successful reduction of circulating antibodies pre-transplant leads to comparable 5 year outcome post transplant, despite increased first year rejection. Therefore, significantly elevated PRAs in the pre-transplant patient should not be a contraindication for heart transplantation.

334 KIDNEY TRANSPLANT PROLONGS SURVIVAL IN PATIENTS WITH END STAGE KIDNEY DISEASE AFTER HEART TRANSPLANTATION

H. Song, M. Kawano, K. Kiyosaki, D. Patel, M. Hamilton, and J. Kobashigawa. David Geffen School of Medicine at UCLA, Los Angeles, CA.

Purpose of Study: End stage renal disease (ESRD) can occur after heart transplantation, mainly due to pre-transplant renal dysfunction as well as nephrotoxicity from chronic calcineurin inhibition (CNI). Non-transplant patients who undergo kidney transplantation have improved survival and quality of life compared with non-transplant patients who remain on dialysis. We sought to determine if the same benefit applies to heart transplant patients who undergo kidney transplantation.

Methods Used: Between 1986 and 2006, 72 post-transplant patients developed ESRD requiring dialysis. The most common causes of ESRD were chronic CNI use (69%) and diabetic nephropathy (8%). Of the 72 ESRD heart transplant patients, 11 ultimately received a kidney transplant, and these two groups were compared.

Summary of Results: The mean time to initiation of dialysis was similar in dialysis versus kidney transplant groups (4.1 years vs. 5.8 years; p = NS). In the 11 patients who received a kidney transplant, the mean time on dialysis pre-kidney transplant was 1.3 years. 5-year survival (conditional on survival 1.3 years on dialysis) in the dialysis and kidney transplant groups was 45.5% and 54.1% (p = 0.49). In the ongoing dialysis group, there were more episodes of non-fatal major adverse cardiac events (14.8% vs. 9.1%) and there was greater development of cardiac allograft vasculopathy (26.2% vs. 18.2%).

Conclusions: Compared with hemodialysis, kidney transplantation after heart transplant is associated with a trend towards improved survival, fewer major adverse cardiac events, and a reduced incidence of cardiac allograft vasculopathy. Whenever possible, kidney transplant should be performed in those heart transplant patients with end stage renal disease in order to improve long term outcomes.

335 HYPERCHOLESTEROLEMIA RESISTANT TO STATIN THERAPY IN FIRST YEAR AFTER TRANSPLANT IS HIGH RISK FOR FUTURE COMPLICATIONS

M. Kawano, J. Kawano, M. Hamilton, and J. Kobashigawa. David Geffen School of Medicine at UCLA, Los Angeles, CA.

Purpose of Study: Hypercholesterolemia is common after heart transplant. The use of statins to lower cholesterol has been suggested to have an immunomodulatory effect. In non-transplant patients, it has been shown that the lower the total cholesterol (TC), the better the outcome. It has not been shown whether lower TC levels result in better outcome after heart transplant.

Methods Used: Between 1994 and 2000 we reviewed 301 patients who survived >1 year after heart transplant. The mean first year TC level was correlated to 5 year outcome of survival, non-fatal major adverse cardiac events (n-MACE), and cardiac allograft vasculopathy (CAV), diagnosed as any angiographic stenosis >30%. Mean first year TC levels were divided into 4 groups: group 1 = TC < 150 mg/dl, group 2 = TC 151–200, group 3 = TC 201–250, group 4 = TC > 250. All patients were maintained on statin therapy and/or ezetimibe.

Summary of Results: Group 4 had significantly less freedom from CAV compared to groups 1–3 (see figure, p = 0.008). Group 1 did not appear to have greater freedom from CAV compared to groups 2 and 3. Among all groups, there was comparable 5 year survival and n-MACE.
Multivariate analysis of risk factors showed TC > 250 to be an independent variable for poor outcome.

**Conclusions:** Despite statin therapy, elevated mean first year total cholesterol level (>250 mg/dl) is associated with higher incidence of CAV. Further modalities to lower statin resistant hypercholesterolemia may be needed. However, achieving lowest TC levels may not provide added benefit.

**ADVERSE EFFECTS OF SIROLIMUS VERSUS EVEROLIMUS AFTER HEART TRANSPLANTATION**

S. Carr, K. Kiyosaki, A. Ankrom, M. Kawano, M. Hamilton, and J. Kobashigawa. David Geffen School of Medicine at UCLA, Los Angeles, CA.

**Purpose of Study:** The newer proliferation signal inhibitors (PSIs) appear to have significant benefit with less rejection and cardiac allograft vasculopathy after heart transplant. However, PSIs also have significant adverse effects (AEs) which may lead to nonadherence. From anecdotal reports, it appears that the newer PSI, everolimus (EV), may have less AEs than sirolimus (SR). In multicenter, randomized trials of EV (Eisen) and SR (Keogh), there appeared to be more freedom from adverse effects (32% vs. 17%) in the EV group in the first year. However, post transplant care was not similar between these studies. The purpose of this study is to assess the AE profiles of these drugs while using a standardized post transplant regimen.

**Methods Used:** Between 1994 and 2006 we reviewed 150 heart transplant patients who were placed on EV (n = 20) and SR (n = 130). Both groups were followed for 1 year from initiation of the medication and assessed for AEs and drop out rate. All EV patients were started at the time of transplant, while SR patients were started on average 4.1 years post transplant.

**Summary of Results:** Fatigue and peripheral edema were significantly more common in the SR group than the EV group (see table). Drop out rate from drug was lower in the EV vs. SR group (5% vs. 31%, p < 0.001).

**Conclusions:** Everolimus appeared to have less AEs compared to sirolimus. This is especially significant since everolimus patients were treated earlier, immediately after transplant when AEs are more common, compared to the sirolimus group. A head-to-head comparison trial is warranted.

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**THE ECONOMIC OUTCOME OF HEART TRANSPLANTATION VERSUS MEDICAL THERAPY OF ADVANCED HEART FAILURE PATIENTS**

M. Yajnik, V. Vaidya, M. Kawano, A. Ankrom, M. Hamilton, and J. Kobashigawa. David Geffen School of Medicine at UCLA, Los Angeles, CA.

**Purpose of Study:** We compared the costs per life year gained with heart transplant vs medical treatment of end stage heart failure. The standard for cost effectiveness is seen in kidney dialysis ($50,000–$100,000 per life year gained).

**Methods Used:** From 1997–2004, 625 patients (pts) were listed for heart transplant as UNOS Status 1 (1A or 1B) or Status 2, at our institution. Listed pts were placed into four groups: Group A = Status 1 Transplanted (n = 164), Group B = Status 1 Medical Treatment (n = 52), Group C = Status 2 Transplanted (n = 245), and Group D = Status 2 Medical Treatment (n = 164). Costs were obtained from hospital accounting. Survival and mean cost after listing were obtained annually for each group over 10 years, and were discounted at a 3% annual rate. The costs per quality-adjusted-life-year gained were calculated by utility scores.

**Summary of Results:** The average follow-up (including death) for groups A,B,C,D was 3.6, 0.5, 5.1, and 3.5 years, respectively. For Status 1 pts, 4.95 life years were gained in transplant (group A) vs medical treatment (group B). For Status 2 pts, 2.05 life years were gained in transplant (group C) vs medical treatment (group D). Due to greater survival benefit, costs per life year gained were lower for transplant of Status 1 vs Status 2 pts ($69,622 vs $123,417) compared to medical management (see table).

**Conclusions:** Heart transplant appears to be more cost effective for Status 1 vs Status 2 pts. Compared to the standard of kidney dialysis, the transplantation of Status 2 patients may not be cost effective.

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**REDUCED IMMUNE RESPONSE IN THE OLDER HEART TRANSPLANT PATIENT**

**Adverse Effects Profile**

<table>
<thead>
<tr>
<th>Adverse Effect</th>
<th>Everolimus (n=20)</th>
<th>Sirolimus (n=130)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anemia</td>
<td>7 (35.0%)</td>
<td>32 (24.6%)</td>
<td>0.79</td>
</tr>
<tr>
<td>Leukopenia</td>
<td>4 (20.0%)</td>
<td>17 (13.1%)</td>
<td>0.48</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>2 (10.0%)</td>
<td>13 (10.0%)</td>
<td>1.0</td>
</tr>
<tr>
<td>GI Disorders</td>
<td>6 (30.0%)</td>
<td>43 (33.1%)</td>
<td>0.79</td>
</tr>
<tr>
<td>Fatigue*</td>
<td>3 (15.0%)</td>
<td>47 (36.2%)</td>
<td>0.03</td>
</tr>
<tr>
<td>Infections</td>
<td>2 (10.0%)</td>
<td>19 (14.6%)</td>
<td>0.54</td>
</tr>
<tr>
<td>Renal Impairment or Increased Creatinine</td>
<td>6 (30.0%)</td>
<td>53 (40.9%)</td>
<td>0.34</td>
</tr>
<tr>
<td>Neurological Disorders</td>
<td>3 (15.0%)</td>
<td>21 (16.2%)</td>
<td>0.89</td>
</tr>
<tr>
<td>Mouth Ulceration</td>
<td>1 (6.0%)</td>
<td>7 (6.4%)</td>
<td>0.94</td>
</tr>
<tr>
<td>Hypercholesterolemia &gt; 250</td>
<td>7 (35.0%)</td>
<td>38 (29.2%)</td>
<td>0.66</td>
</tr>
<tr>
<td>Epistaxis</td>
<td>0 (0.0%)</td>
<td>1 (0.8%)</td>
<td>0.31</td>
</tr>
<tr>
<td>Pleural Effusion</td>
<td>2 (10.0%)</td>
<td>2 (1.5%)</td>
<td>0.22</td>
</tr>
<tr>
<td>Pericardial Effusion</td>
<td>3 (15.0%)</td>
<td>2 (1.5%)</td>
<td>0.11</td>
</tr>
<tr>
<td>Peripheral Edema*</td>
<td>3 (15.0%)</td>
<td>51 (39.2%)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

*P<0.05, statistically significant
Purpose of Study: According to multiple registries, older heart transplant patients (>65 years) have poorer outcome. There appears to be a conundrum in this observation, as older patients appear to have reduced immune response and therefore should have less rejection and better survival. We sought to confirm these observations in a single center study by assessing our older patients (>65 years) and comparing them to those patients in younger age-groups.

Methods Used: Between 1994 and 2006 we evaluated 685 transplant patients and divided them into three groups by age: group 1 (20–40 years) (n = 89), group 2 (41–65 years) (n = 440), group 3 (>65 years) (n = 150). The majority of patients were on tacrolimus and mycophenolate with the rest being on cyclosporine and mycophenolate. There did not appear to be a significant difference in baseline demographics except for age.

Summary of Results: First year survival was decreased in group 3 (>65 years) compared to group 2 (85% vs 92%; p = 0.008) and group 1 (85% vs 91%; p = 0.145). The causes of death in group 3 included the following: malignancy (12), graft failure (6), CAV (3), stroke (2). Group 3 had greater freedom from first year rejection compared to group 1 (93.3% vs. 77.5%, p = .001) and group 2 (93.3% vs. 88.3%, p = .128), reflecting a heightened immune response in the younger age group. Group 3 had lower 5 year freedom from cardiac allograft vasculopathy compared to group 2 and 1 (91%; p = .001) and group 2 (93.3% vs. 79.8%, p = .150, respectively).

Conclusions: Older heart transplant patients appear to have reduced immune response resulting in less first year rejection. However, this benefit appears to be offset by decreased first year survival in these older patients (group 3) due to the usual causes of death associated with this population, including cancer, stroke, atherosclerotic vascular disease. Despite this, the older population appears to have an overall acceptable lifespan and this age group should continue to be considered for transplant.

339 PHOSPHOPROTEOMIC IMMUNE PROFILING IN ORTHOTOPIC HEART TRANSPLANT RECIPIENTS

M. Mossanen1, D. Cruz1, A. Gregson2, and P. Krutzik3. 1David Geffen School of Medicine at UCLA, Los Angeles, CA; 2David Geffen School of Medicine at UCLA, Los Angeles, CA and 3Stanford University Medical Center, Stanford, CA.

Purpose of Study: The efficacy of modern immunosuppressive agents has made orthotopic heart transplantation (OHT) the treatment of choice for end-stage heart failure. The goal of immunosuppressive therapy is to prevent allograft rejection while avoiding complications associated with chronic immunosuppression such as vasculopathy, opportunistic infections, and malignancy. We hypothesize that complications following OHT arise from distinct alterations in innate and adaptive immunity, and that immunologic profiling using a flow cytometry based phosphoproteomic approach will reveal specific alterations of signaling networks among OHT recipients.

Methods Used: A phosphoproteomic approach was used to search for specific alterations of signaling networks among OHT recipients. Our preliminary experiments analyzed cryopreserved peripheral blood mononuclear cells (PBMC) from five different OHT recipients. Of these, four were receiving identical immunosuppressive regimens. PBMC were stimulated with four different cytokines and the phosphorylation of three Stat proteins was analyzed by phosho-flow. Using cell surface markers, six different cell types were identified, generating a 90 point signaling biosignature for each patient.

Summary of Results: Two distinct biosignatures emerged, which differed dramatically in terms of responsiveness of the CD8+ memory T cells to IL-6. Signaling in other cell types remained constant between the groups. The fifth recipient was receiving a distinct immunosuppressive regimen containing everolimus, a recently FDA-approved proliferation signal inhibitor. This biosignature was associated with hyper-responsiveness of the CD8+ memory T cells to IL-6, but the profile was otherwise similar to the other patient samples.

Conclusions: Our preliminary results demonstrate immunologic profiling of signaling networks in OHT recipients is feasible, and distinct alterations in key signaling pathways occur in individual OHT recipients. We predict that the determination of immunologic signaling in OHT recipients will reveal distinct clusters of profiles, which may ultimately yield prognostic and mechanistic insight into disease pathogenesis in OHT recipients.
Endocrinology II
Concurrent Session
8:30 AM
Saturday, February 2, 2008

341 HYPOTHALAMIC ATYPICAL PROTEIN KINASE C MEDIATES ENDOTOXIN-INDUCED INFLAMMATORY ANOREXIA
J. Thaler1, M. Sajan2, M. Matsen3, H. Nguyen1, R. Farese2, and M. Schwartz1. 1University of Washington, Seattle, WA and 2University of South Florida, Tampa, FL.

Purpose of Study: The atypical protein kinase C isoforms, ε and λ, (aPKC) are central effectors in the NF-κB pathway, generating pro-inflammatory cytokines in response to immune challenges. aPKC is also a critical PI-3 kinase target in the insulin pathway, promoting peripheral glucose uptake and liver VLDL synthesis. The inflammatory cascade initiated via NF-κB by the bacterial cell wall component, endotoxin, induces a potent "sickness response" that includes fever, anorexia, and decreased physical activity. These behavioral responses require signaling in the hypothalamus through mechanisms that are not fully characterized. We investigated whether aPKC is expressed in the hypothalamus and serves as a mediator of leptin/PI-3 kinase and/or inflammatory anorexia.

Methods Used: To map the distribution of aPKC in the CNS, we performed immunohistochemistry on rat brain sections. In addition, adult male Wistar rats bearing 3rd ventricle cannulae underwent ICV pretreatment with aPKC inhibitor or its vehicle followed by IP injection of endotoxin or saline. Food intake, body weight, energy expenditure, temperature, and ambulatory activity were assessed in metabolic cages. An identically treated group was sacrificed to measure hypothalamic cytokine gene expression and aPKC activity 2h after endotoxin administration. A similar experiment involved ICV injection of leptin or its vehicle followed by ICV injection of leppin or its vehicle.

Summary of Results: aPKC was expressed broadly in the CNS including within the paraventricular and arcuate nuclei of the hypothalamus, regions involved in energy balance and inflammatory signaling. ICV leptin or insulin increased hypothalamic aPKC activity by ~30% while IP endotoxin increased aPKC activity 2-fold. While ICV pretreatment with the aPKC inhibitor failed to alter leptin-mediated anorexia, it fully reversed food intake suppression induced by systemic endotoxin. Furthermore, endotoxin stimulation of hypothalamic cytokine gene expression was dependent upon intact aPKC signaling.

Conclusions: aPKC is expressed in key brain areas for energy homeostasis and is required for anorexia induced by endotoxin, but not leptin. Thus, aPKC inhibition may provide a therapeutic approach to ameliorate sickness-related anorexia without affecting normal food intake.

342 HYPOGLYCEMIA IN A PATIENT WITH ANTI-INSULIN RECEPTOR ANTIBODIES ASSOCIATED WITH SYSTEMIC LUPUS ERYTHEMATOSIS
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Purpose of Study: Case report.

Methods Used: Case report.

Summary of Results: Background Systemic Lupus Erythematosis (SLE) is an autoimmune disorder which has in rare cases been associated in the development of antibodies to the insulin receptor (IR). x-IR antibodies may antagonize insulin’s physiologic actions, resulting in insulin resistance and hyperglycemia. In addition, x-IR antibodies may confer insulin agonist activity resulting in spontaneous hypoglycemia. We report a case of severe hypoglycemia in a 55 year-old man with SLE that was due to x-IR antibodies.

Case Report: The patient hospitalized at age 53 for “passing out at home” that was associated with severe, recurrent hypoglycemia that required D10 infusion. Insulin and C-peptide levels were 3.6 microgram/ml and <0.5 ng/ml with concurrent serum glucose concentration 51 mg/dl. A diagnosis of SLE was established by having 5 of 11 criteria (serositis; renal-typeIII and V lupus nephritis by biopsy, proteinuria; blood changes-hemolytic anemia, thrombocytopenia; +anti-SM; and +ANA), x-IR antibodies were confirmed by the laboratory of C. Ron Kahn. The patient was treated with corticosteroids and D10 infusion, but recurrent hypoglycemia with intermittent hyperglycemia, requiring insulin therapy, complicated clinical management. Cyclophosphamide and Plasmapharesis were used and resulted in resolution of hyperglycemia and disappearance in x-IR antibody and reappearance of them there after. More recently, the patient has been treated Rituximab, with resolution of hypoglycemia.

Conclusions: The development of x-IR antibodies is a rare phenomenon that may occur in the setting of SLE and result in clinically significant hypoglycemia. Therapies directed toward immune response appeared to be beneficial, with presumed disappearance in x-IR and resolution of life-threatening hypoglycemia.

343 EFFECT OF THIAZOLIDINIDIONE ADMINISTRATION ON BONE TURNOVER MARKERS IN HUMAN SUBJECTS WITH TYPE 2 DIABETES
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Purpose of Study: To determine if changes are present in the bone turnover marker, N-Telopeptide (NTx), when the thiazolidinedione (TZD), Rosiglitazone, is added to the medication regimen in subjects with Type 2 Diabetes. TZDs interact with the peroxisome proliferator-activated receptor-γ (PPAR-γ) nuclear receptors leading to increased insulin sensitivity in Type 2 Diabetes. Activation of PPAR-γ promotes adipocyte differentiation. As adipocytes and osteoblasts share common mesenchymal stem cells essential for osteoblastogenesis, TZDs may directly affect bone metabolism. Rodent and early human data have shown both positive and negative affects of TZD use on bone mineral density. With increased bone turnover, bone mineralization may be lost leading to osteopenia or osteoporosis. NTx, a quantitative measure of the excretion of cross-linked N-telopeptides of type I collagen, is found in urine and correlates with the rate of bone turnover. NTx can thus be used as a marker of bone turnover in patients on TZD therapy, quantifying the TZD effect.

Methods Used: To determine if clinically relevant doses of the TZD, Rosiglitazone, affect bone turnover in humans, this pilot study measured urinary NTx as the primary outcome at 0, 3 & 6 months in subjects treated with 8 mg of Rosiglitazone. Following informed consent, TZD naïve participants had a run in period and were changed from their current oral hypoglycemic monotherapy regimen (sulfonylurea or metformin) to an equivalent dose of the sulfonylurea, glimepiride. After the run in period, baseline urine NTx was performed. Rosiglitazone was then started and titrated to 8 mg QD. Urine NTx was again performed at the study end period and data was analyzed.
Summary of Results: 19 subjects were recruited and completed the protocol. Mean age was 61.3 years (SD 7.6), baseline BMI 30.6 (SD 4.5), and baseline HbA1c 8.4 (SD 0.9). Baseline bone turnover as measured by urine NTx was 30.5 nM BCE/mM Cr and the post treatment NTx was 31.3 nM BCE/mM Cr. This yielded an absolute difference of +0.8% & percent change of 0.8% (p 0.8).

Conclusions: This pilot study showed no statistically significant change in bone turnover after initiation of a TZD. This provides evidence that no early bone damage is occurring with TZD use.

344 DIABETES MELLITUS TYPE 2 AND REACTIVE HYPOGLYCEMIA IN A PATIENT WITH MOSAIC PATTERN TURNER SYNDROME
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Purpose of Study: Case report.
Methods Used: Review of patient history.
Summary of Results: Background Reactive hypoglycemia is characterized by postprandial hypoglycemia, which can be classified as early (within 2–3 hours after a meal) or late (3–5 hours). In early or occult diabetes mellitus, a delay in insulin release after a meal results in initial hyperglycemia, followed by an exaggerated insulin response and late hypoglycemia. In patients with insulin resistance, poor glucose absorption leads to the release of more insulin than is normally required, and hypoglycemia can occur several hours after a meal. A traditional 2-hour oral glucose tolerance test may not detect late reactive hypoglycemia. In these patients, an OGTT lasting 4–6 hours can help diagnose reactive hypoglycemia.

Case: A 29-year-old female with a history of mosaic pattern Turner Syndrome and hypothyroidism was found to have capillary blood glucose measurements above 200 mg/dL during routine evaluation. However, she experienced episodes of lightheadedness, dizziness and sweating and self-monitored blood glucose revealed values less than 30 mg/dL during her symptoms. She did not report any fasting hypoglycemia. Relevant medications include Levothyroxine 25 mcg daily. Her physical exam was normal. Laboratory tests revealed a fasting glucose of 72 mg/dL and a fasting insulin of 15 IUU/mL. Her glucose:insulin ratio was 4.8. She had a normal 24 hour urine free cortisol and cortisol stimulation test. Her proinsulin level was 12.1 (2.1–26.8 pmol/L) and c-peptide value was 1.8 ng/mL (1.1–5 ng/mL). Her glycosylated hemoglobin was 4.7%. The results of an oral glucose tolerance test after a 75 gram glucose load are shown in the table below. The results document hypoglycemia at 240 minutes. She was started on metformin, with improvement in her hyper- and hypoglycemia.

Conclusions: An oral glucose tolerance test was successful in documenting hypoglycemia in a patient with type 2 diabetes mellitus. Patients with diabetes or insulin resistance may have severe hyperglycemic episodes 3–5 hours after a meal, which can be documented using a prolonged OGTT 4–6 hours in length. Further, insulin sensitizers may mitigate reactive hypoglycemia in type 2 diabetes.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>0</th>
<th>60</th>
<th>90</th>
<th>120</th>
<th>150</th>
<th>180</th>
<th>210</th>
<th>240</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dL)</td>
<td>92</td>
<td>229</td>
<td>250</td>
<td>203</td>
<td>124</td>
<td>83</td>
<td>77</td>
<td>40</td>
</tr>
</tbody>
</table>

346 A CASE OF MISSED PHEOCHROMOCYTOMA ONE YEAR AFTER NEPHRECTOMY FOR RENAL TUBERCULOSIS
A.V. Pacheco and P. Kapsner. University of New Mexico Health Science Center, Albuquerque, NM.
Purpose of Study: Case Report.
Methods Used: Review of individual case history.
Summary of Results: A 21 year old man was diagnosed with Von Hippel Lindau at the age of 8 yrs after being diagnosed with a pheochromocytoma following hospitalization for a hypertensive crisis. He underwent a partial right sided adrenalectomy. Two years later, he had a second hypertensive crisis. He underwent resection of a mesenteric pheochromocytoma and a left adrenal pheochromocytoma, with resultant hypoadrenalism. Seven years later he presented with a third hypertensive crisis, a new left sided pheochromocytoma was identified and resected, one year later, a left 6 x 2 cm retro peritoneal mass was noted, and he underwent a fourth surgery. Seven months later a bilobed mass inferior to the most recent resected mass was noted. This mass has been followed over the last year, and has increased in size. Catecholamine levels were significantly elevated.

Meds: phenoxybenzamine 10 mg po bid, Cortef 15 mg q AM 10 mg q PM, Florinef 0.05 mg po qd. FH: Mother: VHL, pheochromocytoma, sister: pheochromocytoma. SH: Patient graduated from HS, no tobacco, ETOH, Illicit Drugs. Currently incarcerated.

Conclusions: We will discuss the epidemiology of pheochromocytoma in distinct genetic disorders, the malignant potential of these tumors, standard interventions and treatments and investigative therapeutic options.

<table>
<thead>
<tr>
<th>g/dl</th>
<th>0</th>
<th>1096</th>
<th>503</th>
<th>504</th>
<th>2000/07</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptomatic/Malignant</td>
<td>HTN</td>
<td>HTN crisis</td>
<td>None</td>
<td>Diuretic, sweating, lable BP</td>
<td></td>
</tr>
<tr>
<td>Labs: none available</td>
<td>Elevated 24h VMA</td>
<td>Plasma metanephrine: (0.4-4) (N&lt;0.40)</td>
<td>Plasma metanephrine: (0.2-6) (N&lt;0.2)</td>
<td>Plasma dopamine: 72 (N&lt;0.20)</td>
<td>Urine normetanephrine: 727 (N&lt;0.45)</td>
</tr>
<tr>
<td>Imaging:</td>
<td>2x3x3cm R adrenal phe</td>
<td>4x5x3cm mesenteric mass, left adrenal phe</td>
<td>Left recurrent adrenal phe</td>
<td>MBG scan: 6x2cm retro peritoneal mass</td>
<td>MRI/CT chest/pelvis: 2x3cm mass at L2-L3, consistent with recurrent phe</td>
</tr>
<tr>
<td>Treatment: Partial R adrenalectomy</td>
<td>Resection of mesenteric phe, left adrenal phe noted, and hemiadrenalectomy done</td>
<td>Both adrenals removed</td>
<td>Mass resected</td>
<td>Pursuing possible surgery, also looking into use of Teflene and 1-13 MBG</td>
<td></td>
</tr>
</tbody>
</table>
discharge. Pathology of the right kidney showed caseating granulomas and she was diagnosed with renal TB. A year later she presented with vague abdominal pain. Further history revealed intermittent mild elevations in blood pressure for which she was not receiving any therapy. She had no headaches, palpitations or sweating episodes and felt well except for the abdominal pain. A repeat CT scan of the abdomen revealed a retrocaval mass, which was thought to possibly be TB or lymphadenopathy; less likely tumor. A CT guided biopsy of this mass was performed without complication, but yielded insufficient tissue for diagnosis. A MRI angio showed a heterogeneously enhancing retrocaval mass with internal cystic complexity near the expected adrenal gland raising the possibility of a primary adrenal lesion. Laboratory evaluation was concerning for a pheochromocytoma. The patient was managed with pre-operative alpha blockers, and tolerated open adrenalectomy. Pathology confirmed the diagnosis of a 5.7 × 4.6 × 2 cm pheochromocytoma with capsular invasion and present in peri-adrenal adipose tissue.

**Conclusions:** Pheochromocytomas are rare and have a variable clinical presentation. Most present with hypertension and episodes of headaches, sweating or palpitations. Even in the absence of the common presentation, consideration should be given to the diagnosis in any patient in which there is a possible lesion involving the adrenal gland. This is especially true in a patient who may require surgical or invasive procedures. This patient did well in spite of the failure to diagnosis her prior to a major surgery and biopsy of the lesion.

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**347 PROLACTINOMA- CORRELATION OF SERUM PROLACTIN LEVELS WITH TUMOR SIZE POST DOPAMINE AGONIST TREATMENT**

R.M. Dawson1, and K. Kapsner2. 1University of New Mexico, Albuquerque, NM and 2University of New Mexico, Albuquerque, NM.

**Purpose of Study:** There are several studies suggesting a positive correlation between serum prolactin level and tumor size prior to treatment with dopamine agonists. Although it is assumed that a decreasing serum prolactin level correlates with a decreased tumor size, no literature exists to define a relationship between serum levels and tumor size during treatment with dopamine agonists. Our purpose is to determine if decreasing serum prolactin level does correlate with decreasing tumor size. If a linear relationship between the two exists, it would make it possible to reduce or eliminate the need for expensive and time consuming follow up radiological testing in the treated patient population.

**Methods Used:** A chart review of patients seen at the University of New Mexico Hospital between 1997–2007 was performed. All patients included in the study had received treatment with dopamine agonists and had received post-treatment MRI of the brain and serum prolactin levels. Patients were recruited using ICD-9 codes rather than serum prolactin levels, so as to reduce bias from excluding patients with prolactinomas and normal serum prolactin levels. Logistic Regression Analysis was then performed to determine if a relationship between serum prolactin level and tumor size existed on dopamine agonist therapy.

**Summary of Results:** A preliminary review of our data demonstrates a relationship between serum prolactin levels and tumor size post dopamine agonist therapy. While the relationship is not directly linear, we are currently in the process of adding additional data points to our study by including another 2 years of patient encounters.

**Conclusions:** Our preliminary results suggest a positive association between serum prolactin level and tumor size. However, there is not a statistically significant linear association allowing us to recommend against repeat MRI to assess tumor size in treated patients. With the addition of more patients to our data pool, we may more firmly establish a linear association. Based on our results, we will discuss recommendations for repeat imaging in prolactinoma patients treated with dopamine agonists.

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**348 1α, 25-(OH)2 D3 (1,25D) INHIBITS CELL PROLIFERATION AND PROMOTES CELL CYCLE ARREST WITHOUT INDUCING APOPTOSIS IN MULTIPOTENT CELLS**

J.N. Artaza1, M. Braga1, and K.C. Norris1, 2. The Charles Drew University, Los Angeles, CA and 3UCLA, Los Angeles, CA.

**Purpose of Study:** The vitamin D receptor (VDR) and its ligand 1,25D (the biologically active form of vitamin D), play an important role in the regulation of cell growth and cell differentiation. In this study we evaluated the role of 1,25D on cell proliferation, apoptosis and cell cycle progression in multipotent cells.

**Methods Used:** Briefly, C3H 10 T1/2 multipotent cells were primed with 5′-azacytidine to induce differentiation for two days, and then treated with 1,25D at different doses (from 10 nM to 500 nM), or with Ethanol <0.1% as vehicle in a time course manner for 4 days. Cell proliferation was evaluated by the formazan assay and for the expression of proliferating cell nuclear antigen (pcNA) by quantitative immuno- cytochemistry (QICC). Apoptosis was evaluated by TUNEL assay. The biological activity of 1,25D was evaluated by change in expression and sub-cellular localization of VDR by IF and Western blot. The expression of genes related to the cell cycle was analyzed by RT2PCF microarrays after total RNA isolation.

**Summary of Results:** Increasing doses of 1,25D (plateau effect noted at 100 nM) 1) inhibits cell proliferation; 2) do not promote apoptosis; and 3) decreased the expression of several genes related to the regulation of the G1 Phase and G1/S Transition: (e.g. Cdk6, Ppp3r1); S Phase and DNA Replication: (e.g. Mcm2/3/4, Mki67 (cell proliferation), Rad51, pCNA [cell proliferation]); G2 Phase and G2/M Transition: (Chek1); M Phase: (e.g. Cdk2, Wee1, Rad21), and 4) repressed several Cell Cycle Checkpoint and Cell Cycle Arrest genes: (e.g. Casp3, Mad2l1, Mdm2); Regulation of the Cell Cycle: (Cena2, Ccnb1/2, Ccn1); and Negative Regulation of the Cell Cycle: (Brc1 [breast cancer 1 gene], Casp3 [an apoptosis inhibitor]).

**Conclusions:** We concluded that the addition of 1,25D, the biologically active form of vitamin D, inhibits cell proliferation and promotes cell cycle arrest without promoting apoptosis in mesenchymal multipotent cells through activation and nuclear translocation of the VDR receptor.
adipogenic markers by IF and western blots at different time points, and by quantitative image analysis (qIA) of fat cells before and after Ohi-Red-O staining after 12 days. The anti-inflammatory/fibrosis effect was evaluated by the expression of pro-inflammatory/fibrosis factors: TGF-β and PAI-1 by quantitative immuno-cytochemistry (QICC) and Western blot. Collagen I and III deposition were also assessed by QICC.

**Summary of Results:** The addition of 1,25D to multipotent cells promotes 1) increased expression and nuclear translocation of the VDR; 2) increased expression of very early adipogenic markers, namely C/EBPβ and C/EBPδ; 3) increased expression of C/EBPα and PPARγ (key adipogenic regulators) and PPARα (+3.82 ratio by QRT/PCR); 4) a 3-fold increase in the number of fat cells per field after 12 days, 5) decreased expression of TGF-β and PAI-1 as well as expression of collagen I and collagen III (pro-inflammatory/fibrosis factors).

**Conclusions:** We concluded that 1,25D, the biologically active form of vitamin D, induces an adipogenic conversion and the promotion of an anti-inflammatory/fibrosis phenotype in multipotent cells suggesting that supplementation of Vitamin D could be a rational anti-inflammatory/ fibrosis strategy in therapeutic treatment of chronic diseases such as renal or cardiac fibrosis.

**350 CEREBROSPINAL FLUID LEAK AFTER CABERGOLINE THERAPY**

W.C. Chapin and M. Fleischer. OHSU, Portland, OR.

**Purpose of Study:** To present a case of cerebrospinal fluid(CSF) leak and pneumoencephalus in a patient with giant prolactinoma after three doses of cabergoline(C). CSF leakage has sporadically been reported in patients with prolactinomas treated with dopamine agonists such as bromocriptine. The occurrence after (C) use has been described just in nine patients to date.

**Methods Used:** We describe the clinical course, biochemical and imaging findings of a 54 year old female patient with MEN1 syndrome and a giant prolactinoma who developed CSF leak and pneumoencephalus shortly after initiating (C).

**Summary of Results:** The patient presented with CSF leak after three doses of (C) and shrinkage of the tumor corresponded with a fall in prolactin from 12000 ng/ml to 1270 ng/ml. Both MRI and surgical findings found the tumor had extended well beyond the sella turcica causing ethmoid and sphenoid bone erosion with prolapse of tumor into the sphenoid sinus. Intraoperatively a CSF leak was identified from both ethmoid and sphenoid sinuses. Intraoperatively a CSF leak was identified from both ethmoid and sphenoid sinuses. Debulking of the pituitary tumor was performed at the time of the CSF leak repair with post-operative complications. CSF leak after the initiation of (C) in patients with prolactinoma is an uncommon complication that has been described in a small number of patients. This complication arises with previously existing skull base erosions from tumor. The onset of CSF leaks varies from days to months after initiation of various doses of (C). Rapid, often large, reductions in tumor size and prolactin levels have been documented in these patients. It is presumed that physical shrinkage in the tumor clinically unmasks these underlying erosions. The management of CSF leaks following dopamine agonist treatment for invasive prolactinomas is difficult and there is no clear consensus for its treatment. Whether one continues (C) to prevent further tumor growth after CSF leak repair and debulking of the tumor is unclear. Furthermore, the risk of recurrent CSF leak upon reintiating (C) after surgical closure of the skull base defect is unknown.

**Conclusions:** This is the first reported case of CSF leak after (C) use in a prolactinoma patient with MEN1. Surgical resection of the pituitary tumor at the time of CSF leak repair is the treatment of choice in patients with macroadenomas and dopamine agonist related CSF leaks. This case underlines that certain clinical settings still require surgical therapy of prolactinomas.
Purpose of Study: Previous studies have demonstrated that African Americans have lower response rates to hepatitis C (HCV) therapy. However, the response of Hispanics to pegylated interferon and ribavirin remains unclear. The aim of this study was to compare the treatment response of Hispanic to non-Hispanic white patients.

Methods Used: A retrospective review was conducted of all treatment-naive Hispanics and non-Hispanic whites (NHW) with HCV who were treated at the University of New Mexico or Albuquerque VA Medical Center between October 2001 and January 2007. Genotype 1 patients received 12 months of therapy with pegylated interferon and ribavirin; genotype 2 and 3 patients received 6 months of treatment.

Summary of Results: A total of 396 patients were included in the analysis, consisting of 179 Hispanics and 217 NHW. Baseline characteristics between the two groups were not significantly different. Of the 179 Hispanic patients, 64% were genotype 1 and 36% genotype 2/3. Of the 217 NHW, 61% were genotype 1 and 39% genotype 2/3. More Hispanics prematurely discontinued therapy compared to NHW (35% vs. 20%, p < 0.001). In genotype 1 patients, early virologic response (EVR), end of treatment response (ETR), and sustained virologic response (SVR) did not significantly differ between the two groups. Results for genotype 2/3 patients are presented in the table below.

Conclusions: Hispanics with genotype 2/3 hepatitis C infection are less likely to achieve ETR and SVR with pegylated interferon and ribavirin therapy compared to non-Hispanic whites. After correcting for treatment discontinuation, SVR in genotype 2/3 Hispanics continued to be lower due to increased relapse and a diminished ability to maintain viral clearance after treatment completion.

<table>
<thead>
<tr>
<th></th>
<th>Hispanics</th>
<th>Non-Hispanic Whites</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early Virologic Response (EVR)</td>
<td>81%</td>
<td>88%</td>
<td>NS</td>
</tr>
<tr>
<td>End of Treatment Response (ETR)</td>
<td>64%</td>
<td>83%</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Sustained Virologic Response (SVR)</td>
<td>45%</td>
<td>75%</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>After correcting for premature treatment discontinuation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sustained Virologic Response (SVR)</td>
<td>66%</td>
<td>87%</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Relapse after achieving End of Treatment Response</td>
<td>25%</td>
<td>8%</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

AN UNUSUAL CAUSE OF LIVER FAILURE IN A 17 YEAR OLD PREGNANT WOMAN
R. Brandes¹, J. Bunning², and J. Dunkelberg¹. ¹University of New Mexico, Albuquerque, NM and ²University of New Mexico, Albuquerque, NM.

Purpose of Study: Case report to review the unique considerations related to liver disease in pregnancy.

Methods Used: Pregnancy can be associated with several hepatic complications, including HELLP syndrome, cholestasis of pregnancy, and acute fatty liver of pregnancy. Each of these entities has unique findings and timing of presentation, and they may be recurrent in subsequent pregnancies. It is important to recognize other causes of liver dysfunction during pregnancy.

Summary of Results: Case: We present a case of a 17 year old woman with a previously uncomplicated, normal pregnancy. She presented to an outside facility at the 27th week of pregnancy with right flank pain, was treated for pyelonephritis, and was transferred to our facility. A severe hepatitis developed and progressed to fulminant liver failure. Urgent cesarean section was performed for presumed chorioamnionitis. Liver biopsy was performed during the cesarean section. Histology showed coagulative necrosis with nuclear inclusions consistent with herpes simplex hepatitis, as well as cytoplasmic clearing and ballooning degeneration suggestive of acute fatty liver of pregnancy. Immunohistochemistry was strongly positive for HSV in both the liver and the placenta. The patient recovered with high-dose Acyclovir and supportive care.

Conclusions: Discussion: HSV is a known cause of fulminant hepatic failure, and HSV-related hepatic failure is more common in pregnant women, neonates and other immunocompromised individuals. In the setting of pregnancy, HSV hepatitis can be a difficult diagnosis to make due to the other known hepatic diseases that are unique to pregnancy. Maternal and perinatal mortality rates are nearly 40%. Liver biopsy is essential to rapid diagnosis, and swift treatment with high-dose Acyclovir is required for good outcomes.

FIVE YEAR EXPERIENCE WITH TENOFOVIR FOR LAMIVUDINE RESISTANT HEPATITIS B VIRUS
A. Dudukula¹, C. Kulig², J. Trotter², and G. Everson¹,². ¹UCHSC, Denver, CO; ²UCHSC, Denver, CO and ³Denver VAMC, Denver, CO.

Purpose of Study: Virologic breakthrough during HBV therapy with lamivudine is often due to development of YMDD mutations (YMDD+). Tenofovir disoproxil fumarate (TDF), a nucleotide analog approved for HIV therapy and has activity against HBV in HIV/HBV co-infection, YMDD+ and wild-type HBV. We sought to describe the efficacy and any evidence of resistance to Tenofovir disoproxil fumarate 150 mg therapy in YMDD+ HBV infection.

Methods Used: An IRB approved retrospective analysis of TDF therapy in YMDD+ HBV infection at UCHSC hepatology clinic was conducted; data collected included HBV DNA levels, mutational analysis, patient demographics and standard biochemistries.

Summary of Results: Four male patients and four female patients had YMDD+ HBV infection. Prior to TDF, they had been treated with lamivudine 75 mg to 150 mg for 1–4 years; age ranged from 33–67 (mean = 47.5) yrs. Mean TDF therapy duration was 59 months (46–69 months). Seven patients were treated with 150 initially, one with 300 mg.

Complete virological suppression was achieved in 3 patients at TDF 150 mg QD in an average of 15.75 months; ALT levels normalized by 12 months. DNA levels decreased by 3.8934 log10 copies/mL and 7.0614 log10 copies/mL at 3 and 12 months, respectively, after starting TDF therapy. These 3 patients also had HBVeAb seroconversion at 11, 12 and 52 months with a mean of 25 months. One patient had complete virological suppression at 12 months followed by resurgence at 43 months not suppressed at TDF of 300 mg QD. One patient has complete virological suppression at 9 months followed by resurgence at 29 months and eventual complete suppression on TDF of 300 mg in 29 months after increasing the dose. Three patients never had complete virological suppression, one had TDF increased to 300 mg at 2.25 months of therapy, one was started on 300 mg QD from beginning and one is on TDF at 150 mg QD.

Conclusions: TDF 150 mg has anti-HBV effects and is effective for some individuals with YMDD+ HBV infection. However, lack of complete DNA suppression in some individuals may be related to mutational virologic resistance, patient compliance and/or altered TDF metabolism. Hence, additional analyses are required to further evaluate the anti-HBV efficacy and potential mutational-based resistance for TDF in HBV infection.

CIPROFLOXacin IN RABBITS INFECTED WITH A SHIGA-TOxin (STX)-PRODUCING E. CoLI (STEC) STRAIN LEADS TO WORSE CLINICAL OUTCOME AND INCREASED TOxin RELEASE

Volume 56, Issue 1 221
Purpose of Study: Antibiotics should not be given to patients with STEC (E. coli O157-H7) infections since patients receiving antibiotics have a worse clinical outcome. This may result from increased shiga toxin synthesis and release due to antibiotic induction of lysogenic Stx-encoding bacteriophages. To test this in an animal model of STEC infection: we infected Dutch Belted Rabbits (DBR) with a rabbit-specific STEC strain; gave antibiotics (ciprofloxacin) at the onset of clinical infection; observed the clinical course of the disease (weight change, stool character, challenge strain shedding); and sacrificed the animals on day 7 to determine toxin and challenge strain concentrations, and minimal inhibitory concentration (MIC) of antibiotic in the cecum.

Methods Used: 5 groups of DBR were challenged with 10 to the 10 E22 Stx 2, an attaching/effacing rabbit pathogen encoding Stx2 on a phage from an O157-H7 strain and a control group received PBS. Ciprofloxacin, at doses of 0.2 to 30 mg/kg/day, was given to 4 groups starting on day 3 post inoculation to achieve a dose yielding sub-inhibitory concentration of antibiotics in the cecum. Rabbits were sacrificed on Day 7 to determine cecal toxin concentrations by HeLa cell cytotoxicity assay, cecal antibiotic levels by MIC, and concentration of the challenge strain as colony forming units (CFU)/gram.

Summary of Results: The rabbits given an infectious dose of an STEC strain with a Stx2-encoding bacteriophage that received the highest dose of the fluorquinolone antibiotic ciprofloxacin (30 mg/kg/day) had the most severe illness and worst outcome as manifest by the least weight gain. This group had the highest level of antibiotics in the cecal contents, the most effective killing of the challenge strain, but also the highest level of free shiga toxin in cecal supernatants.

Conclusions: These results support the prior findings of a prospective study in humans that administration of antibiotics during STEC infection results in a worse outcome. It also supports the hypothesis that this effect of antibiotics is due to toxin release into the intestine as organisms are killed. Since fluorquinolones are known to cause induction of Stx2 bacteriophages from strains of O157:H7, these studies also support this mechanism of antibiotic action.

537 ABSENCE OF PNEUMOCYSTIS CARINII PNEUMONIA IN LIVER TRANSPLANT RECIPIENTS USING A SHORT TERM (3-MONTH) PROPHYLACTIC REGIMEN

J.M. Lancaster1, J. Trotter1, M. Levi1, and T. Steinberg2, 1University of Colorado Health Sciences Center, Denver, CO and 2University of Colorado Hospital, Denver, CO.

Purpose of Study: Immunosuppression, often following organ transplantation or due to human immunodeficiency virus (HIV), predisposes patients to Pneumocystis jiroveci pneumonia (PCP). Current regimens of immunosuppression following liver transplantation are likely much lower than those seen in the 1980s and 1990s. Although common early in the experience of liver transplantation, with infection rates near 10%, PCP incidence has decreased dramatically with the introduction of effective prophylactic therapy. While many centers currently use prophylaxis for 12 months following liver transplantation, we report our experience with a 3-month prophylactic regimen over 10 years.

Methods Used: Medical records of 967 patients undergoing 1040 liver transplants at the University of Colorado Health Sciences Center were reviewed. The clinical microbiology laboratory archives were reviewed for positive PCP diagnoses from 1997 to current. Additionally, the internal liver transplant database was reviewed for PCP infection and immunosuppression regimens from January 1992 to July 2007.

Summary of Results: From 1992 to 2007 we identified on 3 out of 967 liver recipients who developed PCP. Two of these cases occurred in 1997, and there has not been a single case of PCP at our center in the past 10 years.

Conclusions: Using a 3-month prophylactic regimen and steroid avoidance, the incidence of PCP at our center is 0, suggesting that a prolonged course of prophylaxis may not be required in liver transplant recipients.

538 IMMUNOHISTOCHEMICAL STAINING FOR THE TUMOR MARKERS P53, MIB, CAM5.2, CEA, CA19-9 AND alcian BLUE/PAS INCREASES THE DIAGNOSTIC YIELD OFBILE DUCT BIOPSY FROM PATIENTS WITH SUSPECTED MALIGNANCY

A.W. Jahng1, J.M. Nieto3, D. Chung3, S. Reicher2, B. Pham2, R. Venegas1, S. French1, and V. Eysselinck2. 1Harbor-UCLA, Torrance, CA; 2Harbor-UCLA, Torrance, CA; and 3Harbor-UCLA, Torrance, CA.

Purpose of Study: Biliary strictures from malignant and benign pathologies may be difficult to differentiate. Endoscopic cytology and
biopsy samples are highly specific, but lack sensitivity due to the difficulty of the procedure and the inherent pathologies of the strictures, leading to either a suboptimal or a non-diagnostic result. The aim of this study is to improve the accuracy of the endoscopic biopsy samples in differentiating benign and malignant strictures by the addition of immunohistochemical tumor markers for malignancy.

**Methods Used:** 20 cases of biliary strictures were retrospectively reviewed, some of which underwent multiple endoscopies. Final diagnosis was established from clinical follow-up and correlate, or by direct surgical specimens. Biopsy histology with immunohistochemical staining for p53, MIB, CAM5.2, CEA, CA19-9, and Alcian blue/PAS (AB PAS), alone or in combination, were analyzed for their ability to detect and distinguish malignant strictures. For this study, atypical samples were considered to be benign.

**Summary of Results:** 11 benign and 9 malignant biliary strictures were identified. Biopsy histology had sensitivity of 55% with specificity of 100% to detect malignancy. Addition of all 6 immunohistochemical markers increased the sensitivity to 82% with specificity of 20%. In optimizing for accuracy, AB PAS alone had best accuracy with sensitivity of 64% and specificity of 93%; addition of AB PAS staining to histology did not change the diagnostic yield. Addition of p53 staining to histology increased the sensitivity to 64% with a specificity of 87%.

**Conclusions:** Addition of immunohistochemical staining for tumor markers improves the diagnostic yield of bile duct biopsies for suspected malignancies. A larger number of patients is needed to confirm our findings.

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**Hematology and Oncology II**

**Concurrent Session**

8:30 AM

Saturday, February 2, 2008

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**359**

**MAPPING OF A CANDIDATE SUPRATENTORIAL PRIMITIVE NEUROECTODERMAL TUMOR LOCUS ON CHR19Q**

Z.A. Fang, K. Lee, and A. Huang

1 University of British Columbia, Vancouver, BC, Canada; 2 University of Toronto, Toronto, ON, Canada and 3 Hospital for Sick Children, Toronto, ON, Canada.

**Purpose of Study:** Primitive neuroectodermal tumours (PNET) are the most common malignant brain tumors in children, comprising of about 20-30% of all pediatric CNS tumors. PNETs include supratentorial primitive neuroectodermal tumors (sPNET), tumors located in the cerebrum, and medulloblastomas, tumors located in the cerebellum. sPNETs are rare but aggressive tumors that do not respond well to treatment. The poor prognosis and limited treatment options present a need to obtain a better understanding of these tumors on a molecular basis in order to develop better treatment options; however, the rarity of sPNETs has prevented extensive genomic analysis of these tumors.

The goal of my project was to delineate the specific genes that are altered in sPNET tumors within a specific region on chromosome 19q that has been observed to be amplified in a subset of sPNETs.

**Methods Used:** To study the genetics of sPNETs, 29 tumors were analyzed using Affymetrix 500K SNP array. Real-time PCR was then used to validate the copy number changes that were observed in the SNP array data, and to identify focal changes within the region of amplification. RT PCR was also used to examine the expression profile of select candidate genes.

**Summary of Results:** The in silico data demonstrated that 14% of the sPNET tumor samples exhibited chr19q13.41-13.42 amplification. The boundaries of the amplicon were determined to be chr19:58,889,323-59,594,447. Bioinformatic tools were then used to identify the specific genes that are present in this region and their functions, and the list of candidate genes were narrowed down to include a microRNA cluster, PKCG, NALP12, and LILRA4. The real time PCR results for 5 loci showed copy number amplification in tumor samples with copy numbers ranging from 3 to 24. Reverse transcriptase PCR results at the PRKCG loci did not show increased expression in medulloblastoma and sPNET cell lines.

**Conclusions:** A novel amplicon on chromosome 19q13.41-13.42 was identified in 14% sPNET tumor samples. Reverse transcriptase PCR analysis of the loci PRKCG did not show an increase in expression levels in sPNET and medulloblastoma cell lines.

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**360**

**THE ROLE OF NRF2-REGULATED GENES IN CHEMORESISTANCE OF MESOTHELIOMA**

C.L. Nguyen, H. Gao, A. Campbell, A. Pham, X. Bi, L. Guanghong, Z. Hu, J. Abad, and Y. Huang

1 Western University of Health Science, Pomona, CA; 2 Western University of Health Science, Pomona, CA and 3 City of Hope National Medical Center, Duarte, CA.

**Purpose of Study:** Malignant mesothelioma is an aggressive and treatment-resistant tumor; the incidence of this tumor is increasing throughout the world. Several studies have shown that the expression of genes encoding antioxidants (e.g. glutathione) are increased in mesothelioma cells and may play an important role in drug resistance. The synthesis of glutathione requires the rate-limiting cellular uptake of cystine via the cystine-glutamate transporter SLC7A11. Our previous studies showed that SLC7A11 overexpression in lung and ovarian cancers conferred chemoresistance to multiple anticancer agents. The present study investigated the expression and activity of SLC7A11 and related genes such as NADPH quinine oxidoreductase 1 (NQO1), both of which are positively regulated by the transcriptional factor NRF2 and negatively regulated by the NRF2 suppressor KEAP1.

**Methods Used:** We examined the mRNA and protein levels of SLC7A11, NQO1, NRF2 and KEAP1 in eight mesothelioma cell lines using RT-PCR, Dot Blot, Western Blot and immunocytochemistry techniques. We also inhibited the transport activity of SLC7A11 using glutamate or (S)-4-carboxyphenylglycine and examined their effects on the sensitivity of tumor cells to anticancer drugs geldanamycin and L-alanosine.

**Summary of Results:** Our results indicated that SLC7A11 and NQO1 were differentially expressed by the eight mesothelioma cell lines and their expression levels correlated with the drug resistance in these cells: In cell lines with high levels of SLC7A11 expression, SLC7A11 inhibition significantly increased the potency of geldanamycin, while the potency of L-alanosine, an amino acid analog and potential substrate of SLC7A11 was reduced. No somatic mutation in exon-coding regions of the KEAP1 gene was detected in the eight cell lines.

**Conclusions:** These findings indicate a possible role of NRF2-regulated proteins such as SLC7A11 and NQO1 in chemoresistance of mesothelioma. Understanding the function of these proteins will allow more effective pharmacological treatment for this illness.

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**361**

**SURFACE EXPRESSION OF CD40 ON HUMAN MELANOMA AND THE DIRECT IN VITRO GROWTH EFFECTS OF AN ANTI-CD40 AGONIST ANTIBODY, CP-870,893**

A. Kalbasi, B. Chmielowski, and A. Ribai

1 UCLA David Geffen School of Medicine, Los Angeles, CA and 2 UCLA David Geffen School of Medicine, Los Angeles, CA.
Purpose of Study: Whereas in immune cells CD40 seems to play an activating role, engagement of CD40 with CD40L on tumor cells can cause growth inhibition via apoptosis. This makes CD40 an intriguing clinical target; a recent phase I study in solid tumors tested a novel anti-CD40 agonist monoclonal antibody, CP-870,893. Previous studies have shown that 40–60% of human melanoma cells express CD40. Here, we confirm the expression of CD40 on human melanoma cell lines. Then, we examine the direct in vitro effects of CP-870,893 on human melanoma cell viability to test whether it inhibits growth, as seen with CD40L.

Methods Used: Expression was evaluated by flow cytometry, and cell viability was measured by bioluminescence and MTT assays.

Summary of Results: Fifty percent of tested cell lines expressed CD40 (4/12 had high CD40 expression and 2/12 had marginal expression). CD40 expression was upregulated by histone deacetylase inhibitor (HDACi) in all of the CD40+ cell lines, and moreover, HDACi induced CD40 expression in 1/5 CD40- cell lines. Of the CD40+ cell lines, CD40 expression in SKMEL28 is most upregulated by HDACi. Interferon-γ treatment did not significantly change CD40 expression in any cell lines, unlike what had been described in prior studies. Antibody treatment did not significantly change the viability of melanoma cells. After CD40 upregulation of SKMEL28 by HDACi, CP-870,893 treatment of SKMEL28 conferred a survival advantage.

Conclusions: Seemingly paradoxically, high concentrations of CP-870,893 in combination with upregulated CD40 expression may promote growth in melanoma cells. Otherwise, in the cell lines tested, CP-870,893 does not have a direct cytotoxic effect on CD40+ or CD40- melanoma.

362 IDENTIFICATION OF HEMANGIOBLAST FORMATION FROM HUMAN EMBRYONIC STEM CELL

J.S. Lee1, E. Carrier2, and S. Takayasu2. 1Western University of Health Sciences, Downey, CA and 2UCSD, La Jolla, CA.

Purpose of Study: Hemangioblast is the term first introduced by P.D.F Murray in 1932, for early progenitor cells that can differentiate into both blood and blood vessels. The existence of a hemangioblast is inferred from the observation of a parallel generation of endothelial and hematopoietic progenitor cells from a single progenitor. The hemangioblast is important both diagnostically and therapeutically. Some examples for a therapeutic use of the hemangioblasts would be in heart attacks and stroke in the case of patients who suffer from hypoproliferative vasculopathies. Our study aims at delineating various gene expressions during course of hemangioblast formation, as well as understanding molecular mechanism leading to its formation. Our objective is to find methods for confirming hemangioblast formation for future studies.

Methods Used: Generation of hemangioblasts begins with growing and maintaining human embryonic stem cells. Embryoid bodies (EB) were induced in primary cultures and this process took about 2–4 days. EB-derived cells were plated into a secondary differentiation culture to promote early hematopoietic and endothelial development. The specific cytokines that were used to promote the development of the hemangioblast were VEGF (endothelial), SCF (hematopoietic), TPO (both endothelial and hematopoietic) and EPO (hematopoietic). As a result of this exposure, EB-derived cells were differentiated into blast like colony forming unit (BL-CFU), otherwise called hemangioblast. EB were stained using Giemsa for morphological evaluation. These hemangioblasts were then tested for expression of FLK-1, Runx-1, SCF, HEX and GATA-1.

Summary of Results: The result showed a progressive increase in expression of Flk-1, new expression of SCL, and Runx genes, and disappearance of Oct4 gene expression (pluripotent gene).

Conclusions: If confirmed with repeated experiments, the expression of SCL and Runx genes along with loss of Oct4 gene expression may be a marker indicating the presence of hemangioblast. This method may facilitate identification and study of hemangioblasts in the future.

363 IN VITRO DRUG SENSITIVITY AS A PREDICTIVE TOOL OF EARLY CLINICAL RESPONSE IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

F.C. Galderisi, L. Stork, J. Li, M. Mori, S. Mongeau-Tchokote, and J. Huang. Oregon Health and Science University, Portland, OR.

Purpose of Study: Residual disease or rapidity of response to induction therapy is among the most powerful predictors of outcome in pediatric Acute Lymphoblastic Leukemia (ALL). We hypothesize that drug sensitivity at the cellular level predicts rapidity of response in ALL. We recently developed a flow cytometry based cytotoxicity assay for in vitro cellular drug response profiling for pediatric ALL. We report preliminary data that correlate results of this drug sensitivity assay with rapidity of response to induction therapy among patients with ALL.

Methods Used: We performed in vitro tests, applying a flow cytometric drug cytotoxicity assay, on cryopreserved bone marrow (BM) samples of 23 patients with newly diagnosed ALL. Fourteen patients were rapid early responders (RER) and 9 were slow early responders (SER) by Children’s Oncology Group criteria. Drugs were tested at three different concentrations. Leukemic cell survival index (LCSI) was determined at 48 hours after in vitro exposure to individual standard induction agents for pediatric ALL. LCSI differences between RER and SER were compared for each drug and concentration, as well as over the three concentrations (referred to as “averaged concentrations”).

Summary of Results: For dexamethasone, a significantly lower LCSI was seen in the RER compared with the SER cohort at individual and averaged concentrations (p = 0.01, mixed effects model). A trend toward a lower mean LCSI in the RER compared with the SER group was noted for asparaginase and vincristine (p < 0.1). Mean LCSI was not different between the RER and SER groups for daunomycin and prednisone.

Conclusions: This in vitro drug sensitivity assay provides a response profile for dexamethasone that correlates with in vivo response to induction therapy. Research is ongoing with more patient samples in order to achieve a greater statistical power to detect a smaller difference for all drugs tested. These results will determine the potential value of this assay for earlier risk stratification and modification of therapy in de novo or relapsed ALL.

364 THE NOVEL MULTI-TARGETED RECEPTOR TYROSINE KINASE INHIBITOR MP470 INHIBITS SYNOVIAL SARCOMA PROLIFERATION IN VITRO


Purpose of Study: Synovial sarcoma (SS) is a soft tissue cancer primarily of young adults. Despite best treatments of chemotherapy and radiation, the 10-year survival rate is only 10–30%. Obviously, new treatments are desperately needed for this young patient population. In this study, a novel receptor tyrosine kinase (RTK) inhibitor, MP470, was tested against two SS cell lines, HS-SY-II and SYO-I. MP470 has limited side effects in animal studies and is currently being tested for safety in a Phase I clinical trial.

Methods Used: MTS proliferation assays were used to determine MP470 inhibition of SS cell line growth. Flow cytometry with propidium iodide

...
was used for cell cycle studies, while an annexin V and propidium iodide kit was used to measure apoptosis. Western blots were used to examine protein phosphorylation status.

**Summary of Results:** MP470 was found to inhibit proliferation in vitro with an IC50 below 10 IM. MP470 treatment showed a limited ability to induce apoptosis, indicating a primarily cytosstatic effect in SS. This was confirmed by MP470 treatment causing a non-specific cell cycle arrest. With respect to molecular targets, MP470 was observed to inhibit phosphorylation of multiple RTKs known to be expressed in SS: Met, Kit, and Axl. Additionally, the downstream signaling nodes Akt and Erk1,2 showed a loss of phosphorylation.

**Conclusions:** Our results demonstrate the efficacy of the agent against SS in vitro, which most likely occurs through inhibition of a number of RTKs. Animal studies indicating in vivo anti-tumor activity, together with successful Phase I safety testing, would support a Phase II trial of MP470 in patients with synovial or other soft tissue sarcomas.

### 365 DUTASTERIDE EFFECTS ON GENES ASSOCIATED WITH PROSTATE CANCER PROGRESSION: IMPLICATIONS FOR PROSTATE CANCER CHEMOPREVENTION

L. Geng1, E. Mostaghel2, R. Coleman2, and P. Nelson2. 1University of Washington School of Medicine, Seattle, WA and 2Fred Hutchinson Cancer Research Center, Seattle, WA.

**Purpose of Study:** Prostate cancer development is greatly influenced by androgens. 5-a-reductase (SRD5A) inhibitors block the conversion of testosterone to the more potent androgen dihydrotestosterone, and may reduce the risk of prostate cancer. The Prostate Cancer Prevention Trial (PCPT) revealed a remarkable 25% overall reduction in prostate cancer incidence following treatment with a SRD5A inhibitor, but was accompanied by an unanticipated increase in the incidence of high grade tumors. We hypothesized that SRD5A inhibition may lead to the development of high grade prostate tumors by influencing the expression of genes linked to the development of invasive tumors.

**Methods Used:** We examined the expression of genes associated with high grade/invasive prostate cancers in prostate samples from patients treated with the SRD5A inhibitor dutasteride. Prostate samples were obtained from a clinical trial in which men with localized prostate cancer were randomized to treatment with placebo or dutasteride (at 0.5 or 3.5 mg orally daily) for 3 months prior to prostatectomy. Benign epithelial and stromal cell populations were separately isolated by laser-capture microdissection from frozen tissue sections and subject to RNA isolation and amplification. Quantitative RT-PCR was used to assess the mRNA expression of about 40 genes associated with aggressive prostate tumor profiles.

**Summary of Results:** Most of genes we examined were not influenced by dutasteride treatment. Compared to placebo treated samples, dutasteride was associated with lower CXCL12 but higher CXC4 expression in the epithelial samples, and lower EGF but higher CTGF and HGF expression in the stromal samples.

**Conclusions:** Short term treatment with dutasteride altered the expression of very few genes that were identified as important in tumor progression, suggesting that dutasteride does not induce an environment conducive to the development of high grade prostate cancer. Examination of samples from larger studies of longer duration, such as the ongoing REDUCE trial of chemoprevention, will be useful in evaluating the significance of the relatively limited changes in gene expression observed in our study.

### 366 EVALUATION OF FAILURE PATTERNS IN NON-SMALL CELL LUNG CANCER

J. Pakish1, P. Yen1, P. Sarah1, T. Quang1,2, N. Bittner1, R. Martins3, D. Wood4, K. Eaton1, L. Carr3, M. Mulligan3, and S. Patel1. 1University of Washington Medical Center, Seattle, WA; 2Puget Sound Veterans Administration, Seattle, WA; 3Seattle Cancer Care Alliance, Seattle, WA and 4University of Washington Medical Center, Seattle, WA.

**Purpose of Study:** Previous studies have investigated the effectiveness of trimodality therapy in stage IIIA and IIB non-small-cell lung cancer, but the results have been inconclusive and no new standard of care has been defined.

**Methods Used:** 836 patients were identified as being diagnosed with lung cancer between January 1994 and March 2007. Of these 99 were identified as having Stage IIIA and IIB non small cell lung cancer. These patients were analyzed retrospectively based on treatment plans with a focus on failure differences seen in chemoradiation alone (Arm 1) versus neoadjuvant chemoradiation followed by surgical resection (Arm 2). Arm 1 (n = 81) included 28 (34.6%) patients with pathological stage IIIA disease and 53 (65.4%) with stage IIIB disease. Arm 2 (n = 18) contained 17 lobe resections and 1 pneumonectomy with 14 (77.8%) patients identified as pathological stage IIIA disease and 4 (22.2%) as stage IIIB disease.

**Summary of Results:** When comparing patients in Arm 2 versus those in Arm 1, 3 year overall survival was 38.0% v. 16.1%, (p = 0.013 ). Arm 2 in comparison to Arm 1 also demonstrated an improved median survival; median 20 v. 11 months, (p = 0.013).

**Conclusions:** OS of Arm 2 validates the most recent non small lung cancer intergroup study. Our retrospective study however had a lower 3 year survival in Arm 1 when compared to this study. The discrepancy in OS for Arm 1 most likely reflects both the inclusion of patients with more progressive disease (stage IIIB) and those of poor surgical candidacy. In reviewing the literature along with this data, patients with stage IIIA and IIB NSCLC and a high performance status should be considered for trimodality treatment.

### 367 OVARIAN CLEAR CELL CARCINOMA: AN EXAMINATION OF RELAPSES

P. Nguyen1, and F. Wong2. 1UBC, Vancouver, BC, Canada and 2BC Cancer Agency, Surrey, BC, Canada.

**Purpose of Study:** To examine the relapse rate and relapse pattern as influenced by the use of adjuvant whole abdominal radiotherapy.

**Methods Used:** Data from the Cheryl Brown Ovarian Cancer Outcomes Group database and retrospective chart review were used. Between 1994 to 2000, 174 cases of OCCC were identified in BC Cancer Agency, and 149 cases had had pathology re-confirmation. Information was extracted from the reviewed cases only. The sites of first relapse were further reviewed to determine whether they were inside or outside of the pelvis or whole abdomen region (radiation treatment field). The relapse frequency, and relapse free interval were correlated with adjuvant therapy, using Univariate and multi-variate analysis.

**Summary of Results:** 44 patients presented with stage 1C disease and 50 patients with stage 2C. The remaining 45 patients were distributed in stages 1A,B, 2A,B and 3A,B,C. Consequently, the statistical analyses were restricted to stage 1C and 2C disease only. Among stage 1C, 17 patients had chemotherapy alone (Chemo) and 27 had chemotherapy and adjuvant radiotherapy (C/RT). The overall relapse frequency was 37%, with 41% of cases relapsed in the Chemo group versus 37% in the C/RT group (p = 0.55). The relapse free interval (from surgery to relapse) were 26 months and 33 months respectively (p = 0.50). For stage 2C, 25 patients received chemotherapy alone and 25 patients received C/RT. The overall relapse frequency was 34%, with 44% and 24% for the chemo and C/RT groups respectively (p = 0.14). The relapse free interval was 18 months and 46 months respectively (p = 0.28). The relapse sites for stage 1C Chemo group were mainly in the pelvic/abdomen area whereas the C/
RT group occurred more in the abdomen and distant sites. In stage 2C, the C/RT group relapsed mainly in the abdomen and distant sites with no pelvic relapses while the Chemo group relapsed in the pelvic, abdomen and at distant sites. Though the results observed did not show a statistically significant difference, the trend is suggestive of a decrease in total relapse rate, in pelvic relapse and lengthening of relapse free interval when adjuvant radiotherapy is used with chemotherapy, particularly for stage 2C disease.

Conclusions: The use of adjuvant radiotherapy in OCCC for stage 1C and 2C should be evaluated in larger prospective clinical trial.

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### 368 PROGNOSTIC INDICATORS FOR BONE METASTASES IN MEN WITH PROSTATE CANCER

**B. Rezvani**1,2, and E. Yu1,1. 1University of Washington, Seattle, WA and 2Seattle Cancer Care Alliance, Seattle, WA.

**Purpose of Study:** Nearly 250,000 men are diagnosed with prostate cancer annually, making it the most common malignancy in men in the United States. Bone metastases are a major cause of morbidity for these patients and factors that are prognostic for the development of bone metastases are important. Our hypothesis is that patients with osteopenia/osteoporosis, or high bone resorption will have a local environment in their bones that predisposes to the settling of metastases.

**Methods Used:** An institutional database was queried for prostate cancer patients who underwent dual-energy x-ray absorptiometry (DXA) scans or had measured levels of urinary cross-linked N-telopeptides (NTX) at least 3 months before a radiologist documentation of bone metastases by abnormal malignant bone scan. DXA scan is a measure of bone mineral density and NTX is a marker of bone resorption. 102 patients were identified with bone metastases, 84 of them with documented DXA scans, 71 of them with documented NTX levels, and 53 with both. Patients were categorized as having an abnormal DXA scan if they had a T score consistent with osteopenia/osteoporosis. Patients were categorized as having a high NTX level if at any time they had a level greater than 83 nM BCE/mM creatinine. Time from the first DXA scan or NTX level to bone metastases was calculated and compared between the groups.

**Summary of Results:** Refer to table.

**Conclusions:** Patients with increased bone resorption demonstrated by NTX levels have an abbreviated median interval to the development of bone metastasis when compared to patients who do not have abnormal NTX levels. Osteoporosis/osteopenia by DXA scans do not have prognostic value for bone metastases. Formal statistical analysis is pending.

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### 369 DEPRESSION IN WOMEN RECEIVING CHEMOTHERAPY FOR BREAST CANCER

**J. Reece**1,2, J. Herbert2, and J.R. Fann1,1. 1University of Washington School of Medicine, Seattle, WA; 2Seattle Cancer Care Alliance, Seattle, WA and 3Fred Hutchinson Cancer Research Center, Seattle, WA.

**Purpose of Study:** There is evidence that depression impacts quality of life, treatment adherence, and possibly survival among breast cancer patients. Nevertheless, it is often under-recognized and under-treated. Little is known about the epidemiology of depression during chemotherapy for breast cancer. This study aims to improve the understanding of depression and its treatment during the course of chemotherapy for breast cancer.

**Methods Used:** Women with stage I-III breast cancer who were receiving neoadjuvant or adjuvant chemotherapy were interviewed during consecutive chemotherapy infusions. The survey included the Patient Health Questionnaire-9 (PHQ-9) depression scale and Generalized Anxiety Disorder-7 (GAD-7), recent use of psychological services, and depression treatment preferences.

**Summary of Results:** Of the 35 patients in the study, 4 (11%) were stage I, 16 (46%) were stage II, and 15 (43%) were stage III. The mean PHQ-9 sum score for those receiving neoadjuvant chemotherapy was 4.0, and for those receiving adjuvant chemotherapy was 5.4. Among all patients, 12 (34%) scored for at least one assessment in the moderately depressed range (PHQ-9=10–14), 6 (17%) in the moderately severe range (15–19), and 1 (3%) in the severely depressed range (20+). Among the 13 (37%) patients scoring a 10 or above for at least one assessment, the mean percentage of assessments in this range for each patient was 40%. The top 3 preferred treatments for depression among all the patients in our study were “wait and see” (51%), individual counseling alone (46%), and a combination of individual counseling with medication (43%).

**Conclusions:** Over one third of breast cancer patients receiving chemotherapy experience at least moderate depression during their treatment. Higher depression scores were seen in those receiving adjuvant chemotherapy, and only 695 of those with moderate or greater depression receiving depression treatment. Oncology clinicians should regularly screen for depression and refer to treatment if necessary. Our results indicate that most patients, if feeling depressed, would prefer to either watchful waiting or to seek individual counseling with the possibility of medication.

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**Immunology and Rheumatology II**

**Concurrent Session**

Saturday, February 2, 2008

### 370 EXOGENOUS Reactive Oxygen SPECIES (ROS) FAIL TO INDUCE Neutrophil EXTRACELLULAR TRAP (NET) FORMATION IN PMNs ISOLATED FROM NEWBORN INFANTS

**C.C. Yost**1,2, M.J. Cody1, N.L. Thornton1,2, K.H. Albertine1, A.S. Weyrich2, and G.A. Zimmerman2. 1University of Utah, Salt Lake City, UT and 2University of Utah, Salt Lake City, UT.

**Purpose of Study:** Impaired NET formation in polymorphonuclear leukocytes (PMN) isolated from newborn infants, both term and preterm, represents a newly described deficiency of innate immunity in newborn infants and results in decreased extracellular bacterial killing. PMNs isolated from patients with chronic granulomatous disease also fail to form NETs, but treatment with exogenous ROS rescues NET formation in vitro. Whether NET formation in LPS-stimulated PMNs is impaired or to seek individual counseling with the possibility of medication.

**Summary of Results:** Refer to table.

**Conclusions:** Patients with increased bone resorption demonstrated by NTX levels have an abbreviated median interval to the development of bone metastasis when compared to patients who do not have abnormal N

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**Extracellular Reactive Oxygen Species (ROS) Fail to Induce Neutrophil Extracellular Trap (NET) Formation in PMNs Isolated from Newborn Infants**

**C.C. Yost**1,2, M.J. Cody1, N.L. Thornton1,2, K.H. Albertine1, A.S. Weyrich2, and G.A. Zimmerman2. 1University of Utah, Salt Lake City, UT and 2University of Utah, Salt Lake City, UT.

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**Summary of Results:** Refer to table.

**Conclusions:** Patients with increased bone resorption demonstrated by NTX levels have an abbreviated median interval to the development of bone metastasis when compared to patients who do not have abnormal NTX levels. Osteoporosis/osteopenia by DXA scans do not have prognostic value for bone metastases. Formal statistical analysis is pending.

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**Extracellular Reactive Oxygen Species (ROS) Fail to Induce Neutrophil Extracellular Trap (NET) Formation in PMNs Isolated from Newborn Infants**

**C.C. Yost**1,2, M.J. Cody1, N.L. Thornton1,2, K.H. Albertine1, A.S. Weyrich2, and G.A. Zimmerman2. 1University of Utah, Salt Lake City, UT and 2University of Utah, Salt Lake City, UT.

**Purpose of Study:** Impaired NET formation in polymorphonuclear leukocytes (PMN) isolated from newborn infants, both term and preterm, represents a newly described deficiency of innate immunity in newborn infants and results in decreased extracellular bacterial killing. PMNs isolated from patients with chronic granulomatous disease also fail to form NETs, but treatment with exogenous ROS rescues NET formation in vitro. Whether NET formation in LPS-stimulated PMNs is impaired or to seek individual counseling with the possibility of medication.

**Summary of Results:** Refer to table.

**Conclusions:** Patients with increased bone resorption demonstrated by NTX levels have an abbreviated median interval to the development of bone metastasis when compared to patients who do not have abnormal NTX levels. Osteoporosis/osteopenia by DXA scans do not have prognostic value for bone metastases. Formal statistical analysis is pending.
+/- DPI [10–40 \text{ mM}], a potent inhibitor of NADPH-oxidase, the enzymatic source of endogenous ROS. We assessed NET formation both qualitatively and quantitatively using live cell imaging via confocal microscopy and determination of extracellular DNA content via fluorimetry, respectively.

**Summary of Results:** LPS fails to induce NET formation in PMNs isolated from newborn infants, but does induce NETs in vitro in PMNs isolated from healthy adults. While pre-treatment with DPI blocks NET formation following LPS stimulation of PMNs isolated from healthy adults, treatment with glucose oxidase restores NET formation in DPI-treated PMNs isolated from the same donors. However, in assays performed in parallel, no concentration of glucose oxidase tested induces NET formation in PMNs isolated from newborn infants, either term and preterm.

**Conclusions:** We conclude that exogenous ROS fail to induce NET formation in vitro in PMNs isolated from newborn infants while inducing NET formation in PMNs isolated from healthy adults. We speculate that the decreased endogenous generation of ROS via NADPH-oxidase documented in PMNs isolated from newborn infants as compared to PMNs isolated from healthy adults does not fully explain failed NET formation in PMNs isolated from newborn infants, whether term or preterm.

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**371 PLATELETS ALTER NEUTROPHIL EXTRACELLULAR TRAP FORMATION**

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**Purpose of Study:** Polymorphonuclear leukocytes (PMNs) generate neutrophil extracellular traps (NETs) which are lattices of PMN extracellular chromatin decorated with PMN antimicrobial enzymes and degradative proteins. NETs trap bacteria and provide a high local concentration of antimicrobial activity to effect bacterial killing. In addition to their role in primary hemostasis, platelets also interact with PMNs allowing for platelet regulation of PMN function. Whether there is an optimal platelet concentration to induce in vitro PMN NET formation is unknown. We hypothesized that the addition of increasing numbers of platelets to PMNs would result in increased in vitro NET formation in LPS-stimulated PMNs isolated from both adults and term newborn infants.

**Methods Used:** Adult and term PMNs were isolated from peripheral and umbilical whole blood via positive immunoselection for CD15 expression. Platelets were purified from platelet rich plasma from the same adult or newborn donor, via negative immunoselection for CD45 expression. Platelets were added at increasing concentrations to a single concentration of PMNs and then stimulated with LPS. Cells were incubated on poly-L-lysine coated glass coverslips in standard conditions for 60 minutes. We then assessed for NET generation via live cell imaging using confocal microscopy.

**Summary of Results:** LPS-stimulated adult PMNs generate more NETs than PMNs isolated from term newborns consistent with unpublished data from our lab. There is an inverse correlation between the concentration of platelets added and PMN NET formation, with platelets, added at a ratio of 100 to 1 PMN, almost completely inhibiting LPS-stimulated NET generation when compared to LPS-stimulated PMNs alone.

**Conclusions:** Contrary to our hypothesis, increasing numbers of platelets results in decreased PMN NET formation in both adult and term newborn PMNs. While beneficial in effecting bacterial killing, NET associated tissue damage is likely with the extracellular release of PMN degradative proteins. Regulation of NET formation to maximize bacterial killing while minimizing tissue damage is necessary. As platelet counts often rise with severe inflammation, we speculate that platelet inhibition of NET formation could minimize NET associated tissue damage.

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**372 CELLULAR PENETRATION AND LOCALIZATION OF THE ANTI-GUANOSINE ANTIBODY 4H2**

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**Purpose of Study:** Systemic lupus erythematosus is characterized by anti-nuclear antibodies including antibodies to individual nucleosides of DNA. Of the four commonly occurring nucleosides, guanosine has been demonstrated to be the most immunogenic. Human polyonal anti-guanosine antibodies and the murine monoclonal anti-guanosine antibody (4H2) exhibit the same binding specificity for guanosine indicating a conserved epitope. Human serum anti-guanosine antibody levels correspond well with the disease activity as determined by SLEDAI scores of systemic lupus erythematosis (SLE) including nephritis, polyserositis, arthritis, CNS lupus and the hematologic features of SLE.1–2. Our work aims to characterize 4H2 cellular penetration and intracellular pathogenic effects.

**Methods Used:** We incubated live HTori-3-LLU thyroid epithelial cells with Alexa flour-594 labeled 4H2. In addition, we added the mitochondrial dye, Mitotracker.

**Summary of Results:** By fluorescent microscopy, we established that 4H2 penetrates the cell membrane in live and fixed cells and localizes in the mitochondria arresting mitochondria dehydrogenase activity.

**Conclusions:** This mitochondrial localization provides a unique mechanism in which lupus may exert pathogenic effects.

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**373 REGULATORY T CELLS FROM TRANSGENIC MICE EXPRESS MULTIPLE T CELL RECEPTORS, CONFIRMING STUDIES OF THEIR ANTIGEN SPECIFICITY**

J.S. Kurche\(^1\), S.M. Weber\(^1\), J.B. Buhman\(^1\), R.M. Kedl\(^1\), and R.G. Gill\(^2\).

\(^1\)University of Colorado at Denver and Health Sciences Center, Denver, CO and \(^2\)University of Alberta, Edmonton, AB, Canada.

**Purpose of Study:** CD4+ CD25+ FoxP3+ regulatory T cells (Tregs) have been studied in the context of autoimmunity, transplantation, and cancer and can dampen the effector function of lymphocytes. However the specificity of Treg-mediated suppression remains uncertain. Under-
standing the nature of recognition between Tregs and their ligands is critical for the manipulation of these cells in disease. Previous studies suggest Tregs are contact-dependent and recognize antigen through their T cell receptors (TCRs), consistent with the behavior of antigen-specific effector T cells. However, several model systems have demonstrated that Tregs suppress in an antigen-nonspecific fashion. These studies primarily used cells derived from TCR transgenic animals. As development of Tregs in TCR transgenic animals is Rag recombinase-dependent, we hypothesized transgenic Tregs, but not transgenic effectors, express endogenous receptors that may account for antigen-nonspecificity.

**Methods Used:** We analyzed primary cells obtained from B6 mouse spleen and lymph nodes by flow cytometry and flow-cytometric fluorescence microscopy. Furthermore, we sorted B6 CD4+ CD25+ T cells and used them to suppress proliferation of effector T cells stimulated with various antigens in culture.

**Summary of Results:** Effector T cells from TCR transgenic mice show antigen specificity whereas Tregs from the same mice react to both cognate antigen and alloantigen equivalently. We demonstrate that Tregs from TCR-transgenic mice differentially express secondary Vx chains than effector T cells. Using single-cell FACS imaging, we were able to show that Vx and transgene double-positive Tregs are real cells with nuclear FoxP3 and overlapping cell surface TCRs. Furthermore, we show that stimulation through either T cell Vx leads to internalization of transgenic VB in dual-receptor cells.

**Conclusions:** Our data support the idea that the failure of Treg generation in Rag-deficient TCR transgenic mice is due to lack of endogenous TCR recombination and that the selective pressure for endogenous TCRs on Tregs is sufficient to favor expression of multiple TCRs in transgenic mice, thus complicating studies of Treg-ligand interactions.

**374 THE EFFECT OF LEUKOCYTE IMMUNOGLOBULIN-LIKE RECEPTOR A2 ON CYTOKINE/CHEMOKINE PRODUCTION IN HUMAN PERIPHERAL BLOOD MONOCYTES**

J. Lee,1,2 R.J. Carbone3, R.L. Modlin,2 and D.J. Lee.1 1Charles R. Drew University of Medicine and Science/David Geffen School of Medicine at UCLA, Los Angeles, CA and 2David Geffen School of Medicine at UCLA, Los Angeles, CA.

**Purpose of Study:** Leukocyte immunoglobulin-like receptor A2 (LILRA2) is an activating receptor that is thought to play an immunomodulatory role. In leprosy, patients with lepromatous leprosy (L-lep) express higher levels of LILRA2 compared to tuberculoid leprosy (T-lep) (L-lep) express higher levels of LILRA2 compared to tuberculoid leprosy (T-lep) expressed higher levels of LILRA2 compared to tuberculoid leprosy (T-lep). We hypothesized that LILRA2 activation stimulates cytokine/chemokines, including IL-23, from monocytes which may contribute to the immunomodulatory role. In leprosy, patients with lepromatous leprosy (L-lep) express higher levels of LILRA2 compared to tuberculoid leprosy (T-lep) expressed higher levels of LILRA2 compared to tuberculoid leprosy (T-lep) expressed higher levels of LILRA2 compared to tuberculoid leprosy (T-lep) expressed higher levels of LILRA2 compared to tuberculoid leprosy (T-lep) expressed higher levels of LILRA2 compared to tuberculoid leprosy (T-lep).

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**Summary of Results:** Effector T cells from TCR transgenic mice show antigen specificity whereas Tregs from the same mice react to both cognate antigen and alloantigen equivalently. We demonstrate that Tregs from TCR-transgenic mice differentially express secondary Vx chains than effector T cells. Using single-cell FACS imaging, we were able to show that Vx and transgene double-positive Tregs are real cells with nuclear FoxP3 and overlapping cell surface TCRs. Furthermore, we show that stimulation through either T cell Vx leads to internalization of transgenic VB in dual-receptor cells.

**Conclusions:** Our data support the idea that the failure of Treg generation in Rag-deficient TCR transgenic mice is due to lack of endogenous TCR recombination and that the selective pressure for endogenous TCRs on Tregs is sufficient to favor expression of multiple TCRs in transgenic mice, thus complicating studies of Treg-ligand interactions.

**375 INTEGRATION OF SAFETY TECHNOLOGIES INTO RHEUMATOLOGY AND ORTHOPEDIC PRACTICE: A RANDOMIZED, CONTROLLED TRIAL**


**Purpose of Study:** The Joint Commission and OSHA mandate integration of new safety technologies into medical facilities to improve the safety of both patients and health care workers. Despite this, adoption of safety technologies by rheumatology and orthopedic services has been minimal. The purpose of the present study was to identify and integrate new safety technologies into outpatient musculoskeletal procedures.

**Methods Used:** Using national resources for patient safety and literature review, these safety technologies were identified: a) a safety needle to reduce inadvertent needle sticks to health care workers, and b) the reciprocating procedure device (RPD) to improve patient safety and to reduce pain, tissue trauma, and adverse outcomes. 480 musculoskeletal syringe and needle procedures were randomized to the RPD-safety needle vs. the conventional syringe, and quality, safety, and physician acceptance were measured.

**Summary of Results:** With the safety needle there were no accidental needle sticks (0 sticks/480 procedures). The RPD resulted in a 36.1% reduction in patient pain (RPD VAPS score: 3.17 ± 2.67; Syringe: 4.96 ± 3.25; p < 0.001, CI: 14% to 48% decrease) and a 52.5% reduction in significant pain (VAPS ≥ 5; p < 0.001, CI 23% to 68% decrease). Physician acceptance and satisfaction with the combined RPD-safety needle was excellent.

**Conclusions:** As mandated by the Joint Commission and OSHA, safety technologies can be successfully integrated into rheumatologic and orthopedic practices. The combination of a safety needle to reduce needle stick injuries to health care workers and the RPD to improve safety and comfort of patients is effective and well accepted by physicians.

**376 THE ROLE OF TGF-ALPHA AND VITAMIN D IN KERATINOCYTE HOST DEFENSE**

C.G. Senavsky,1,2 L.S. Miller,1,2, and R.L. Modlin.1,2 1UCLA, Los Angeles, CA and 2UCLA, Los Angeles, CA.

**Purpose of Study:** The expression of antimicrobial peptides by epithelial cells represents a first line of defense against microbial pathogens. TGF-alpha, an important growth factor involved in wound healing, can increase the expression of human cathelicidin antimicrobial peptide (LL-37). In addition, LL-37 can also be induced by activation of the Vitamin D receptor (VDR) pathway. The purpose of our study was to examine if TGF-alpha can augment the VDR-mediated induction of LL-37 in human epithelial keratinocytes.

**Methods Used:** To study the expression of LL-37 in human skin, we used air-lifted organotypic keratinocyte cultures that form layers resembling normal human skin and the HaCAT keratinocyte cell line derived from immortalized adult epithelium. We first examined if these
keratinocytes were capable of responding to active vitamin D (1,25D3), by measuring downstream Vitamin-D pathway gene activity (cathelicidin and CYP24) by quantitative PCR (qPCR). After confirming these cell were responsive to 1,25D3, we measured the expression of CYP27, the enzyme that converts inactive vitamin D to the active form, in the presence of TGF-alpha by qPCR. Finally, we challenged both models with inactive Vitamin D (25D3) and TGF-alpha to determine if the increased expression of cathelicidin seen in the presence of TGF-alpha was due to the upregulation of the Vitamin D pathway.

Summary of Results: TGF-alpha stimulation, in the absence of VDR activation, resulted in increased mRNA expression of CYP27, which converts inactive 25D3 (25-hydroxyvitamin D3) to active 1,25D3, and also increased expression of VDR. CYP24 and LL37 mRNA were up-regulated in the presence of both 25D3 and TGF-alpha to a greater extent than that of 25D3 alone. In summary, these results provide evidence that TGF-alpha increases expression of CYP27, which can convert inactive 25D3 to active 1,25D3, resulting in VDR-mediated up-regulation of CYP24 and LL-37 and enhanced host defense mechanisms at epithelial surfaces.

Conclusions: TGF-alpha, increases the expression of CYP27 and VDR of the Vitamin D pathway, allowing greater conversion of active Vitamin D, which results in increased expression of LL-37, a potent antimicrobial peptide. This may be an important mechanism in keratinocyte host defense against pathogens.

377 CYTOMEGALOVIRUS (CMV) INFECTION IN LARGE VESSEL VASCULITIS: IS IT A CAUSATIVE AGENT?
S. Mehdi, C. Leehealey, B. Andrews, and J. Tencati. UCI Medical Center, Orange, CA.

Purpose of Study: Previous studies have attempted to demonstrate a direct relationship between CMV infection and large vessel vasculitis. None have demonstrated serologic evidence of acute CMV infection, or DNA evidence of CMV by PCR. We have demonstrated serologic evidence of acute CMV infection preceding the clinical onset of large vessel vasculitis in a human, most likely giant cell arteritis.

Methods Used: Ours is a case report - We report a 61 year old Caucasian female who presented with a nine-day history of fever, chills, myalgia, fatigue, and a history of breast cancer ten years earlier. Physical exam was negative, and diagnostic studies revealed a normal WBC and its major branches, suggestive of arteritis. With prednisone therapy her symptoms and anemia rapidly improved and the ESR fell to <20 mm/hr. Angiography showed aneurysmal dilatation in the left subclavian artery, and alternating areas of band-like stenosis followed by areas of luminal enlargement in the aorta and multiple vessels. A temporal artery biopsy was not performed.

Summary of Results: We have demonstrated serologic evidence of acute CMV infection preceding the clinical onset of large vessel vasculitis in a human, most likely giant cell arteritis.

Conclusions: Possible causative relationship between CMV and vasculitis.

378 CIRCULATING ANGIogenic AND ANGIOstatic FACTORS IN SYSTEMIC SCLEROSIS
K. Tran1,2, A. Meredith2, B. Whalen2, S. van Eeden2,3, and J. Dunno3.
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Purpose of Study: To determine the relationship of circulating angiogenic factors, vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), and angiostatic factor, endostatin, with clinical parameters of disease severity and activity in patients with Systemic Sclerosis (SSc).

Methods Used: Sixty-five consecutive SSc patients were evaluated, of which 46 patients had limited disease and 19 had diffuse disease. Thirty-five healthy subjects were included as age and sex matched controls. Quantitative colorimetric sandwich ELISAs were used to measure plasma levels of VEGF, bFGF, and endostatin.

Summary of Results: Plasma levels of VEGF were significantly higher in patients with SSc (49.0 ± 6.8 pg/mL), compared to healthy controls (25.3 ± 5.2 pg/mL). Similarly, plasma concentrations of bFGF were markedly increased in SSc patients (34.6 ± 3.0 pg/mL) compared to healthy volunteers (19.8 ± 3.4 pg/mL). Circulating levels of angiotropic factor, endostatin, did not differ significantly from controls and showed no correlation with clinical parameters. Circulating VEGF levels showed a significant correlation with global disease measurement of severity (r = 0.44, p = 0.0005), disease activity scores (r = 0.40, p = 0.002) and diffusing lung capacity (r = 0.34, p = 0.01). In contrast, circulating levels of bFGF correlated significantly with disease duration (r = 0.38, p = 0.004) and Rodman skin score (r = 0.33, p = 0.02), but not with disease activity or severity.

Conclusions: Patients with SSc have increased levels of circulating angiogenic factors, despite having chronic vasculopathies and tissue ischemia. Interestingly, angiogenic factor, VEGF, correlated with global assessment of disease severity and activity in subjects with SSc. Furthermore, VEGF may be a useful marker in assessing pulmonary involvement in SSc patients.

Metabolism III Concurrent Session
8:30 AM
Saturday, February 2, 2008

379 CENTRAL MELANOCORTIN BLOCKADE ATTENUATES CARDIAC CACHEXIA IN A RAT MODEL OF CHRONIC HEART FAILURE
A.K. Batra1, J.M. Scarlet1, D.D. Bowel1, X. Zhu1, W.F. Grant1, and D.L. Marks1,2, 1Oregon Health and Science University, Portland, OR and 2Oregon Health and Science University, Portland, OR.

Purpose of Study: Anorexia, wasting of fat, and wasting of lean body mass (LBM) are salient features of cardiac cachexia. Recently, studies in
our lab and others have demonstrated that experimental cachexia in rodents is attenuated by blockade of central melanocortin signaling (a key neuronal circuit in the regulation of energy homeostasis) at the melanocortin-4 receptor (MC4-R). To investigate the contribution of the central melanocortin system in the transduction of cardiac cachexia, we developed a rat model of CHF and measured the effect of blockade of melanocortin signaling on accumulation and maintenance of fat mass and LBM.

Methods Used: We induced CHF in 3-4 week-old male Wistar rats by aortic banding. Six weeks after surgery, rats received intracerebroventricular injections of agouti-related peptide (AgRP), an endogenous antagonist of the MC4-R, or artificial cerebrospinal fluid (aCSF) over a two-week period. Accumulation of fat mass and LBM over the treatment period was measured by dual x-ray absorptiometry analysis.

Summary of Results: Sham-aCSF, Sham-AgRP, and Band-AgRP rats gained significant amounts of body weight over the treatment period, but Band-aCSF rats failed to gain weight. AgRP treatment increased the percent increase in LBM per animal in banded rats (Band-aCSF 3.39% + 2.50% vs Band-AgRP 17.90% + 2.29%, P < 0.001). Fat mass accumulation was significantly increased by AgRP treatment in banded rats (Band-AgRP 44.04 ± 3.61g vs Band-aCSF 18.46 ± 2.50g; P < 0.05). Organ hypothyropathy in banded rats was reversed by AgRP treatment (Band-AgRP kidney 1.230 ± 0.07g, liver 12.48 ± 0.78g vs Band-aCSF kidney 1.049 ± 0.03g, liver 9.07 ± 0.47g, P < 0.05).

Conclusions: Rats with CHF display an impaired ability to accumulate and maintain fat mass and LBM. Our data demonstrate that this pathology can be prevented by pharmacologic blockade of central melanocortin signaling. Weight gain, LBM, fat mass accumulation, kidney weights and liver weights were significantly increased by AgRP treatment in banded rats. These results suggest that the central melanocortin system may represent an important therapeutic target for the treatment of cardiac cachexia.

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**380 NITRIC OXIDE PRODUCTION IN HUMAN CORPUS CAVERNOSAL ENDOTHELIAL CELLS: ROLE OF ADIPONECTIN AND ADIPONECTIN RECEPTORS**


**Purpose of Study:** Adiponectin, a potential nitric oxide (NO) stimulator in endothelial cells (EC), is decreased in type 2 diabetic men and may contribute to their higher rates of erectile dysfunction. Studies suggest that adiponectin acts either directly as a key mediator or indirectly through suppressing hyperglycemic inhibition of insulin-stimulated NO production. However, it is unknown if adiponectin can enhance NO production. Therefore, it is important to determine the relative androgenic hormonal milieu in female macaques to a predominant androgen milieu will result in development of physical and biochemical symptoms of MBS. Our specific aim is to determine if exposure of peripubertal female macaques to elevated testosterone (T) levels leads to physical and biochemical features of MBS.

**Methods Used:** This project uses monkeys from a study whose long-term goal is to determine whether raised peripubertal T levels in female macaques affect the hypothalamic-pituitary-ovarian sequelae of PCOS. To raise T levels, Silastic tube implants were placed subcutaneously starting at age 12 months. The implants of controls (n = 6) contained cholesterol (C) and treated monkeys (n = 8) contained T and C mixture. Weekly serum T measurements guide the implants' replacement.

**Summary of Results:** The mean circulating T in the experimental group has been maintained between 1.2 and 1.6 ng/ml for over 50 days (mean 1.6 ng/ml) with no overlap with the control group’s T (0.57 ng/ml). DEXA scanning revealed no difference between body fat mass of control monkeys (58.8 ± 8.7 g) and T-treated animals (70.5 ± 11.0 g). The control animals’ ratio of high-molecular weight (HMW) to Total Adiponectin was 0.71 ± 0.03 versus 0.67 ± 0.02 in the T-treated animals. There was no statistically significant difference between the leptin levels in controls (1.53 ng/ml ± 0.11) and the T-treated animals (1.79 ng/ml ± 0.18).

**Conclusions:** After 16 months of elevated T levels in lean peripubertal female macaques, androgen exposure has not altered some physical (DEXA) and hormonal measures of MBS. Ongoing studies will determine any differences in insulin sensitivity and other study parameters. This work is supported by a gift from the Circle of Giving, OHSU Center for Women’s Health and NIH grants HD18185, DK007674-13, and RR00163.

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**381 METABOLIC PARAMETERS IN FEMALE MONKEYS CHRONICALLY EXPOSED TO HYPERANDROGENIC HORMONAL MILIEU**

A. Bahar1, J. Cameron1,2, and R.L. Stouffer1,2. 1Oregon Health & Science University, Portland, OR and 2Oregon National Primate Research Center, Beaverton, OR.

**Purpose of Study:** Observational studies demonstrate a positive association between the relative androgenic hormonal milieu in postmenopausal women and the occurrence of the metabolic syndrome (MBS). If androgen exposure and action lead to the development of MBS, then chronic exposure of female macaques to a predominant androgen milieu will result in development of physical and biochemical symptoms of MBS. Our specific aim is to determine if exposure of peripubertal female macaques to elevated testosterone (T) levels leads to physical and biochemical features of MBS.

**Methods Used:** This project uses monkeys from a study whose long-term goal is to determine whether raised peripubertal T levels in female macaques affect the hypothalamic-pituitary-ovarian sequelae of PCOS. To raise T levels, Silastic tube implants were placed subcutaneously starting at age 12 months. The implants of controls (n = 6) contained cholesterol (C) and treated monkeys (n = 8) contained T and C mixture. Weekly serum T measurements guide the implants' replacement.

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**Conclusions:** After 16 months of elevated T levels in lean peripubertal female macaques, androgen exposure has not altered some physical (DEXA) and hormonal measures of MBS. Ongoing studies will determine any differences in insulin sensitivity and other study parameters. This work is supported by a gift from the Circle of Giving, OHSU Center for Women’s Health and NIH grants HD18185, DK007674-13, and RR00163.
Purpose of Study: Describe early growth and energy intakes of infants with SLOS.

Methods Used: Seven infants diagnosed with SLOS by 6 wk were evaluated by 6 mo and again by 14 mo. Weight, length, weight/length and BMI Z-scores were calculated using CDC (2000) and WHO (2006) reference data. Energy intake was calculated and compared to the Dietary Reference Intake (DRI). Repeated measures ANOVA was used to compare differences in Z-scores at the 1st and 2nd evaluations. Paired t-tests were used to compare differences in Z-scores calculated using CDC and WHO data.

Summary of Results: Growth parameters and energy intake were below sex- and age-matched reference values at both evaluations. Mean energy intake was less than 90% of the DRI at both evaluations and did not improve significantly over time or with the use of a G-tube in 5 of 7 infants. Mean weight, height, weight/height and BMI Z-scores did not improve over time and were different when calculated using CDC and WHO data.

Conclusions: Some infants with SLOS grow appropriately with nutrition support while others exhibit early and significant impairments in growth due in part to insufficient energy intake. It is not clear if infants with impaired growth are unable to demonstrate catch-up growth due to SLOS or whether nutritional support is insufficient to optimize growth potential.

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383 PREVALENCE OF METABOLIC SYNDROME IN CANADIAN FIRST NATIONS CHILDREN LIVING ON THE REMOTE PACIFIC COAST


Purpose of Study: The purpose of this study was to determine the prevalence of obesity, metabolic syndrome and its components in children living in three remote Tsimshian Nation communities of British Columbia.

Methods Used: Measurements of height, weight, blood pressure, and waist circumference were performed on children aged 6 to 18 years. Body mass index (BMI) was standardized for age and sex. Overweight was defined as a BMI =85th %ile, and obesity was defined as a BMI =95th %ile for age and sex. An oral glucose tolerance test was administered following a 12-hour fast. Baseline and 2-hour levels of glucose and insulin were measured. The International Diabetes Federation criteria for metabolic syndrome in children and adolescents were used.

Summary of Results: 194 (85%) of the 229 eligible children participated. Of these 194 children, 37 (19%) were overweight and 51 (26%) obese. 67 (34.5%) children had a waist circumference ≥90th percentile, 38 (19.6%) children had impaired fasting glucose, 13 (6.7%) elevated triglycerides, 17 (8.8%) low HDL, 7 (3.6%) systolic hypertension (=130 mmHg) and none had a diastolic blood pressure =85 mmHg. All of the eight children found to have metabolic syndrome were overweight or obese. The overall rate of metabolic syndrome was 4.1% (8/194); however, the prevalence in overweight/obese children was 9.1% (8/88). In overweight/obese children, 40% had one component of metabolic syndrome and 25% had two. One child <10 years met the criteria for metabolic syndrome but was excluded from prevalence rates because of age. Another child was not included in prevalence calculations because of normal fasting glucose but met criteria for impaired glucose tolerance (2 hr glucose =7.8 mmol/L) and had two other components of MS.

Conclusions: Current criteria for metabolic syndrome may underestimate prevalence because there are no criteria for children <10 years, and impaired glucose tolerance with normal fasting glucose is currently not a component of the definition. Nevertheless, the high prevalence rates of the components of the metabolic syndrome as well as overweight/obesity documented in these Canadian Tsimshian Nation children raise concerns about their future diabetes and cardiovascular disease risk.

384 A PREDICTIVE MODEL FOR TYPE 1 VS. TYPE 2 DIABETES AT PRESENTATION IN PEDIATRIC POPULATIONS

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Purpose of Study: The incidence of type 2 diabetes (T2D) is increasing in the pediatric population. The clinical management of T2D differs greatly from type 1 diabetes (T1D), but both T2D and T1D can have similar initial presentations. African American (AA) and Latino children are at especially high risk for T2D. Thus, the need for a prompt and accurate diagnosis in these populations is critical. This study aims to confirm and validate a pediatric T2D vs. T1D diagnosis model developed from data obtained at the Children’s Hospital and Research Center Oakland (CHRCO) with patient data from the University of California, Davis Medical Center (UCDMC).

Methods Used: Charts for 130 AA diabetes patients were abstracted and reviewed at CHRCO. Diagnoses of T1D (n = 73) or T2D (n = 57) were confirmed by clinicians. Variables that differed significantly (p < 0.05) between the groups were age, BMI, gender, C-peptide, anti-GAD autoantibodies, current use of oral diabetes medications, hyperpigmentation, and family history of T2D. Regression analysis was done, and recursive partitioning was used to optimize predictors and cut-points. UCDMC data from AA and Latino patients were entered into the CHRCO model, which uses four variables easily gathered at diagnosis, to validate its predictive accuracy.

Summary of Results: The traits of older age, BMI greater than 85th percentile, female gender, and hyperpigmentation favored a diagnosis of T2D. The CHRCO model predicted the diagnosis of T2D and T1D with 100% and 89% sensitivity in a combined CHRCO and UCDMC AA patient data set (n = 52). In a pooled AA and Latino data set (n = 35) from UCDMC, 90% and 94% sensitivity for T2D and T1D resulted.

Conclusions: Our model seems to effectively predict the diagnosis of T2D vs. T1D in pediatric AA patients based on variables obtained at presentation. Also, our tests testing the CHRCO model’s applicability to Latino children with an initial UCDMC patient data set show promising results. Using our model, we hope to create a user-friendly, data-entry interface readout to indicate a mathematical probability that a patient has T2D or T1D. This tool should aid in the early differential diagnosis of T2D vs. T1D and has implications for the clinical management of those children.
85 MEASURED AND NON-HDL BASED LDL IN HYPERTRIGLYCERIDEMIA: WHICH ONE TO TARGET?
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patients with excessive hypertriglyceridemia. Designed to attain optimal LDL targets affect cardiovascular outcomes in parameters. It is more important to determine if therapeutic strategies should be treated more aggressively. Alternatively, further studies are patients did not reach eLDL target. This implies that these patients dLDL is significant lower than that of eLDL and the majority of this group. However, dyslipidemia was underdocumented and under-predictors (R2 = 0.565).

Purpose of Study: Although LDL is the critical therapeutic target for cardiovascular risk reduction, it cannot be calculated when triglyceride (TG) exceeds 400 mg/dl. Estimated LDL from non-HDL (eLDL) was proposed as an alternative goal. However, eLDL was much less targeted in statin trials. Little is known about the clinical significance of directly measured LDL (dLDL) in excessive hypertriglyceridemia. The purpose of this study was to determine the relationship between dLDL and eLDL.

Methods Used: SYNCHRON LX® System was used to measure dLDL in 266 VA outpatients with TG between 400 and 1300 mg/dl. Other clinical data included demographics, vital signs, and diagnoses. Chi-square, ANOVA, and linear regression statistics were used.

Summary of Results: Ninety-eight percent of patients were male with a mean age of 59 years, BMI of 32 kg/m2, and BP of 130/73 mmHg. Co-existing diagnoses (%) included hypertension 60.5, diabetes 48.5, coronary artery disease 13, cerebral vascular accident 9, peripheral vascular disease 9, alcohol misuse 14, and hypothyroidism 7. Diagnosis of dyslipidemia were not documented in almost 1/3 of the cases. Only 31% of patients were on statins, 22% on fibrates, and 14% on both agents. The mean lipid values were: cholesterol 223 mg/dl, HDL 31 mg/dl, TG 584 mg/dl, dLDL 92 md/dl, eLDL 131 mg/dl. There was a statistically significant difference in means between dLDL and eLDL (P < 0.005). Sixty-five percent of patients had dLDL > 100 mg/dl while only 7.2% had targeted eLDL (P < 0.05). Values of dLDL can be derived from a linear regression model with TG, HDL, TG, and age as predictors (R2 = 0.565).

Conclusions: Concomitant cardiovascular risks are highly prevalent in this group. However, dyslipidemia was underdocumented and undertreated. Although dLDL is calculable in our regression model, the mean dLDL is significantly lower than that of eLDL and the majority of patients did not reach eLDL target. This implies that these patients should be treated more aggressively. Alternatively, further studies are needed to re-define the eLDL target threshold based on all non-HDL parameters. It is more important to determine if therapeutic strategies designed to attain optimal LDL targets affect cardiovascular outcomes in patients with excessive hypertriglyceridemia.

386 DIABETES MANAGEMENT IN METLAKATLA, ALASKA
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Purpose of Study: Metlakatla, Alaska has a high incidence of diabetes, much of it poorly controlled. The clinic recently hired a community health educator (a Native Alaskan retired schoolteacher born and raised in Metlakatla), as well as a registered nurse who is the new Diabetes Coordinator. She is planning a diabetes management program to improve continuity of care and begin patient education. The purpose of this project was to write a preliminary plan for this management program, the scope of which includes medical management and patient education for the diabetic population.

Methods Used: A search of the literature (using Cochrane database and PubMed) identified diabetes management techniques which are most likely to give successful patient outcomes, and this project focused on adapting those techniques to the health care resources and patient population in Metlakatla. Because this project is the preliminary step in a long-term management plan to be organized and overseen by the diabetes coordinator, the plan was created in cooperation with her. Potential members of the diabetes management team, and Indian Health Service (IHS) providers with extensive diabetes management experience in Anchorage were also consulted.

Summary of Results: The final draft of this plan included the program goals, actions and oversight, and the general outline for a curriculum. The published ADA Standards of Care and the IHS curriculum for diabetes self-management education are the basis for medical management and patient education. Patient feedback is built in to the preliminary plan as surveys and knowledge pre and post tests, in addition to monitoring medical results. A key component is follow-up with patients and modification of curriculum or methods based on feedback. First steps for implementation of the plan include organizing a diabetes management committee, evaluating patients’ current level of control, and designating a primary care provider for each patient.

Conclusions: Evidence shows that improving prevention and management of diabetes requires improvements in continuity and follow-up between providers and patients, and in education for patient self-management. This project created a dynamic and outcome-oriented plan for the diabetic patient population of Metlakatla, Alaska.

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387 UTEROPLACENTAL INSUFFICIENCY DOWN-REGULATES KIDNEY VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF)

Purpose of Study: The interplay between hypoxia and glucocorticoid-mediated cellular responses in nephrogenesis is intriguing. Both IUGR individuals as well as rats rendered IUGR by uteroplacental insufficiency are exposed to increased corticosterone levels and hypoxia, and ultimately, have decreased nephron number. The vascular endothelial growth factor (VEGF) has been shown to be critical for normal kidney development. Under hypoxic conditions, VEGF is up regulated stimulating angiogenesis. In contrast, glucocorticoids have angiostatic properties. To date, the effects of UPI, hypoxia and elevated corticosterone levels upon kidney VEGF expression are unknown. We hypothesize that IUGR and resultant glucocorticoid overexposure of the fetus leads to decreased kidney VEGF expression in spite of hypoxic conditions.

Methods Used: Bilateral uterine artery ligation was performed on day e19 pregnant Sprague-Dawley rats and pups were harvested at term (e21.5). Levels of hypoxia-inducible factor (HIF-1α) and VEGF mRNA were quantified using real-time RT-PCR from whole kidneys on P0 and P21 of life (juvenile rat).

Summary of Results: On P0, IUGR was associated with decreased kidney VEGF mRNA levels in both males and females (81 ± 7% and 77 ± 5% of control). Of interest, persistently elevated corticosterone levels on P21 were associated with decreased VEGF mRNA levels in IUGR juvenile rat kidneys (males: 75 ± 8% females: 76 ± 2% of control) (*p < 0.05). Kidney HIF-1α mRNA levels were not significantly different between groups.

Conclusions: In our animal model characterized by decreased nephron number and adult onset hypertension, UPI decreased fetal VEGF
expression during a period of active nephrogenesis in the IUGR rat. We speculate that increased glucocorticoid levels could mediate inhibition of hypoxia-induced VEGF expression, and that this cross-talk between the 2 signaling cascades may have a significant role in kidney angiogenesis and development. (Supported by the University of Utah Department of Pediatrics CHRC).

**388 IN UTERO NICOTINE EXPOSURE DISRUPTS PARATHYROID HORMONE-RELATED PROTEIN (PTHrP) SIGNALING IN THE DEVELOPING KIDNEY AND IMPAIRS NEPHROGENESIS**

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**Purpose of Study:** Although cigarette smoking is extremely harmful, especially during pregnancy, the full extent of damage that it causes to the developing fetus is not known. Nicotine, the main constituent of cigarette smoke, alters the growth and development of many organs, and though the effect of *in utero* nicotine exposure on the developing kidney is not known, exposure of the fetus to cigarette smoke is associated with hypertension as an adult. The underlying mechanism for this association remains undetermined. We hypothesize that *in utero* nicotine exposure alters the PTHrP signaling pathway known to be critically involved in nephrogenesis and that we can prevent the effects of *in utero* nicotine exposure by using PTHrP pathway agonists.

**Methods Used:** Pregnant Sprague Dawley rat dams received placebo (diluent) or nicotine (1 or 2 mg/kg) intraperitoneally in 100 μl volumes daily from embryonic day 6 until term (term = 22 days). Pups were allowed to deliver spontaneously and feed ad libitum. Animals were sacrificed at postnatal day 1, 8, 15, 21, and 60, and kidneys were allowed to deliver spontaneously and feed ad libitum. Animals were sacrificed at postnatal day 1, 8, 15, 21, and 60, and kidneys were collected for analysis using morphometry, immunohistochemistry, RT-PCR, and Western hybridization. The functional markers examined included PTHrP, Peroxisome Proliferator-Activated Receptor γ (PPARγ), Glial cell line-Derived Neurotrophic Factor (GDNF) and its receptor Ret, Pax 2, and bone morphogenetic protein-7 (BMP7), all signaling intermediates involved in kidney development.

**Summary of Results:** We found significant reductions in kidney weights and glomerular numbers at all age points examined in the nicotine exposed vs control group (*p < 0.05*). The expression of PTHrP, PPARγ, GDNF, Pax2, and BMP-7 mRNA decreased significantly in the nicotine exposed group at all ages examined. Concomitant treatment with a PTHrP/PPARγ agonist, PGJ2, blocked the decrease in the signaling intermediates involved in nephrogenesis as well as in the nephron number.

**Conclusions:** We conclude that *in utero* nicotine exposure down-regulates PTHrP/PPARγ signaling in the kidney and nicotine-induced kidney damage can be prevented by concomitant treatment with PTHrP/PPARγ pathway agonists. Supported by TRDRP (14RT-0073 & 15IT-0250).

**390 INTRAUTERINE GROWTH RESTRICTION ALTERS PPARγ1 AND γ2 EXPRESSION AND PROTEIN LEVELS IN THE RAT LUNG**


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**Purpose of Study:** Intrauterine growth restriction (IUGR) is associated with an increased risk of chronic lung disease in both humans and rats. Peroxisome proliferator-activated receptor gamma (PPARγ), a member of the nuclear receptor family of transcription factors, is localized to the conducting airway epithelium of murine lungs and is necessary for normal postnatal lung maturation. Despite the known importance of PPARγ in lung homeostasis, the expression of PPARγ splice variants, PPARγ1 and γ2, in the IUGR rat lung is currently uncharacterized. We hypothesized that IUGR would result in altered expression of PPARγ1 and γ2 mRNA transcript and protein levels in the rat lung.

**Methods Used:** IUGR (induced through utero-placental insufficiency) rat lungs were compared to control lungs. PPARγ1 and γ2 mRNA transcript levels were measured using real-time RT-PCR at birth (d0), day 7 (d7) day 21 (d21) and day 120 (d120) of life. Levels of PPARγ protein were measured at birth and d7 using Western Blotting.

**Summary of Results:** Results are expressed as % of control ± SEM. IUGR decreased PPARγ1 and γ2 mRNA levels in male lungs at d0 (γ1-71.9 ± 14.3%, γ2-58.2 ± 8.2%) and at d7 (γ1-84.3 ± 16.2%, γ2-86.5 ± 23.4%), with levels returning to that of controls by d21. In female lungs IUGR resulted in a decrease in mRNA levels of both
isoforms at d0 (γ1:61·5 ± 9·1%, γ2:61·1 ± 6·8%), followed by an increase in PPARγ1 levels by d7 (120·4 ± 9·4%), persisting through d120 (d21:122·5 ± 7·2%, d120:113·6 ± 9·4%). No significant differences were seen in protein levels at d0 in either gender; however IUGR increased levels of PPARγ1 protein in female d7 lungs (154% ± 32%).* p = 0.05.

Conclusions: We conclude that IUGR results in decreased PPARγ1 and γ2 mRNA at birth, followed by an increase in PPARγ1 mRNA and protein in females. We speculate that decreased expression of PPARγ variants in the IUGR rat lung during periods of active lung development may play an important role in defining lung morphology and subsequent susceptibility to disease. The gender specific difference in PPARγ mRNA and protein variants later in life may reflect an improved compensatory mechanism in female compared to male rat lungs. CHRC HD41075.

391 POSTNATAL STRESS AND MORPHINE INTERACT TO AFFECT CEREBRAL N-METHYL-D-ASPARTATE RECEPTOR SUBTYPE AND MU-OPIATE RECEPTOR-1 EXPRESSION AT ADULTHOOD

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Purpose of Study: Though both are common in neonatal intensive care, little is known about the long-term effects of neonatal stress and/or morphine. Using a rat model of neonatal stress, our group has shown impaired adult learning and increased adult forced swim behavior after neonatal morphine exposure. NMDA receptors are key substrates of learning and behavior. NMDARs consist of NR1 and NR2 subunits, the latter of which includes NR2A and NR2B. Morphine binds to Mu-opioid receptor-1 (MOR-1), which is also implicated in learning. Thus, altered NMDAR, or MOR-1 expression may occur in this model. We hypothesized that postnatal morphine and/or stress would affect cerebral MOR-1, NR1, NR2A, and NR2B subunit expression in adult rats.

Methods Used: Neonatal rats were treated from P3 to P7: CC (dam reared), CV (plus vehicle), CM (plus morphine), and SV (stress and vehicle). Stressors were artificial feeding, daily maternal separation plus isolation, hypo/hyperoxia and cold exposure. Protein was quantified by Western blotting using membrane fractions, and mRNA by real time RT-PCR (n = 6 per group).

Summary of Results: Protein expressed as arbitrary densitometry units ± SEM: morphine exposure alone decreased NR2A (CM 0.7 ± 0.1 vs CC 1.1 ± 0.2, p = 0.04). Stress alone decreased NR2B (SV 0.9 ± 0.1 vs CC 1.5 ± 0.2, p = 0.02), mRNA data (a percent of control ± SEM): morphine increased NR1 (CM 155 ± 22% and SM 144 ± 10%) over CC. CM, CV and SV increased NR2B over CC (p < 0.005). SM decreased MOR-1B2 (an isoform) relative to CV (55 ± 5%).

Conclusions: Effects of morphine on NR2A levels are blunted by stress, and effects of stress on NR2B levels are blunted by morphine. Interestingly, NR2A knockout mice display increased swimming behavior as well as impaired learning and memory. No conditions changed total MOR-1 mRNA, but SM decreased the expression of one of its isoforms. NR1 mRNA, but not protein, was increased by morphine. These results are intriguing because they support the premise that many early life insults experienced by neonates can have long-lasting effects on cerebral development and function. NMDARs as well as MOR-1 are potentially important mediators of these changes. (Supported by CHRMC, CHRCDA).

392 NEONATAL STRESS REDUCES CEREBRAL CYP19A1 EXPRESSION

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Purpose of Study: Preterm infants in neonatal intensive care units (NICU) are exposed to a variety of stressors. Exposure to NICU stressors occurs during key periods in brain development, yet the effect of such stress on brain development is unknown. Brain development is dependent upon the concentrations and actions of cerebral sex steroids. Cerebral steroid concentrations are largely mediated by the CYP19a1 gene, which converts androgens into estrogens. Actions of sex steroids are mediated by the androgen receptor (AR) and estrogen receptors (ERα and ERβ). We hypothesized that neonatal stress would alter the expression of CYP19a1, AR, ERα and ERβ in the rat brain. In addition, we hypothesized that treatment with erythropoietin (EPO) would normalize gene expression in stressed animals.

Methods Used: Expression of CYP19a1, AR, ERα and ERβ was measured in the whole brain of control rats (CC), vehicle-treated controls (CV), EPO-treated controls (CE), vehicle-treated and stressed rats (SV), and EPO-treated and stressed rats (SE) (n = 5). Stressed rats were isolated, hand-fed, exposed to 100% nitrogen and 100% oxygen, cooled to 4°C, warmed to 32°C, and returned to dams. Stress protocols were repeated daily from postnatal day 3-7 (P3-P7) and followed by injections of saline vehicle or EPO. Brains were harvested and flash frozen on postnatal day 10 (P10) and postnatal day 114 (P114) of life. Real-time RT PCR and western blots were used to measure mRNA and protein expression respectively.

Summary of Results: At P10 mRNA and protein data indicate CYP 19a1 is reduced in CE, SV, and SE (p < 0.0001 and p < 0.01). While CYP19a1 mRNA expression is unaltered at P114, there is a significant reduction in protein expression in CV, SV, and SE groups (p < 0.05) with a trend towards reduction in the CE group (p = 0.08). Expression of AR, ERα and ERβ were not affected by stress or EPO treatment at either age.

Conclusions: Our results suggest that at P10, cerebral androgens may be elevated and cerebral estrogens reduced in both stressed and EPO treated animals. We speculate that underexpression of CYP19a1 and resultant alteration of cerebral androgen and estrogen concentrations are likely to impact cerebral development in neonatally stressed animals. CHRC HD41075.

393 IUGR ALTERS THE DNA METHYLATION AND THE HISTONE CODE IN THE PROMOTER REGION OF THE HIPPOCAMPAL IGF1R


Purpose of Study: We have previously shown that IUGR rat exhibits gender specific changes in cerebral IGF1 downstream signaling. Cerebral expression of IGF1R could provide a mechanistic explanation for these changes. Our previous results showed significantly decreased IGF1R mRNA and protein levels in the brain of d21 IUGR males and females rats. Alterations in IGF1R expression could be due to changes in the epigenetic determinants such as DNA methylation, histone methylation and acetylation. We hypothesize that in the hippocampus of d21 rats IUGR alters IGF1R DNA methylation and the histone code in the promoter region of the gene.

Methods Used: To test our hypothesis we used hippocampal tissue from d21 IUGR and control rats (n = 4/group). We performed bisulfite
diagnosis. In RhD negative women, the detection of the D gene in DNA pregnant women has opened a new avenue for non-invasive prenatal screening. The discovery of free fetal DNA in plasma of Moines University, Des Moines, IA.

Methods Used: The growth factor-I (IGF-I) and glucose homeostasis in rat pups. Maternal Zn deficiency resulted in elevated insulin and IGF-I levels, glycemia, hyperinsulinemia and insulin resistance in the offspring. Additionally, liver IGF-I and IGFBP-3 protein levels were higher in ZnD pups than in control pups. HOMA index (measure of insulin resistance) was noted, pups from ZnD dams weighed significantly more than control animals by postnatal day (PD) 10 (+20%) and PD 20 (+50%). Serum insulin, C-peptide and glucose were significantly higher than in control animals on PD 10 (+50%).

Summary of Results: On d21 IUGR males present with DNA hypermethylation at CpG site 19 (p = 0.02), 200 bases upstream the exon 1 and with H3K4 hypomethylation in the same region (53% of the control). In contrast, IUGR females present with H3K4 hypermethylation (56%), associated with H3K9 hypoaacetlylation (54%) of the promoter.

Conclusions: IUGR leads to significant changes in postnatal IGF1R expression associated with gender specific changes in DNA methylation, histone methylation and acetylation. Interestingly, on d21 the neuronal gene expression is decreased in both genders, while the epigenetic modifications we demonstrated are different. We speculate that in d21 hippocampus, IUGR activates gender specific pathways that alter the epigenetic characteristics of the IGF1R and subsequent decreases its expression.

394 MATERNAL ZINC DEFICIENCY AFFECTS GROWTH, INSULIN-LIKE GROWTH FACTOR-I, AND GLUCOSE HOMEOSTASIS IN RAT PUPS

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Purpose of Study: The interaction between zinc (Zn), insulin, and glucose metabolism is complex. Mild to moderate maternal Zn deficiency affects maternal carbohydrate metabolism and fetal growth and development, but the mechanisms behind changes in fetal glucose homeostasis previously observed are not well understood. We examined the effects of maternal moderate Zn deficiency on growth, insulin-like growth factor-I (IGF-I) and glucose homeostasis in rat pups.

Methods Used: Rats were fed Zn deficient diet (ZnD, 7 K\textsuperscript{2}g/g) ad libitum for 3 weeks, bred and kept on Zn deficient diet during pregnancy and lactation. Postnatally, rat pups were nursed by their original mothers, and weights of pups were recorded daily. Serum insulin, glucose, and C-peptide concentrations, and hepatic IGF-I, and IGF-binding protein-3 (IGFBP-3) were measured.

Summary of Results: Although no difference in birth weight between the two groups was noted, pups from ZnD dams weighed significantly more than control animals by postnatal day (PD) 10 (+20%) and PD 20 (+50%). Serum insulin, C-peptide and glucose were significantly higher in ZnD pups than in control pups. HOMA index (measure of insulin resistance) of ZnD pups was also higher than in control pups. Additionally, liver IGF-I and IGFBP-3 protein levels were higher in ZnD pups compared with controls.

Conclusions: Maternal Zn deficiency was associated with hyperglycemia, hyperinsulinemia and insulin resistance in the offspring. Maternal Zn deficiency resulted in elevated insulin and IGF-I levels, suggesting that maternal Zn status may affect insulin and glucose homeostasis postnatally in the offspring, resulting in excessive postnatal weight gain.

395 QUANTITATIVE MEASURE OF EPSILON GLOBIN GENE EXPRESSION AS A MARKER FOR FETAL NUCLEIC ACID

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Purpose of Study: The discovery of free fetal DNA in plasma of pregnant women has opened a new avenue for non-invasive prenatal diagnosis. In RhD negative women, the detection of the D gene in DNA derived from maternal plasma by quantitative PCR has been used to assist in prenatal management, however a negative result (no D gene detected) must be further evaluated for adequacy of fetal DNA sample. We hypothesized that significant epsilon globin gene expression (a component of embryonic hemoglobin) in the form of epsilon globin mRNA could serve as a positive control for the presence of fetal sample.

Methods Used: We measured epsilon globin mRNA in fetal blood, liver and marrow, and compared epsilon globin mRNA concentrations with that measured in adult blood. Total RNA was isolated from marrow, liver and blood of fetal samples (11–22 weeks gestation), and from adult peripheral blood samples. RNA isolation was performed using a commercial reagent (Trizol, Invitrogen, CA). Total RNA was measured spectrophotometrically, and reverse transcribed to cDNA using a commercial reverse transcriptase kit (Applied Biosystems, Inc. ABI, Foster City, CA). Quantitative polymerase chain reaction (PCR) was performed on a 7500 Fast Real-Time PCR System (ABI) using epsilon globin primers and probe (ABI). Quantitative expression of 18S ribosomal RNA was used as an internal control to normalize starting quantities of sample cDNA.

Summary of Results: Epsilon globin mRNA was detected in all fetal samples, but was negligible in all adult samples. Gene expression was 300 to 400 fold greater in the fetal blood samples than in the adult blood samples (387.4 ± 79.4 vs 1.4 ± 0.6, p = 0.005). Epsilon globin mRNA was four-fold greater in fetal peripheral blood as compared to fetal liver and marrow (p = 0.035, blood vs. liver and marrow).

Conclusions: Fetal epsilon globin gene expression can be differentiated from adult gene expression, and may serve as a positive control when determining the presence of fetal RNA in total RNA isolated from maternal plasma. We speculate that abundant epsilon globin gene expression may serve as evidence for the presence of fetal RNA.

396 INTRAUTERINE GROWTH RESTRICTION ALTERS EXPRESSION OF TRANSFORMING GROWTH FACTOR BETA ISOFORMS AND RECEPTORS IN A GENDER DEPENDENT MANNER

A. J. Zabrocki, R.H. Lane, R.A. McKnight, S. O’Grady, D. Caprau, C. Callaway, M.A. Hale, X. Yu, and H. Fu, University of Utah, Salt Lake City, UT.

Purpose of Study: Intrauterine growth restriction (IUGR) in both humans and rats alters brain histology, with males being more affected. One mechanism which may account for the altered histology is aberrant gene expression associated with gender specific changes in DNA methylation, histone methylation and acetylation. Interestingly, on d21 the cerebral cortex, hippocampus, IUGR activates gender specific pathways that alter the epigenetic modifications we demonstrated are different. We speculate that in d21 hippocampus, IUGR activates gender specific pathways that alter the epigenetic characteristics of the IGF1R and subsequent decreases its expression.
82%, and 74%) as compared to sham males. In contrast, IUGR increased mRNA levels of the TGFβ receptors (107%, 122%, 123%), as compared to sham males.

**Conclusions:** We conclude that IUGR increases mRNA levels of TGFβ receptors in the male rat brain. In contrast, mRNA levels of many of receptors and isoformal were generally decreased in the IUGR female brain. These preliminary findings are intriguing, because in other model systems, over exuberant expression of genes involved in TGFβ signaling are associated with decreased neuron and synapse numbers. Both of which we have observed as key characteristics of the male IUGR brain at day 0. Furthermore, IUGR increases neuron numbers in the female brain. We speculate that gender specific differences in expression of TGFβ signaling components contribute to the disparate histological response we observe between male and female IUGR brains.

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**397 IUGR DECREASES PGC-1 ALPHA mRNA AND PROTEIN EXPRESSION IN D0 RAT BRAIN**

N. Mitchell, S. O’Grady, D. Caprau, Y. Contreras, M. Hale, X. Yu, C. Callaway, X. Ke, R. McKnight, and R. Lane. University of Utah, Salt Lake City, UT.

**Purpose of Study:** IUGR is associated with long-term neurodevelopmental impairment in the human. However, the exact mechanisms of such disabilities are unclear. Previous studies from our group have demonstrated that IUGR decreases brain mitochondrial number. The transcription factor peroxisome proliferator-activated receptor gamma (PPAR gamma) coactivator 1 alpha, or PGC-1α, is involved in mitochondrial biogenesis. Furthermore, recent literature has also indicated that PGC-1α exerts a neuroprotective effect in the brain of rats. PGC-1α appears to activate the anti-ROS systems in the brain, producing a neuroprotective effect. The impact of IUGR upon PGC-1α expression is unknown. We therefore hypothesize that PGC-1α will be decreased in the brains of IUGR rats compared to sham rats.

**Methods Used:** Bilateral uterine artery ligation and sham procedure was used to induce IUGR and control animals, respectively. Whole brain tissue was obtained from D0 rats. We used real-time PCR to analyze the expression of PGC-1α in both IUGR and sham rat pups, and Western blots were used to analyze protein levels.

**Summary of Results:** In the newborn rat pups, IUGR significantly decreased PGC-1α mRNA levels to 52 percent (+/- 12) of control values (P < 0.05). Similarly, IUGR also decreased PGC-1α protein levels to 65 percent (+/-15) of control values (P < 0.05).

**Conclusions:** We conclude that IUGR decreases PGC-1α expression in D0 newborn rat pups. These findings are consistent with our previous findings of decreased mitochondrial number, as well as increased ROS content, in the IUGR brain. Furthermore, PGC-1α null mice experience more ROS-induced neuronal damage versus wild type mice. We speculate that the decrease in PGC-1α expression contributes to two of the previous pathologies we have described in the IUGR rat pup, decreased mitochondrial number and increased ROS content.

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**399 IUGR ALTERS HIPPOCAMPAL DUAL SPECIFICITY PHOSPHATASE 5 GENE EXPRESSION IN NEWBORN AND JUVENILE RATS**

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**Purpose of Study:** Background: IUGR alters hippocampal histology in both human and rats. In rats, these changes are associated with epigenetic determinants of gene expression. Previous studies from our group have determined that epigenetic determinants of DUSP5 gene are vulnerable to IUGR in non-CNS tissue. DUSP5 dephosphorylates Erk1/Erk2, which are members of the important MAPK cascade, a pathway critical to memory.

**Objective:** It is unknown whether IUGR affects DUSP5 expression and epigenetic characteristics in the hippocampus. We hypothesized that hippocampal DUSP5 gene expression would be affected by IUGR at d0 and d21 of life and that these changes will correlate with hippocampal Erk1/Erk2 phosphorylation.

**Methods Used:** We compared IUGR and control hippocampus at d0 and d21 of life. We used real-time RT-PCR to measure DUSP5 mRNA levels. Western blotting was used to quantify DUSP5 and phosphorlated ERK1/2 protein levels. DUSP5 expression in the brain was localized by fluorescence in situ hybridization (FISH) and immunohis-
toehistone acetylation (H3K). Bisulfite sequencing analysis was used to determine DNA methylation of DUSP5 promoter Sp1 site and exon2.

Summary of Results: IUGR significantly decreased hippocampal DUSP5 mRNA and protein levels in males at d0 and d21 of life while DUSP5 protein levels remained unchanged in female at d0 but decreased at d21 of life. In contrast, Erk2 phosphorylation was significantly increased in d0 IUGR male hippocampus. Furthermore, Erk1 phosphorylation was significantly increased in both genders at d21 of life. Similar to the direct measures of gene expression, FISH and IHC showed that DUSP5 expression decreased in the male hippocampus. Interestingly, DNA hypermethylation within DUSP5 promoter Sp1 site was observed in female hippocampus but not in male at both d0 and d21.

Conclusions: IUGR affects hippocampal DUSP5 gene expression and epigenetic determinants in a gender specific manner. Our findings underlie the complex and gender specific nature of genetic regulation of gene expression. We speculate that the dysregulation of Erk phosphorylation and MAPK cascade may contribute to the impaired hippocampal memory we have previously demonstrated in the IUGR rat (CHRC; HD41075).

400 HISTONE ACETYLATION OF VENTRICULAR WHITE MATTER IS AFFECTED BY VENTILATION MODE IN PRETERM LAMBS


Purpose of Study: Mechanical ventilation (MV) of the premature human neonate is associated with brain injury. We have shown that MV injures the brain of preterm lambs, and that the injury is reduced by using nasal CPAP, as an alternative mode of ventilation, as well as by administering the histone deacetylase inhibitors, valproic acid (VPA) or trichostatin A (TSA), during MV. Those findings suggest that ventilation mode affects epigenetic regulation of gene expression in the brain. We hypothesized that histone acetylation in ventricular white matter will be affected by ventilation mode in preterm lambs.

Methods Used: Preterm lambs (~132d gestation; term~148d), treated with antenatal steroids and postnatal surfactant, were managed by MV, nasal CPAP, MV+VPA, or MV+TSA (n = 4 each). At the end of 3d, ventricular white matter was isolated and analyzed by immunoblot for acetylated H3K14 and acetylated H3K18.

Summary of Results: Ventricular white matter from the MV group had significantly less normalized abundance of acetylated H3K14 and acetylated H3K18 proteins (15.3 ± 0.5 and 3.3 ± 0.4, respectively, X ± SD; p < 0.05) than the nasal CPAP (32.4 ± 1.9 and 7.5 ± 1.1, respectively), MV+VPA (30.7 ± 2.9 and 7.4 ± 0.6, respectively), and MV+TSA (26.5 ± 3.2 and 7.5 ± 0.6, respectively) groups.

Conclusions: We conclude that ventilation mode affects acetylation of histones (histone modifications) in the preterm lamb brain. These results are consistent with our previous observations that MV leads to moderate ischemia of the brain, as evidenced by increased apoptosis among glia in the brain. We speculate that a specific subset of genes is expressed in the brain due to MV-mediated changes in cytokine/chemokine release from the injured lung and limited cerebral vascular autoregulation in the premature newborn. (HL62875, HL56401, HD41075, CHRC).

401 HIND LIMB RECOVERY OF SOLEUS MUSCLE IN T8 TRANSECTED RATS WITH PULSE FIELD MAGNETIC STIMULATION TREATMENT

A. Ehsan1, Y. Lee1,2, and V. Lin1,2. 1Long Beach Veterans Affairs, Long Beach, CA and 2UC Irvine, Irvine, CA.

Purpose of Study: To demonstrate reduction of soleus muscle atrophy with pulse field magnetic stimulation in T8 transected rats.

Methods Used: Eighteen rats were used in three groups (n = 6 each) including T8 laminectomy-only (group 1), T8 spinal cord transection (group 2), and T8 spinal cord transection with magnetic stimulation (group 3). Levels of soleus atrophy were analyzed by two approaches: 1) Cross-Sectional Area of Soleus: Animals were terminated at one month following spinal cord surgery to collect the soleus muscle. Muscles were sectioned into 10 mm and stained. Areas of 300 fibers from each muscle fiber type were measured. 2) Western Blot Analysis of Phosphorylated Akt Protein: Soleus muscles were collected from each animal up to 30 days post-surgery and homogenized in lysis buffer. The supernatant was analyzed via western blot and was probed with a serine phosphorylated Akt antibody and later probed with a total Akt antibody. Blots were analyzed by scanning densitometry and ratios of phosphorylated Akt/total Akt were compared for each animal and each leg to evaluate level of atrophy.

Summary of Results: 1) Cross-sectional area of muscle measurement: Of the three groups, the average soleus cross sectional area of group 1 was 2869 ± 30.6 μg², group 2 was 1758 ± 17.3 μg², and group 3 was 2030 ± 25.1 μg². In addition the average body weights of the groups 1–3 were 296 ± 3.6 grams, 295 ± 4.5 grams, and 292 ± 2.8 grams respectively.

2) Western Blot analysis of phosphorylated Akt protein: Magnetic stimulation ranged from 1 day to 1 month followed by termination. Our results had a significant amount of variation among animals in the same time period treatment group and also among the left and right leg of the same animal. Thus no distinct expression pattern of phosphorylated Akt/total Akt was discernable.

Conclusions: In evaluating the level of atrophy with pulse field magnetic stimulation in T8 transected rats, magnetic stimulation increased the cross sectional area and mass of the soleus muscle while stimulation did not show a discernible pattern of phosphorylated Akt/total Akt. In conclusion, magnetic stimulation did in fact cause a decrease in atrophy detectable by evaluating the morphology and mass of the soleus, but not by the measure of phosphorylated Akt expression.

402 HELICAL COMPUTED TOMOGRAPHY ANGIOGRAPHY IN THE PRE TREATMENT EVALUATION OF PATIENTS WITH CEREBRAL ANEURYSMS - COMPARISON TO ROTATIONAL ANGIOGRAPHY

J. Chung1,2, G. Duckwiler1, R. Jahan1, F. Vinuela1, J. Sayre1, and P. Villablanca1. 1David Geffen School of Medicine at UCLA, Los Angeles, CA and 2UCLA Medical Center, Los Angeles, CA.

Purpose of Study: 3D CTA is a sensitive and specific modality for detecting and characterizing cerebral aneurysms as compared to 2D DSA. More recently, studies indicate that 3D DSA is superior to 2D DSA. This study’s purpose was to compare 3D CTA to 3D DSA in detecting and characterizing cerebral aneurysms. Dictated treatments based on each imaging modality, actual treatment employed, and final outcomes were also evaluated.

Methods Used: Fifty-two patients (35 F/17 M) who received both 3D CTA and 3D DSA imaging studies between November 2004 and July 2007 at the UCLA Medical Center were included in this retrospective comparative analysis. Inclusion criteria for patients with known or suspected intracranial aneurysms were 1) a 3D CTA study 2) a 3D DSA study and 3) an iscan interval < 1 month. Dictated neuroradiology reports were retrospectively reviewed using an online reporting database at the UCLA Medical Center. Information regarding aneurysm detection, lesion characterization, dictated and actual treatments, and final treatment outcomes were extracted. All CTA/DSA scans were reviewed
ADHESION KINASE (FAK) IN EYES AFFECTED BY PROLIFERATIVE VITREORETINOPATHY (PVR)

S.C. Kim, L. Gordon, and M. Wadehra. David Geffen School of Medicine at UCLA, Los Angeles, CA.

Purpose of Study: Proliferative vitreoretinopathy (PVR) occurs in 10% of patients with retinal detachment. A multi-phase process involving cell migration and membrane formation in an aberrant wound-healing strategy, PVR may result in loss of vision. The tetraspan protein epithelial membrane protein 2 (EMP2) is highly expressed in retinal pigment epithelium (RPE), and has been shown to co-immunoprecipitate with focal adhesion kinase (FAK). In ARPE-19 cells, a RPE cell line, EMP2 modulates collagen gel contraction through activation of the FAK-Src pathway. As this in vitro phenotype mimicked PVR, we speculated that EMP2 may play a critical role in this disease process. Thus, the purpose of this study was to investigate the relative expression of EMP2 and FAK in retinal membranes from ocular tissue affected by PVR.

Methods Used: To assess the role of EMP2 and activated FAK in PVR, an in vivo rabbit model of PVR was created through intraocular injection of ARPE-19 cells. Immunohistochemistry was performed to semi-quantitatively identify EMP2 and FAK expression.

Summary of Results: In both human and murine eyes, EMP2 and activated FAK are co-expressed in corneal epithelium and RPE cells. In APRE-19 cells, EMP2 is predominantly expressed on the plasma membrane. Strikingly, in the in vivo PVR model, EMP2 and activated FAK are translocated and co-localize in the nuclei of RPE cells.

Conclusions: These findings suggest that these proteins may play a critical role in PVR. Defining the underlying biological mechanisms of this process may provide insight on methods of intervention, leading to new strategies in the therapy of PVR.

A POTENTIAL ANIMAL MODEL TO STUDY THE PHYSIOLOGY OF AUTISM

L.A. Heise and A. Allan. UNM, Albuquerque, NM.

Purpose of Study: The drastic increase in the autism incidence during the past decade suggests that epigenetic modulations, such as DNA methylation, may play a significant role in the etiology. DNA methylation represses gene expression by recruiting a family of methylated-CpG binding proteins (MBDs), including MBD1 and MeCP2. Recently a mutation of MeCP2 has been shown to be responsible for majority cases of Rett Syndrome, an autism related syndrome. Dr. Zhao (from University of New Mexico) has developed an MBD1 mutant (Mbd1−/−) mouse line. The mice appear normal, both anatomically and developmentally; however, they exhibit behaviors that are often seen in autistic patients (social avoidance, impaired prepulse inhibition, heightened anxiety). While there are few specific physiological hallmarks of autism among patients, a few studies have noted increased levels of adrenocorticotropic hormone and lower plasma corticosterone, which mice exhibit. The project aims to look at the central deregulation of the corticosterone releasing factor-hypothalamic-pituitary-adrenal axis by comparing the presence of receptors for corticosterone releasing factor-1 in the hippocampi of knock-out and wildtype mice.

Methods Used: Six knock-out mice and 6 wildtype mice were euthanized and the hippocampi removed and homogenized. A western
406 CHANGES IN PRO-BRAIN DERIVED NEUROTROPHIC FACTOR (BDNF) LEVELS IN MICE PRENATALLY EXPOSED TO ETHANOL

N. Spence, M. Roehlk, and A. Allan. University of New Mexico School of Medicine, Albuquerque, NM

Purpose of Study: Prenatal Alcohol Exposure (PAE) in humans has been associated with an increased incidence of depressive disorders. Postmortem brain tissue samples from patients with major depressive disorder have been found to have lower levels of brain-derived neurotrophic factor (BDNF). In addition, antidepressant drugs have been shown to elevate BDNF levels. This has lead to the suggestion that depression is the result of lower BDNF in the brain. In a previous study using a mouse model of PAE, this laboratory found an increased degree of depression, as measured by learned helplessness behavior, and lower levels of BDNF in the medial frontal cortex (MFC), but not the hippocampus (HPC). BDNF is produced in neural tissue by the enzymatic cleavage of the pro form of the protein, or pro-BDNF. Our purpose is to extend studies from UNM laboratories (Allan, 2003) and use a PAE mouse model displaying Fetal Alcohol Spectrum Disorders (FASD) to explore the relationship between PAE, depression, and changes in brain pro-BDNF levels.

Methods Used: Female mice were selected from a colony and half were provided with set amounts of alcohol immediately pre-natal and throughout pregnancy. After birth and weaning, the offspring were put through learned-helplessness training, a previously established protocol to identify depressive behavior in the mice, to assess differences in depressive behavior in offspring of mothers with alcohol exposure. Mice with a history of prenatal alcohol exposure did show greater learned helplessness behavior. Mice from each group were then euthanized and the medial frontal cortex and hippocampus from each was removed over the weekend. The levels of the immunolabeled secondary antibodies were detected using a chemiluminescent kit.

Summary of Results: pro-BDNF protein levels were reduced by a significant amount in the medial frontal cortex of the mice exposed to prenatal alcohol but not in the hippocampus of these mice.

Conclusions: These results identify reduced pro-BDNF protein levels in the medial frontal cortex as potential mediators of depressive disorders associated with prenatal ethanol exposure.

Pulmonary and Critical Care II
Concurrent Session
8:30 AM
Saturday, February 2, 2008
mechanical ventilation. 

The carbon cost of breathing index (C-COBI) was used to determine the work of breathing during spontaneous breathing trials. The C-COBI was calculated using the difference in minute ventilation (VE) and carbon dioxide production (VCO2) between the start and end of the spontaneous breathing trial, divided by the VCO2 in the minute prior to the trial. This index helps to quantify the effort required to breathe during mechanical ventilation.

**Summary of Results:** In the 13 trials included, 10 tolerated the SBT by pre-established criteria (successful group) and 3 did not (failure group). The mean C-COBI reached a stable value within the first 10 minutes of each SBT. The mean C-COBI for the failure group was 16% (±0.13) compared to 0.06% (±0.13) for the successful group.

**Conclusions:** A clinically relevant increase in work of breathing, as measured by the C-COBI, is apparent within 10 minutes of spontaneous breathing trials in patients who are unlikely to tolerate liberation from mechanical ventilation.

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**409 A DYNAMIC CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD) SEVERITY SCORE PREDICTS LONGITUDINAL HEALTH-CARE UTILIZATION**


**Purpose of Study:** To prospectively validate a chronic obstructive pulmonary disease (COPD) severity score.

**Methods Used:** Using a population-based sample of 267 adults with self-reported physician-diagnosed COPD, we examined the relationship between a previously-developed COPD severity score and future respiratory hospitalizations, emergency department (ED) visits, and outpatient visits. This COPD severity score (range 0–35) is comprised of 5 domains: respiratory symptoms, systemic corticosteroid use, other COPD medications, prior hospitalization or intubation, and home oxygen use. Structured telephone interviews were conducted at baseline and on an annual basis in 2 subsequent years (total of 3 waves). These interviews determined baseline and follow-up COPD severity scores and health-care utilization. Generalized estimating equations were used for repeated measures in a multivariate model (controlling for sociodemographics, tobacco history, and medical comorbidities) to estimate the effect of both the baseline COPD severity score and the annual change in the severity score on subsequent health-care utilization.

**Summary of Results:** The mean baseline COPD severity score was 7.8 ± 6.3. The mean two-year change (Δ) in the score was +0.65 (SE = 0.29), in the direction of more severe disease (Δ deviating from 0, p = 0.03). Both the baseline severity score and the annual change in score were prospectively associated with respiratory hospitalizations, ED visits, and outpatient visits (Table).

**Conclusions:** The baseline COPD severity score predicts health-care utilization. Moreover, the change in the severity score is itself associated with these outcomes, suggesting that the score is longitudinally responsive to changes in COPD status. This severity score may be used as a study outcome or to adjust for disease severity.

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**410 TRANSITION TO ORAL VASODILATOR THERAPY FOR SEVERE PULMONARY ARTERIAL HYPERTENSION (PAH) - A SINGLE CENTER SERIES**

M.S. Sidhu, R. Saggi, J.A. Belperio, R. Saggi, J.P. Lynch III, S. Weigt, D.A. Zisman, and D.J. Ross. David Geffen School of Medicine, Los Angeles, CA.

**Purpose of Study:** Severe PAH portends a grave prognosis if left untreated. Parenteral prostacyclin (PGI2) has been long considered most appropriate for severe, functional class (FC) III/IV PAH. However, intravenous PGI2 is associated with potential catheter related infections. Experience with transition from intravenous to oral therapy is limited. Herein, we report our single center clinical experience for patients who requested transition to oral therapies after parenteral PGI2 complications.

**Methods Used:** A retrospective review of seven patients with PAH [idiopathic (4), anorexigen (2), ASD (1)] who were determined during Phase I of our protocol, to be “clinically stable.” In Phase II, PGI2 was decreased by 0.5–1 ng/kg/min weekly during close monitoring of physical examination, transthoracic echocardiography, six-minute walking distance (6MWD), FC (I-IV) and B-type natriuretic peptide (BNP) until discontinuation. Concurrently, oral vasodilator therapies were introduced with the combination of bosentan and sildenafil. This combination was selected to target both the endothelin-1 and phosphodiesterase-type 5 pathways in PAH. In Phase III, after discontinuation of PGI2, surveillance was maintained to detect and intervene for potential clinical worsening.

**Summary of Results:** For the seven patient group during phase I, the optimal achieved FCs were: Classes I (2), II (4), and III (1). At the end of phase III, the FCs were: I (3) and II (4). One patient later experienced right ventricular failure after five months, resulting in requirement for therapy with subcutaneous treprostinil and return to “active listing” for lung transplant. The mean duration (mos) ± SD for phase I was 15.9 ±[4.8], phase II 7 ±[3.3], and phase III 10.9 ±[7.4]. Data for 6MWD and BNP is summarized in the TABLE.

**Conclusions:** Selected patients with severe PAH at initial presentation, may be transitioned from parenteral PGI2 to oral therapies during strict clinical monitoring. However, longer term results and a larger series will be necessary to ascertain the efficacy of this strategy.

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**411 HYDROCORTISONE ADMINISTRATION IN THE HYPOTENSIVE VERY LOW BIRTH WEIGHT INFANT: THE EFFECT OF STUDY METHODOLOGY ON MEASURED ASSOCIATIONS**

S. Higgins, I. Seri, and P. Friedlich. CHLA, University of Southern California, Los Angeles, CA.

**Purpose of Study:** A growing body of research has examined the use of hydrocortisone (HC) to increase blood pressure and reduce vasopressor use in hypotensive very low birth weight (VLBW) infants. Studies examining these relationships have used varying designs and HC dosage regimens. A significant amount of variance exists in the effect sizes reported in these studies. The purpose of the present study is to examine the relationship between HC therapy, hypotension and vasopressor weaning by study design and dosage regimen.
Methods Used: Ovid Medline, EBM-Cochrane Database of Systematic Reviews, EBM-Cochrane Central Register of Controlled Trials and EBM-Database of Abstracts of Reviews of Effects were used to identify studies including the terms HC, hypotension and VLBW infant. Studies were coded for the presence or absence of: 1) a placebo control group and 2) titration of HC dosage to achieve the desired hemodynamic response.

Summary of Results: A fixed effects meta-analytic approach found studies without a placebo control group reported a greater blood pressure increase (N = 187, r = .28, 95%CI = .14-.41) and a greater ability to wean VLBW infants from vasopressor use (N = 61, r = .51, 95%CI = .30-.68) than studies employing a placebo control group. A fixed effects meta-analytic approach found studies in which HC dose was titrated to achieve the desired hemodynamic response reported a greater blood pressure increase (N = 187, r = .35, 95%CI = .22-.47) and a greater ability to wean VLBW infants from vasopressor use (N = 61, r = .43, 95%CI = .20-.61) than studies that used fixed doses of HC.

Conclusions: Differences in study design and dosage regimens were found to moderate the relationship between HC therapy, hypotension and vasopressor weaning. To prevent underestimation of the efficacy of HC for increasing blood pressure and decreasing vasopressor use in hypotensive VLBW infants, it is recommended that future studies titrate HC dosage to achieve the desired hemodynamic response. The results of this meta-analysis may have broader applications for research methodology in the area of drug efficacy.

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DYSPHAGIA FROM EXTERNAL LYMPH NODE COMPRESSION OF ESOPHAGUS IN SARCOIDOSIS
H. Bhaktihavatsalam, S. Kaushik, A. Matin, and S. Niranjani. Coney Island Hospital, Brooklyn, NY.
Purpose of Study: Dysphagia from sarcoidosis can be from gastric involvement or neuropathy or external lymph node compression of esophagus.
Methods Used: Case Report.
Summary of Results: 54 year old lady presented with intermittent solid food dysphagia, weight loss, generalized malaise for about 3 months. Patient was a non smoker , had no significant past medical history or family history.Chest Xray revealed hilar adenopathy.Esophagogram showed narrowing and mucosal irregularity near the GE junction.Chest CT showed esophageal compression at subcarinal level by enlarged hilar lymphnodes. EGD revealed GE junction narrowing and gastric ulcer.-Malignancy and H.Pylori infection were ruled out by biopsy.Biopsy of the paratracheal lymphnode showed noncaseating granulomas suggestive of sarcoidosis.Pulmonary function tests were normal.Gallium imaging showed increased uptake in the hilar lymphnodes but no lung involvement.Patient was diagnosed with stage1 sarcoidosis and the dysphagia was from external compression by the hilar lymphnodes. Patient was clinically followed without steroid treatment.At followup by two months, patient was well and reported no dysphagia. Repeat chest CT showed regression of the hilar lymphnodes.
Conclusions: Clinically GI sarcoid is recognized in 0.1% to 0.9%.It commonly involves the stomach but esophagus,appendix,colon, rectum and small bowel involvement have been described. Dysphagia is secondary to gastric/esophageal sarcoid or external compression from hilar lymphnodes or from neuropathy. The diagnosis is made by biopsy detecting noncaseating granulomas.It has been documented that stage1 disease has 60-80% spontaneous remissions without active treatment like our patient.

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LEVEL OF CYTOKINE PRODUCTION IS ASSOCIATED WITH THE CLINICAL COURSE OF CYSTIC FIBROSIS (CF) PATIENTS
A.M. Yu, T. Chin, E. Nuussbaum. 1University of California, Irvine, CA and 2Miller Children’s Hospital, Long Beach, CA.
Purpose of Study: The purpose of the study is to determine the clinical course of CF patients who have had assessment of their cytokine production by peripheral blood mononuclear cells (PBMC) ten years ago.
Methods Used: After informed consent, PBMC from 20 CF patients were isolated and stimulated with Pseudomonas aeruginosa (PSA) antigen (PA-I) for 3 days in 1995-1996. Tumor necrosis factor (TNF-alpha), interleukin 6, and interleukin 8 were measured by ELISA assay (R&D). Five patients were identified as having the highest levels of cytokine production (both spontaneously and after PA-I stimulation) and compared with five patients with the lowest levels.
Summary of Results: Examination of their spirometry measurements over the subsequent 11-12 year period showed the average decline of both FEV1 and FVC were greater in the high cytokine producer group. However, there was no difference in the decline of the average FEF25-75. In addition, the frequency of hospitalizations were greater in the high cytokine producer group (1.5 hospitalizations/patient/year compared with 0.9 hospitalizations/patient/year).
Conclusions: This retrospective pilot study suggests that determination of systemic inflammatory cytokine production can be used as a predictor of clinical outcome in CF patients. It is further evidence for the role of inflammation in the pathogenesis of CF lung disease.

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PSEUDOMONAS AERUGINOSA ADAPTATION TO AIRWAY INFLAMMATION IN CYSTIC FIBROSIS
B. Staudinger, H. Rosen, P. Singh. 1University of Washington, Seattle, WA and 2University of Washington, Seattle, WA.
Purpose of Study: Bacterial airway colonization is associated with significant morbidity in patients with cystic fibrosis (CF). Early colonizing populations, dominated by H. influenza and S. aureus, are typically supplanted by P. aeruginosa (PA) marking for many patients the onset of a progressive decline in respiratory function. Even when first detected, the population of PA in CF airways is heterogeneous with respect to colony morphology and mucoidy and differs from that of most
other clinical and environmental isolates. Chronic colonization is associated with genetic drift suggesting adaptation to selective pressures. Among these may be a hostile environment generated by the inflammatory response in general, and by polymorphonuclear leukocytes (PMN) in particular. We hypothesized that PA would evolve to become more resistant to antimicrobial host-factors involved in the inflammatory response.

Methods Used: This study compared the response of 2 fully sequenced PA isolates, from a CF patient at ages 0.5 (early) and 8 (late), to microbicidal systems of intact PMN and to PMN-related cell-free antimicrobial systems (myeloperoxidase (MPO), hydrogen peroxide and/or reagent hypochlorous acid).

Summary of Results: We observed that PA had become MORE SUSCEPTIBLE to killing by serum factors and reagent hydrogen peroxide, but MORE RESISTANT to killing by an MPO-mediated antimicrobial system. Preliminary studies with intact PMN suggest that the late PA isolate may be more resistant to PMN-generated extracellular antimicrobial factors but more susceptible to antimicrobial systems generated within the phagocyte.

Conclusions: The above observations require confirmation and extension, especially with respect to other paired CF isolates of PA. They generate the conjecture that CF airway conditions select for strains that generate the conjecture that CF airway conditions select for strains that

415 THE EFFECT OF HYDROCORTISONE ADMINISTRATION ON HYPOTENSION AND VASOPRESSOR ADMINISTRATION IN VERY LOW BIRTH WEIGHT INFANTS: A META-ANALYSIS

S. Higgins, P. Friedlich, and I. Seri. CHLA, University of Southern California, Los Angeles, CA.

Purpose of Study: The use of hydrocortisone (HC) has been reported to increase blood pressure and reduce the reliance upon vasopressor therapy in hypotensive very low birth weight (VLBW) infants. However, a quantitative review of the effectiveness of HC for the treatment of hypotensive VLBW infants has not yet been performed. The purpose of the present study is to provide a quantitative review of the research examining HC administration in hypotensive VLBW infants.

Methods Used: This study compared the response of 2 fully sequenced PA isolates, from a CF patient at ages 0.5 (early) and 8 (late), to microbicidal systems of intact PMN and to PMN-related cell-free antimicrobial systems (myeloperoxidase (MPO), hydrogen peroxide and/or reagent hypochlorous acid).

Summary of Results: We observed that PA had become MORE SUSCEPTIBLE to killing by serum factors and reagent hydrogen peroxide, but MORE RESISTANT to killing by an MPO-mediated antimicrobial system. Preliminary studies with intact PMN suggest that the late PA isolate may be more resistant to PMN-generated extracellular antimicrobial factors but more susceptible to antimicrobial systems generated within the phagocyte.

Conclusions: The above observations require confirmation and extension, especially with respect to other paired CF isolates of PA. They generate the conjecture that CF airway conditions select for strains that

416 COMPARISON OF BONE INGROWTH INTO SCAFFOLDS WITH SIMPLE AND NOVEL BIOMIMETIC POROUS ARCHITECTURES


Purpose of Study: Osteoarthritis is a debilitating joint disease affecting millions of people in the US. Current standards of care for the treatment of focal defects have had only limited success in the reduction of pain and restoration of mobility in patients. The purpose of this study was to investigate the bone ingrowth into polymer scaffolds used to repair cartilage defects. Tissue-engineered cartilage is grown on the articulating surface of the polymers, which are then placed into the defect site to replace degenerated cartilage. Bone attachment to the scaffold is essential to ensure that the tissue-engineered cartilage has a solid support structure in order to allow it to integrate with surrounding healthy cartilage tissue.

Methods Used: In this study bone growth was compared between scaffolds with a simple porous structure or a biomimetic inverse trabeculated porous structure using histology, histomorphometry and μCT. The biomimetic scaffolds were produced using negative μCT images of trabecular bone to guide the formation of the polymer PBT scaffolds. This resulted in the narrow space of the trabecular bone being PBT polymer while the bone was turned into the porous space of the scaffold. Bone growth and bone formation rate were measured using histology and histomorphometry, 5 months after scaffold placement.

Summary of Results: Using a biomimetic scaffold resulted in a 506% (p = .09) increase in bone volume within the scaffold pores, a 40% (p = .005) increase in bone volume adjacent to the scaffold, and 95% (p = .028) increase in bone volume deep to the scaffold, compared to simple porous scaffolds. Additionally, use of a biomimetic scaffold resulted in a 66% and 70% (p = .002 and .034) decrease in soft tissue adjacent to and deep to the biomimetic scaffold. Histomorphometry showed a 22% (p = .031) decrease in labeled surface found in the area adjacent to the biomimetic scaffold.

Conclusions: The increase in bone volume within and around biomimetic scaffolds indicates that a biomimetic construct enhances bone formation. Previous studies have shown that biomimetic materials enhance osteoconductivity, and biomimetic shapes and surfaces increase osteoblast phenotype and mineralization. Future studies will look at inverse trabecular porous structures that are patient specific to further study the effect porous structures have on bone formation.

417 POLYMETHYL METHACRYLATE BONE CEMENT PARTICLES INHIBIT MC3T3-E1 OSTEOPROGENITOR DIFFERENTIATION IN VITRO

R. Chiu, R.L. Smith, and S.B. Goodman. Stanford University School of Medicine, Stanford, CA.

Purpose of Study: Osteolysis induced by orthopedic wear debris may result from reduced osteoprogenitor differentiation and proliferation.

Summary of Results: Using a biomimetic scaffold resulted in a 506% (p = .09) increase in bone volume within the scaffold pores, a 40% (p = .005) increase in bone volume adjacent to the scaffold, and 95% (p = .028) increase in bone volume deep to the scaffold, compared to simple porous scaffolds. Additionally, use of a biomimetic scaffold resulted in a 66% and 70% (p = .002 and .034) decrease in soft tissue adjacent to and deep to the biomimetic scaffold. Histomorphometry showed a 22% (p = .031) decrease in labeled surface found in the area adjacent to the biomimetic scaffold.

Conclusions: The increase in bone volume within and around biomimetic scaffolds indicates that a biomimetic construct enhances bone formation. Previous studies have shown that biomimetic materials enhance osteoconductivity, and biomimetic shapes and surfaces increase osteoblast phenotype and mineralization. Future studies will look at inverse trabecular porous structures that are patient specific to further study the effect porous structures have on bone formation.
Previous studies have shown that particulate materials of polymethylmethacrylate (PMMA), a bone cement material used in joint replacement surgery, inhibit the differentiation of osteoprogenitors in heterogeneous murine marrow stromal cell cultures. These cultures contain hematopoietic cells that may potentially influence the response of osteoprogenitor cells to particles. To determine whether the inhibitory effects of PMMA bone cement particles are observed with pure osteoprogenitor populations, we challenged MC3T3-E1 cells, a murine preosteoblast cell-line, with PMMA particles, and examined the dose- and time-dependent effects of these materials on the ability of these osteoprogenitors to differentiate into osteoblasts.

**Methods Used:** Murine MC3T3-E1 osteoprogenitors in vitro were challenged with PMMA particles (1-10 μm) at concentrations of 0.038, 0.075, 0.150, 0.300, and 0.600% vol on their initial day of differentiation in osteogenic medium containing 50 μg/ml ascorbic acid and 10 mM β-glycerophosphate. After 20 days, the quantity of mineralization, proliferation, alkaline phosphatase activity, and osteocalcin production was measured. These outcome parameters were also measured once every 4 days for cultures challenged with a high dose (0.300% vol) and low dose (0.075% vol) of PMMA particles.

**Summary of Results:** MC3T3-E1 cultures showed a dose-dependent decrease in mineralization, proliferation, and alkaline phosphatase activity. MC3T3-E1 cultures challenged with a high dose (0.300% vol) of PMMA particles showed no rise in these outcome parameters over time, while those challenged with a low dose (0.075% vol) of PMMA particles showed a delayed or reduced rate of increase compared to the control. Osteocalcin production was unaffected by particle treatment.

**Conclusions:** This study has demonstrated that PMMA particles inhibit the differentiation of MC3T3-E1 osteoprogenitor cells. The inhibitory effects of PMMA particles observed with heterogeneous marrow stromal cell cultures are therefore reproducible in pure osteoprogenitor populations. Periprosthetic osteolysis is therefore due in part to the inhibition of osteoprogenitor differentiation by orthopedic wear debris.

### 418 ULTRAHIGH MOLECULAR WEIGHT POLYETHYLENE WEAR DEBRIS SUPPRESSES OSTEOSTROBLASTIC DIFFERENTIATION OF BONE MARROW OSTEOPROGENITORS AND MC3T3-E1 PREOSTEOBLASTS IN VITRO

R. Chiu, R.L. Smith, and S.B. Goodman. Stanford University School of Medicine, Stanford, CA.

**Purpose of Study:** Wear debris generated from polyethylene implants induces osteolysis by stimulating osteoclast activity and impairing osteoblast function. Polyethylene wear debris may potentially contribute to osteolysis by suppressing the proliferation and differentiation of osteoprogenitor cells. Previous studies have shown that the osteogenic activity of osteoprogenitors and mesenchymal stem cells is inhibited by polyethylene methacrylate bone cement and titanium particles, but whether polyethylene wear debris also inhibits osteoprogenitor differentiation is unknown. In this study, we examined the effects of ultrahigh molecular weight polyethylene (UHMWPE) particles on the ability of primary murine bone marrow osteoprogenitors and MC3T3-E1 preosteoblasts to differentiate into osteoblasts in vitro.

**Methods Used:** UHMWPE particles (0.5 μm ± 0.2 μm) generated from wear simulator tests were coated on the surfaces of culture plates at concentrations of 0.038, 0.075, 0.150, 0.300, and 0.600% vol with a layer of type I collagen. Primary murine bone marrow cells and MC3T3-E1 cells were cultured on these plates in osteogenic medium containing 50 μg/ml ascorbic acid and 10 mM β-glycerophosphate (medium for MC3T3-E1). The mice recovered from surgery and were load bearing within thirty minutes. The day after surgery two of the implanted strain gauges were functional, providing resistance measurements in a mouse model. CPC coated strain gauges could be used to collect in vivo strain measurements in a mouse model.

**Summary of Results:** Bone properties are dynamic and change due to physiologic strains generated during loading. Physiologic strains have been measured in dogs and rats with calcium phosphate ceramic (CPC) coated strain gauges. The ability to measure in vivo bone strains in mice will provide a powerful research tool because it will allow for the study of genes that regulate strain mediated bone remodeling through the use of transgenic mice. The goal of this study was to test whether miniature CPC coated strain gauges could be used to collect in vivo strain measurements in a mouse model.

**Methods Used:** Four C57BL6 mice were implanted with uniaxial strain gauges. The strain gauges were trimmed, waterproofed, coated with micron-diameter hydroxyapatite particles, and sterilized using ethylene oxide. One strain gauge was implanted on the right femur in each mouse and the wires were led subcutaneously to an incision on the back of the neck, leaving 0.5 cm transcutaneous leads.

**Summary of Results:** The mice recovered from surgery and were load bearing within thirty minutes. The day after surgery two of the implanted strain gauges were functional, providing resistance measurements of 121.0 Ω and 120.8 Ω. One week following surgery the transcutaneous leads on one of the mice were damaged, preventing measurement collection.

In vivo strain measurements were collected from the remaining mouse one week after gauge placement while the animal walked around its cage and on a treadmill. While the average peak strains of 516 ± 67 με measured during cage walking, and 510 ± 98 με measured during treadmill walking were similar, the strain patterns generated during these activities were different. The peak strains increased to 1040 ± 181 με when the animal stood on its hind legs. High-resolution μCT imaging demonstrated that bone growth and attachment to the CPC particles have anchored the implanted strain gauge to the femur.

**Conclusions:** This study shows that in vivo strain measurements can be collected from CPC coated strain gauges within one week of sensor placement in a mouse model and that the measured peak strains are similar to those predicted from previous studies. Future studies will focus on improving the success rate of collecting measurements from implanted gauges and using the strain sensors to measure differences in the in vivo mechanical properties of bone tissue in transgenic mice.

### 420 OSTEOGENIC POTENTIAL OF BMP2 AND BMP6: A COMPARATIVE STUDY OF IN-VITRO DIFFERENTIATION OF pMSC GENETICALLY MODIFIED BY A NON-VIRAL APPROACH


**Purpose of Study:** Bone properties are dynamic and change due to physiologic strains generated during loading. Physiologic strains have been measured in dogs and rats with calcium phosphate ceramic (CPC) coated strain gauges. The ability to measure in vivo bone strains in mice will provide a powerful research tool because it will allow for the study of genes that regulate strain mediated bone remodeling through the use of transgenic mice. The goal of this study was to test whether miniature CPC coated strain gauges could be used to collect in vivo strain measurements in a mouse model.

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**Conclusions:** This study shows that in vivo strain measurements can be collected from CPC coated strain gauges within one week of sensor placement in a mouse model and that the measured peak strains are similar to those predicted from previous studies. Future studies will focus on improving the success rate of collecting measurements from implanted gauges and using the strain sensors to measure differences in the in vivo mechanical properties of bone tissue in transgenic mice.
421 COMBINED PH-IMPEDEANCE MONITORING OF REFLUX IN PATIENTS WITH RESPIRATORY DISEASE

L. Ding, A.V. Martin, and R. Tatum. University of Washington School of Medicine, Seattle, WA.

Purpose of Study: Pharyngeal reflux has been implicated as an exacerbating factor in respiratory symptoms and respiratory disease. Patients who have gastroesophageal reflux in the setting of respiratory disease often do not significantly improve with proton pump inhibitor (PPI) therapy. However, a subset of patients do respond to anti-reflux surgery. In this study, we used combined pH-impedance monitoring to investigate the role of reflux, both acid and nonacid, in patients with respiratory disease. We hypothesized that respiratory disease patients have more reflux episodes that reach the level of the pharynx, both acid and nonacid, compared to other patients referred for workup of reflux.

Methods Used: We retrospectively reviewed 228 pH-impedance studies of patients referred to the University of Washington Swallowing Center for workup of reflux. Ninety-nine patients who were studied off PPIs and had not undergone prior anti-reflux surgery met inclusion criteria. Thirty-five of these patients had respiratory disease (asthma: 26, COPD: 3, IPF: 6) and 64 did not.

Summary of Results: The two groups of patients, with and without respiratory disease, were similar in lower esophageal sphincter pressure, percent normal peristalsis, bolus transit time, DeMeester score, distal acid exposure, and acid clearance time. No significant differences in the number of reflux events in the distal esophagus between the two groups were observed. However, patients with respiratory disease typically experienced more than twice the total number of reflux episodes (both acid and non-acid) reaching the level of the pharynx than those patients without respiratory disease (mean = 7.3 ± 2.0 vs. 3.7 ± 0.7; p = 0.048).

The difference was even greater for the subset of patients with asthma (8.3 ± 2.6 vs. 3.7 ± 0.7; p = 0.036).

Conclusions: Combined pH-impedance monitoring, which demonstrates both acid and non-acid reflux events up to the level of the pharynx, may be a more sensitive modality for the workup of reflux in patients with respiratory disease.

422 OUTCOMES OF LAPAROSCOPIC ASSISTED TRANSHIATAL ESOPHAGECTOMY FOR ADENOCARCINOMA OF THE ESOPHAGUS

K. Chambers1, M.I. Montenovo2, C.A. Pellegrini3, and B.K. Oelschlager1. 1University of Washington School of Medicine, Seattle, WA and 2University of Washington School of Medicine, Seattle, WA.

Purpose of Study: The purpose of this study is to assess short-term and long-term outcomes for laparoscopic assisted transhiatal esophagectomy (LA-THE) for adenocarcinoma of the esophagus over a 12-year period at a major university hospital.

Methods Used: This is a retrospective analysis of 72 patients undergoing LA-THE for adenocarcinoma. The patients were also given a follow-up survey (Gastrointestinal Quality of Life Index) in order to assess long-term quality of life.

Summary of Results: Patient characteristics and treatment: Mean patient age was 63.9 +/- 9.6 years; mean body mass index was 27.5 +/- 3.8. Thirty-nine patients (54%) underwent neoadjuvant chemoradiotherapy. LA-THE was used in all patients. The mean operative time was 321 +/- 73 minutes; mean blood loss 318 +/- 239 ml. All margins were free of cancer and a mean of 11.0 +/- 6.7 lymph nodes were retrieved. The median ICU stay was 1 day (range 1–35) and hospital stay was 9 days (range 7–58). One patient (1.4%) died within 30-days postoperatively.

Complications: Minor: atrial fibrillation 8 (11.1%) patients; pleural effusion 9 (12.5%); wound infection 7 (9.7%); transient recurrent nerve palsy 6 (8.3%); pneumothorax requiring no intervention 12 (16.7%). Major complications: Anastomotic leak requiring intervention in 7 (9.7%) patients; anastomotic leak requiring no intervention in 7 (9.7%); pneumonia in 7 (9.7%); pneumothorax requiring intervention in 6 (8.3%); deep vein thrombosis in 4 (5.5%); pulmonary embolism in 3 (4.1%); myocardial infarction in 2 (2.7%). Late complications included anastomotic stricture in 13 (18.0%) patients.

Survival: Table 1 depicts Kaplan-Meier survival rates.

Conclusions: Our study suggests that LA-THE may reduce morbidity and mortality of esophagectomy and with good survival rates. LA-THE should be considered an alternative to open esophagectomy in the treatment of esophageal adenocarcinoma.

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<th>1-Year</th>
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<tr>
<td>Stage I (n=22)</td>
<td>100%</td>
<td>100%</td>
<td>80%</td>
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<td>Stage IIa (n=21)</td>
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<td>57%</td>
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<td>Stage III (n=24)</td>
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<tr>
<td>Overall (n=72)</td>
<td>85%</td>
<td>68%</td>
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Methods Used: Charts on all patients who received rFVIIa following pediatric cardiac surgery between November 2004 and April 2006 at BC Children’s Hospital were collected. The volume of blood lost via chest tube drainage, the volume of blood products transfused, incidence of thrombosis and clinical status were collected on each patient. Summary of Results: The average dose of rFVIIa given was 143 μg/kg, an average of 1.8 times per patient. The average blood loss after rFVIIa decreased to 8.3 ml/kg/hr compared to 21.9 ml/kg/hr prior to rFVIIa treatment. The incidence of thrombosis was 20% or 3 out of 15 patients. Two of the thrombotic complications were deep venous thromboses while the other was a sagittal sinus thrombosis. The mortality rate was 20% or 3 out of 15 patients. Conclusions: It can be concluded that treatment with rFVIIa appears to reduce blood loss following pediatric cardiac surgery. The rate of thrombosis indicates that this may be a common complication and patients receiving rFVIIa following pediatric cardiac surgery should be carefully monitored. 

HYPOTENSIVE ANESTHESIA REVISITED: IS IT WORTH THE RISK?
S. Gardner1, M. Eagan1, 2, A. Scaduto1, 2, and R. Bowen1, 2. 1 David Geffen School of Medicine, UCLA, Los Angeles, CA and 2 Shriners Hospitals for Children, Los Angeles, CA.

Purpose of Study: This study aims to determine if deliberate, moderate hypotension was reliably achievable and resulted in decreased blood loss and reduced transfusion requirements in pediatric spinal deformity surgery.

Methods Used: A retrospective cohort study was performed in 107 consecutive patients undergoing posterior spinal fusion surgery of 5 or more levels. All patients had hypotensive anesthesia as their major perioperative blood loss reduction technique. Data collected included age, diagnosis, magnitude of deformity, surgery experience, number of thoracic pedicle screws placed, fusion including the pelvis, revision vs. primary surgery, decrease in mean arterial pressure (MAP) and heart rate, estimated blood loss, and intraoperative transfusions (cell saver + autotransfusion). A stepwise multiple linear regression analysis was performed between the dependent variables and the estimated blood loss per kg per level fused as well as intraoperative transfusion volume per kg per level fused.

Summary of Results: Hypotensive anesthesia was variably achieved (mean 19.3% decrease in MAP over baseline; range from 1% elevation to 35% decrease). Heart rate reduction was not reliably achieved (mean 6.4% increase over baseline; range 40% elevation to 27% reduction).

There was no significant correlation between the degree of hypotensive anesthesia and estimated blood loss per kg per level. The transfusion rate with all blood sources (cell saver and allogeneic blood) was 60% and the allogeneic blood product transfusion rate was 22.3%. There was significant correlation between the degree of hypotensive anesthesia and the amount of blood transfused. There was a significant decrease in blood transfused in patients with MAP decrease of greater than 10% (p < 0.05). There was a trend toward significance between surgical time and blood transfusion requirements as well as surgery for kyphosis and estimated blood loss.

Conclusions: Hypotensive anesthesia significantly decreased the amount of intraoperative blood transfusions but not the estimated surgical blood loss during pediatric spinal deformity surgery. There was a trend toward increased blood loss with increased surgical time and a trend toward increasing transfusion requirements in patients undergoing surgery for kyphosis.

ANTECEDENT SIMULATION TRAINING ACCELERATES LEARNING OF SHOULDER ARTHROSCOPY

Purpose of Study: To determine whether antecedent simulation training accelerates the ability of a novice medical student to learn how to perform a diagnostic arthroscopy on a cadaveric shoulder.

Methods Used: Twenty-four medical students with no arthroscopy experience were recruited and divided randomly into two groups. The first group (experimental group) trained on the Procedicus VA shoulder arthroscopy simulator for a total of six hours divided into six sessions, and then performed three abbreviated diagnostic arthroscopies on a cadaveric shoulder, divided into three separate sessions. The second group (control group) performed the same arthroscopies as the first group, but did not receive any simulation training. The cadaveric arthroscopies were videotaped and graded in a blind manner. Performance was measured by several objective parameters including efficiency of probe movement (number of probe misdirections to route to an anatomical structure), total time, number of scope collisions, proper structure identification, and number of scope pullout incidents.

Summary of Results: Students who trained on the simulator were able to locate anatomical structures with significantly fewer probe misdirections (better efficiency) than those students without any simulation training. Simulation-trained subjects were also less likely to leave out structures during their initial trial on the cadaver. Conversely, total time, number of scope collisions, and number of scope pullout incidents were no different between the two groups.

Conclusions: As simulation training continues to develop in all medical fields, it is important to critically evaluate the effectiveness of such training. This is the first prospective, randomized study exploring whether simulation training can enhance the ability of a novice to learn shoulder arthroscopy. Based upon our results, it appears that the Procedicus VA simulator can teach students how to move the probe more efficiently and identify structures more accurately. However, simulation training does not appear to enhance other performance measures. This is likely due to the inherent limitations of simulation in mimicking the reality of actual arthroscopy. Further studies are needed to continue to evaluate the utility of simulation training in development of arthroscopic skills.

CLINICAL FOLLOW-UP OF THROWERS UNDERGOING ULNAR COLLATERAL LIGAMENT RECONSTRUCTION USING THE KERLAN-JOBE ORTHOPAEDIC CLINIC SHOULDER AND ELBOW SCORE
A. Brooks1, B. Domb2, K. Mohr3, N. ElAttrache1, L. Yocum3, F. Jobe3, and J. Davis3. 1 Keck School of Medicine of USC, Los Angeles, CA; 2 Western Regional Meeting Abstracts Volume 56, Issue 1 245
Purpose of Study: The purpose of this paper is to compare the Kerlan Jobe Orthopaedic Clinic (KJOC) Shoulder and Elbow Score to other validated upper extremity scores as outcome assessment tools for ulnar collateral ligament (UCL) reconstruction in overhead athletes. The hypothesis was that the KJOC Score would correlate with the other scores but would be more sensitive in evaluating outcomes in throwing athletes.

Methods Used: 50 professional baseball players who underwent UCL reconstruction were asked to complete the KJOC score, the Disabilities of the Arm, Shoulder and Hand (DASH) score, and the DASH sports module. Pearson (parametric) and Spearman rank (non parametric) correlations among the 4 scoring systems were conducted in order to validate the KJOC score. Paired comparisons of the KJOC score were conducted versus the other two scores (DASH, DASH sport) where all were standardized to a 100 point scale.

Summary of Results: Significant correlations were found between the KJOC Score and all three other scores. 28 of the 50 players had returned to their prior level of competition for at least 12 months. The KJOC score for these 28 players averaged 90.98 (SD 9.45), the DASH score averaged 1.30 (SD 1.87), and the DASH sports module averaged 2.78 (SD 8.33). There was a significant difference between the KJOC score and the DASH score (p = .0245) and the DASH sports module (p = .0050). In all cases the KJOC score was lower than the other scores.

Conclusions: The results of this study validate the use of the KJOC Score for evaluation of overhead athletes undergoing UCL reconstruction. The players who succeeded in returning to play for at least one year scored consistently lower on the KJOC Score than on the DASH or DASH sports module scores, suggesting that the KJOC score is the most sensitive score for detecting subtle changes in performance in the throwing athlete.

428 KINEMATIC ANALYSIS OF ADJACENT SEGMENT DEGENERATION IN THE CERVICAL SPINE USING DYNAMIC MRI

R.M. Martin, Y. Morishita, S.W. Hong, and J.C. Wang. University of California at Los Angeles, Los Angeles, CA.

Purpose of Study: Cervical disc degeneration is an important clinical problem that often leads to surgical treatment to alleviate symptoms. Vertebral fusion, an effective treatment option, is thought to place excessive stress on adjacent disc segments, promoting degeneration and resulting in adjacent segment disease (ASD). With an annual incidence of 3%, ASD is a major contributor to the development of new clinical symptoms that may lead to additional surgery. Currently no predictive criteria exist to determine which discs may be most vulnerable to ASD in the pre-operative patient. To address this issue, mid-sagittal dynamic MRIs of the cervical spine were obtained from 226 symptomatic patients.

Methods Used: The kinematic properties of disc segments adjacent to the level(s) of worst degeneration were analyzed using MRAnalyzer software and compared with patients without degeneration.

Summary of Results: The angular motion of disc segments was somewhat variable and dependent on the level of worst degeneration, the most dramatic effect being a 37-74% increase in angular motion compared to controls at C6/7 when the level of worst degeneration was at C4/5-C5/6. Conversely, degeneration at C6/7 tended to decrease angular motion at C4/5 and C5/6 by as much as 39%. Translational motion of disc segments increased one to two levels inferior to the level(s) of worst degeneration.

Conclusions: We suggest that adjacent disc segments exhibiting increased mid-sagittal dynamic pre-operatively may be most susceptible to ASD, and such knowledge should help direct the surgeon when deciding which disc segments to include in the fusion to limit the incidence of ASD.
Economically disadvantaged women, especially those of Hispanic origin, are less likely to receive cancer screening than other women, more likely to be diagnosed with advanced disease, and have higher mortality rates. 1 of every 3 patients at Kent Community Health Center (KCHC) is Hispanic, and 34% of patients have no insurance. 59% of patients are below the poverty level and 26% are at or below 200% of the Federal Poverty Level. Patients below 250% of the Federal Poverty Line, age 40–65, are eligible for the Breast and Cervical Health Program (BCHP) funded by the State and Federal governments. The purpose of this intervention was to increase the awareness of patients enrolled in BCHP at KCHC, especially Hispanic patients, regarding the need for preventative cancer screening through a culturally appropriate educational intervention.

**Methods Used:** Primary source literature was reviewed and two handouts produced describing breast and cervical cancer prevalence, risk factors, prevention strategies and helpful organizations. These, in addition to existing literature, were translated and provided to Spanish-speaking patients at Mammogram Day (once monthly day for BCHP patients to receive mammograms). A packet was created containing the handouts as well as mammogram information, Breast Self-Exam instructions, and information on BCHP in Spanish and English. Handouts were evaluated and approved by the Patient Care Coordinator, Marketing Director, and all healthcare providers in the clinic.

**Summary of Results:** Packets were given to 18 women on Mammogram Day and continued to be given out to new enrollees in BCHP at the clinic, addressing a need expressed by the health care providers and Patient Care Coordinator, and increasing patient awareness regarding breast and cervical cancer screening.

**Conclusions:** Patients and healthcare providers continue to provide positive feedback regarding the information presented and the manner of presentation. As a result, the packets have become a permanent installment for distribution to new BCHP enrollees at the Kent Community Health Center, to increase awareness of the general patient population, and of the most at-risk patient populations.

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**430 HPV VACCINE EDUCATION IN LEWISTOWN, MT**

M.C. Wilson. University of Washington, Seattle, WA.

**Purpose of Study:** Lewistown, MT is a small town in rural Montana whose citizens are under-informed about the role of Human Papilloma Virus in the development of cervical cancer in females, and the availability of an HPV vaccine. A community project was developed to educate the population about HPV and increase the administration of the HPV vaccine.

**Methods Used:** A literature search was conducted to determine common barriers to the administration of the HPV vaccine, and to establish currently accepted data on HPV infection and the vaccine. Community physicians were questioned about the reasons that patients have declined the vaccine. Concerns about the cost, age of receipt, and utility of the vaccine were identified as barriers to vaccine administration. A newsletter detailing the HPV disease and vaccine with instructions on how to receive the vaccine was published in the town newspaper. A handout was developed for patients outlining instructions to ensure insurance coverage or coverage through federal programs for the vaccine and was distributed to physicians’ offices. An information booth was set up at the local Relay for Life event to reach the community with this information directly.

**Summary of Results:** The response of the community to my project was mixed. The town physicians were very receptive to having more information in the clinic about the vaccine. The booth at the Relay for Life event was minimally visited. This may indicate that the controversial nature of the information combined with the conservative stance of the local population made viewing information about HPV in a public setting uncomfortable. This correlates with the greater success of the newspaper article, which could be read in private. The newspaper article can probably be credited with most of the reported increase in interest about the vaccine, and the actual administration of vaccine in the town clinics.

**Conclusions:** Community education can empower people to receive needed medical services and to seek assistance through existing federal and state programs. Patient education seems to be of paramount importance in rural areas where physicians frequently lack the energy and time to effectively guide patients through new services and programs. With the publishing of key information about the HPV vaccine in the local paper, people were given the information and motivation to seek the vaccine.

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**431 DOMESTIC VIOLENCE IN JUNEAU, AK: CONNECTIONS TO THE CLINIC**

S. Reed. University of Washington, Seattle, WA.

**Purpose of Study:** In both Alaska and in Native American/Alaskan populations, domestic violence (DV) is disproportionately high, and many victims of DV do not use clinic resources. Working at SEARHC, a clinic for Native Americans/Native Alaskans in Juneau, AK, and AWARE, the domestic violence shelter in Juneau, the researcher sought to identify some of the barriers between women in DV situations and their physicians.

**Methods Used:** After an extensive review of literature about DV screening in the clinical setting and DV in Native American/Alaskan communities, conversations were held with women at AWARE, asking the women to tell about their experiences with doctors and the health care system.

**Summary of Results:** From the results of the review and the interviews, several barriers to medical care were identified, and an educational presentation for medical staff on domestic violence screening and issues surrounding domestic violence in the clinical setting was developed. A brochure for victims of domestic violence outlining the resources available within the healthcare system, legal issues surrounding disclosure, and the choices available to a victim within the clinic was also developed.

**Conclusions:** Domestic violence, like many social problems, is deeply ingrained in history and culture, has no simple solutions, and is often not discussed. The very act of having someone listen and ask for suggestions based on the women’s experiences seemed to be empowering for many of the women at AWARE. Because of the scarcity of DV discussion within the medical field in proportion to the prevalence of the problem, all effort to foster conversation around domestic violence and break down barriers to care is beneficial.

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**432 DECREASING PRESCRIPTION DRUG ABUSE BY TEENAGERS IN ST. MARIES, IDAHO THROUGH COMMUNITY EDUCATION**

B. Byrne. University of Washington School of Medicine, Seattle, WA.

**Purpose of Study:** St. Maries, Idaho is a rural community in northern Idaho. Local physicians, pharmacists, and educators agree that prescription drug abuse has become an increasing problem in high school students in St. Maries. A community education program was designed to increase awareness and decrease the frequency of prescription drug abuse.

**Methods Used:** An extensive literature search was completed about prescription drug abuse in the teenage population. A four-part
community education program was designed and targeted to four specific community groups. Interactive presentations were provided for high school counselors and teachers; community high school students; and community senior citizens. Educational material was obtained from the Food and Drug Administration and made available for patient education at the clinic and hospital.

Summary of Results: High school counselors and teachers were educated about the prevalence of abuse in the community and signs of prescription drug abuse among teens. High school students were provided accurate, current information about prescription drug abuse in a setting that allowed for questions and answers. Because research has shown that most prescription drugs are being taken from family members, 20 senior citizens were given a presentation on the proper storage and disposal of controlled medications. The educational materials from the FDA filled a void in current patient education.

Conclusions: Misconceptions about safety compared to street drugs, ease of access, and availability of drugs such as opioids, CNS depressants, and stimulants have lead to an increase in abuse of these drugs nation wide. Educating teachers and students on the dangers of these drugs in addition to discussion with adults in the community on the prevalence of their abuse may be an effective way to decrease their illicit use. In addition, educating local seniors and other adults on proper control and disposal of their medication is necessary to decrease availability of the drugs. Patient education material to be distributed later will help sustain the efforts of this project.

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DESCRIPTIVE ANALYSIS OF MEDICAL WARD ADMISSIONS AT BELAU NATIONAL HOSPITAL
N. Nielsen1, R. Schneeweis1, and S. Lalabula2. 1University of Washington School of Medicine, Seattle, WA and 2Belau National Hospital, Koror, Palau.

Purpose of Study: To describe the reasons for medical ward admissions (adult and pediatric) in the Northern Pacific island of Palau over a two year period for 2005 and 2006 and identify areas for future research on the topic.

Methods Used: Descriptive analysis was carried out using the medical ward admissions database at Belau National Hospital.

Summary of Results: The main ICD-9-CM category for admission in all age groups was respiratory. The main diagnoses for these admissions were asthma and pneumonia. Gastrointestinal and cardiovascular categories were also important. Amongst teenagers, the rather large number of attempted suicide admissions deserves further study. In pediatrics, male admissions were over represented compared to female (61% vs 39%, p value). The number of readmissions was significant. Overall, the total number of admissions has increased by 21% present from 2005 to 2006 (p value).

Conclusions: The systematic collection of admitting data is essential to identify preventable reasons for admission to hospital and to reduce readmission rates.

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PSYCHOSOCIAL STRESS DURING PREGNANCY
S. Madden Woods1,3, J. Melville1,2, A. Gavin1,4, Y. Guo1, A. Cesaitis1, and W. Katon1. 1University of Washington, Seattle, WA; 2University of Washington, Seattle, WA; 3NIH, Bethesda, MD and 4NIH, Bethesda, MD.

Purpose of Study: The presence of psychosocial stress during pregnancy has been proposed as a risk factor for adverse pregnancy outcomes (e.g., low birth weight, preterm labor). Current Practice Guidelines of the American College of Obstetrics and Gynecology (ACOG) have newly endorsed screening for the presence of psychosocial stress, as stress may contribute to a woman’s “attentiveness to personal health matters, her use of prenatal services, and the health status of her offspring”. Levels of psychosocial stress may change throughout pregnancy, and ACOG currently recommends assessment of psychosocial risk in all women throughout pregnancy. The proposed project will identify the prevalence of, significant factors associated with, and course of antenatal psychosocial stress during pregnancy.

Methods Used: Analysis of the ongoing Obstetric Mental Health Registry, which contains self-reported information on over 1,000 pregnant subjects, will be undertaken to determine the prevalence of and factors associated with antenatal psychosocial stress. Stress will be measured using the stress scale of the Prenatal Psychosocial Profile (PPP). Logistic regression will be used to determine which biomedical, psychosocial, and behavioral variables are significantly associated with psychosocial stress. The majority of patients completed one antenatal screen; for the 38% with >1 antenatal screen, we will also examine the course of psychosocial stress during their pregnancies.

Summary of Results: Using established cut-offs of the PPP, we will report prevalence rates of psychosocial stress during pregnancy. We will also calculate adjusted odd ratios for biomedical, psychosocial, and behavioral factors which are significantly associated with antenatal psychosocial stress. The course of psychosocial stress during pregnancy will be reported as PPP stress scores plotted by gestational age for women with >1 antenatal screen.

Conclusions: This study has potential to establish clinically significant factors associated with psychosocial stress in pregnancy that can be easily identified by health care providers. By identifying these factors, health care providers may work to provide interventions with the goal of decreasing stress and potentially decreasing adverse pregnancy outcomes.

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EVALUATION OF A COMMUNITY HEALTH WORKER PROJECT IN THREE GUATEMALAN VILLAGES
S. Leonard, S. Hartman, J. Hooper, L. Ponce, and P. Simpson. University of New Mexico School of Medicine, Albuquerque, NM.

Purpose of Study: While community health care workers are beginning to be implemented in several developing countries, there is little information as to their effectiveness in reducing stunting, and wasting, as well as child mortality rates.

Our study aims to evaluate the effectiveness of community health care workers trained in three villages in Guatemala by comparing anthropomorphic measures, mortality rates, and reported feeding practices of children under five taken before the implementation of the health care worker program with those found in March of 2006. Additionally, we intend to investigate trends in utilization of the CHCWs and whether or not they are correlated with either the socioeconomic status of the caregiver or the care receiver as it may have implications for how CHCWs are chosen in the future.

Methods Used: Guatemala is a developing nation with the third highest child mortality rate in the Western hemisphere—47/1,000 people. In an attempt to improve child health, Dr. Angelo Tomedi of the University of New Mexico School of Medicine along with several medical students trained several community health workers in Guatemala. On two separate occasions, interviews were conducted with families with children under the age of 5 living within the villages of Loma Linda, Pasac Segundo and Chuiziribal, Guatemala regarding feeding habits and socioeconomic status. Additionally, anthropometric data was collected for the children under age five.

Summary of Results: Data is currently being analyzed.
436 DEVELOPMENT AND EVALUATION OF A PATIENT EDUCATION PROGRAM FOR THE VERTICAL AUTO PROFILE CHOLESTEROL TEST

K.D. Eaton. University of Nevada School of Medicine, Reno, NV.

Purpose of Study: The objective of this study was to develop and evaluate a paper-based Vertical Auto Profile (VAP) cholesterol test education program designed for use by health professionals to guide patients with abnormal lipid values through lifestyle changes and potential drug therapies. Patients will receive educational materials customized to their personal lab results and NCEP-defined risk level through the Center for Nutrition and Metabolism in Reno, NV.

Methods Used: The program is based on a monograph of expanded lipid testing developed by Paul Ziajka, M.D. at the Florida Lipid Institute, and received input from an endocrinologist, exercise specialist, and multiple dieticians to develop algorithms that would translate to clinical practice. The readability, clarity, and utility of the patient education materials were evaluated by a focus group of eight nutritionists and physicians using online questionnaires.

Summary of Results: All participants in the focus group assessed the materials as informative and well-organized. Seventy-five percent thought the comprehensibility of the materials was directed at university educated individuals, and half of the participants thought the materials could use more explanation of technical terms. Half of the participants thought that the assembly of customized information packets was straightforward using two flowcharts that organized the different combinations of lab values into individual treatment plans.

Conclusions: This evaluation of the patient education program indicated that the VAP materials will be comprehensive and meaningful to the patient as long as the treatment plan is explained by a provider in a clinical setting. While the materials were found to be thorough and informative, professionals rated the reading level of the materials to be at the university level, and suggested that less technical, less thorough materials might benefit patients without higher education. Further refinement of these materials will allow patients and providers to work together in determining the health education needs of each patient based on the VAP results. The potential impact of the program is to support the new VAP cholesterol test in identifying all individuals who are at risk of cardiovascular disease, and to make the treatment options easy to understand and adhere to by both patients and providers.

437 SCREENING FOR MELANOMA IN HIGH-RISK PATIENTS: USEFUL OR USELESS?

K.D. Eaton1, K. Sa2, and C. Lamerson1,2. 1University of Nevada School of Medicine, Reno, NV and 2Nevada Center for Dermatology, Reno, NV.

Purpose of Study: Past studies have shown that melanomas are more often self-identified than physician-identified, but none of these studies examined the influence of family or personal skin cancer history on melanoma detection. We hypothesized that melanomas self-identified by patients would have a higher Clark’s level and worse prognosis than those identified by dermatologists at routine screening visits, and that individuals with a history of skin cancer would be more likely to self-diagnose a melanoma than those with no prior history.

Methods Used: We performed a retrospective chart review of melanoma diagnoses among high-risk patients (n = 129) over the course of five years by a board-certified dermatologist in Reno, NV. We investigated Clark’s level at diagnosis, and personal and family history of skin cancer in patients who self-reported the lesion, and in patients who were diagnosed with melanoma during routine 3, 6, or 9-month screening visits by the dermatologist. The patients were men (n = 53) and women (n = 76) ranging in age from 7 to 91 (mean age was 60 ± 16 and 55 ± 16, respectively).

Summary of Results: Dermatologist-identified melanomas in high-risk patients at routine screening visits were significantly milder and had lower Clark’s levels than those identified by the patient (P = 0.013). In a five-year period, 41.1% (53) of the melanomas were physician-identified, and 58.9% (76) were patient-identified. Of those found by the physician, 85% were atypical melanocytic proliferation (AMP) or in situ, 9% were Clark’s Level II, 4% were Clark’s Level III, and 2% were Clark’s Level IV. Of those found by patients, 59% were AMP or in situ, 24% were Clark’s Level II, 12% were Clark’s Level III, and 5% were Clark’s Level IV. Patients with no history of skin cancer were more likely to initially identify a melanoma than those with a past history of skin cancer (54 vs. 22 patients, P = 0.003).

Conclusions: Having a past history of skin cancer does not increase a person’s likelihood of self-identifying a cancerous lesion. This is due to the fact that patients with a history of skin cancer are more likely to see a dermatologist for routine screening visits, while patients with no past history are less likely to see a dermatologist. All high-risk persons should see a physician for routine skin cancer screening exams throughout the year.

438 IMPLEMENTATION OF A NEW UNDERGRADUATE RADIOLOGY CURRICULUM: EXPERIENCE AT THE UNIVERSITY OF BRITISH COLUMBIA


Purpose of Study: The purpose of this study was to review and revise the undergraduate radiology curriculum at the University of British Columbia to improve radiology education to medical students and to meet the needs of a medical program with province-wide distribution.

Methods Used: We identified the radiology content of the curriculum from the Curriculum Management and Information Tool online database, from personal interviews with curriculum heads, and from published information. Undergraduates’ and recent graduates’ opinions were solicited by means of surveys. Information on radiology curricula at medical schools across Canada was gathered from email surveys and personal contacts with members of the Canadian Heads of Academic Radiology (CHAR).

Summary of Results: Review of our curriculum indicated that lack of a unified syllabus resulted in redundant content, gaps in knowledge, and lack of continuity in the curriculum. Results from the survey of programs across Canada indicated that most schools also lacked a formal radiology curriculum for medical students. By adapting the guidelines from the Association of Medical Student Education in Radiology, we revised our undergraduate radiology curriculum to emphasize integration and self-learning. The modified curriculum includes a combination of instructional technology, focused lectures in preclinical years, and in-context seminars in clerkship rotations.

Conclusions: Most medical schools in Canada do not have a formal radiology curriculum for medical students. A structured curriculum is required to improve the quality of radiology teaching for medical students.
GLOBAL HEALTH: ARE COMMUNITY DEVELOPMENT AND STUDENT EDUCATION MUTUALLY EXCLUSIVE?


Purpose of Study: To evaluate a student led global health program, the UBC Student’s Global Health Initiative (GHI) after its inaugural year. GHI is a multi-faceted global health education program that provides hands-on learning experiences through skill-building workshops, international health projects and peer mentorship.

Methods Used: Success was measured using three indicators: 1. Student involvement 2. Faculty support 3. Community development. Student involvement was based on the number of students who participated in workshops and international projects. Faculty support was determined by the number of faculty members who assisted GHI in a variety of initiatives. Community development was assessed by the number of project objectives that were achieved.

Summary of Results: 1. An average of thirty students attended each workshop. Four students participated in the Honduras project, seven in the Uganda project and nine in the India project. 2. All three projects have physician advisors who are members of the UBC Faculty of Medicine. GHI’s fundraising initiatives have been supported by the Dean of the Faculty of Medicine and the Head of the Department of Family Practice. A number of faculty members have served as advisors to GHI including the Director of the Division of International Health 3. GHI’s global health program involves three international projects in Uganda, India and Honduras. All three projects ran successfully this year and made significant contributions to the communities with whom they were collaborating. During the project planning stages all three projects highlighted four main objectives they would like to achieve and all were successful. For example, the India team began treatment of common illnesses such as anemia, the Uganda team assessed the oral health of children in four different communities and the Honduras team conducted educational workshops on the spread of diarrhoeal disease.

Conclusions: GHI was deemed a successful global health program based on student participation, faculty support and community development. The continuation of this valuable program ensures that undergraduate medical students at UBC have the opportunity to expand their knowledge and experience in global health while simultaneously helping to address the health care needs of three international communities.

PALLIATIVE CARE SERVICES IN FAMILIES OF YOUNG MEN WITH DUCHENNE MUSCULAR DYSTROPHY


Purpose of Study: The American Academy of Hospice and Palliative Medicine’s definition of palliative care includes specialized services that address physical pain and symptoms, as well as psychosocial and spiritual needs, to enhance quality of life. These services may benefit individuals with progressive diseases such as Duchenne muscular dystrophy (DMD). To analyze patterns of palliative care service utilization among families of young men with DMD.

Methods Used: A telephone interview was administered to families of young men with DMD born prior to January 1, 1982, and residing in one of four sites participating in the Muscular Dystrophy Surveillance, Tracking, and Research Network (MD STARnet).

Summary of Results: Thirty-four families completed the questionnaire. Twenty-five were White, non-Hispanic (73.5%) and nine were Hispanic (26.5%). Thirty-five percent of the young men for whom an interview was conducted were deceased. Most families (85%) had never heard of the term palliative care. Of the palliative and other services analyzed, only Respiratory Care and Skilled Nursing Services showed much usage, with 67% and 50% of respondents indicating receipt of these services, respectively. Other services were received much less frequently: Respite Care (18%), Pain Management (12%), and Hospice Care (6%). Less than 25% of respondents reported having any type of legal directive document in place for their child, including such documents as the Living Will, Advanced Directives and Durable Medical Care Power of Attorney. Results of a binary logistic regression analysis indicate that for Attendant Care and for Case Manager Services, respectively, the receipt of services was 1.4 and 1.7 times more likely in young men who are older, all other factors having been considered. Respondents reported receipt of Attendant Care and Case Manager Services at 44% for each service category.

Conclusions: The age of young men with DMD was the major predictor of receipt of palliative care services. The data suggest that improvements in education about palliative care services and improved access to the services are needed for these families.

COMPARING LONG-TERM OUTCOMES OF GASTRECTOMY AND REDO-FUNDOPICATION FOR RECURRENT GASTROESOPHAGEAL REFLUX DISEASES

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Purpose of Study: To evaluate long-term quality-of-life and symptom outcome of severe GERD patients with one prior failed fundoplication who underwent gastrectomy and redo-fundoplication. We aimed to show that gastrectomy is a satisfactory surgical alternative to redo-fundoplication in face of higher failure rates with multiple prior fundoplications.

Methods Used: Retrospective cohort of 84 patients (gastrectomy = 23, redo = 61) who had at least one prior failed fundoplication, underwent gastrectomy or redo-fundoplication for recurrent gastroesophageal reflux disease at UWMC between 1994–2006. Preoperative laboratory values and primary symptoms were retrieved retrospectively from medical records. Quality-of-life was measured postoperatively with structured-interview using Gastrointestinal Quality of Life Index (GIQLI). Mean follow-up length was 4.5 years. Krustal-Wallis and Student T-Test were used for non-parametric and parametric continuous variables, respectively. Person’s chi-square was used for dichotomous variables. A multivariate regression model was constructed to test for statistic significance of GIQLI between groups. Statistic analyses were done using STATA-9.

Summary of Results: The most common indication for surgery was recurrent reflux (70%) for gastrectomy group, and dysphasia (40%) for redo-fundoplication groups. The most common anatomic abnormality was a herniated wrap for both groups. GIQLI indicated an equivalent overall quality-of-life between gastrectomy (85) and redo-fundoplication (93) groups after adjusting for age, BMI, and number of prior failed
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THE USE OF NEOADJUVANT CHEMOTHERAPY FOR TREATMENT IN SURGICALLY RESECTED SOFT-TISSUE SARCOMAS

Purpose of Study: There are over 9000 adult soft tissue sarcomas diagnosed each year and these are most commonly found in the extremities. The administration of chemotherapy before definitive resection of a sarcoma, termed neoadjuvant chemotherapy, is a relatively recent development in the treatment of sarcomas. In this study, our primary research question was to assess the disease-free survival of patients with soft-tissue extremity sarcomas who received neoadjuvant chemotherapy and were marginally resected.

Methods Used: Patients have been prospectively enrolled into a large clinical registry for sarcoma patients. The inclusion criteria for this study included those patients with an intermediate to high grade soft-tissue sarcoma of the extremity who received at least two cycles of neoadjuvant chemotherapy. Tumors determined to have a poor response to chemotherapy, assessed by imaging, underwent definitive resection with no additional chemotherapy. Tumors responding adequately to chemotherapy received two additional cycles with subsequent resection followed by adjuvant chemotherapy + radiation therapy. Tumors were resected with surgical margins assessed by the Enneking classification of intralesional, marginal, wide, and radical.

Summary of Results: Result will include Kaplan-Meier survival curves for disease-free survival, time to metastases, and time to recurrence. Chi-square analysis and multivariate analysis will be used to analyze tumor characteristics that have prognostic significance, such as tumor subtype (histology), tumor grade, and tumor size, and tumor response to chemotherapy.

Conclusions: Several questions will be answered, including: What is the survival of marginally resected sarcoma patients with extremity tumors who receive neoadjuvant chemotherapy? Do different histologic tumor subtypes respond differently to neoadjuvant chemotherapy? Since we are unable to provide a control group, those patients who are marginally resected without neoadjuvant chemotherapy, we will compare our findings to the published literature where survival is assessed in a comparable control group.

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FUNCTIONAL OUTCOME AND SEXUAL DYSFUNCTION FOLLOWING RESECTION OF THE SACRUM
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Purpose of Study: Determine the incidence of the sexual and other orthopaedic dysfunctions that occur following sacral resections with respect to the level of nerve root sacrifice. Report survival rates of patients following sacral resection.

Background: Sacral resection is a relatively rare surgery for primary and metastatic neoplasm of the sacrum. The level of sacral nerve roots sacrificed is an important determinant of complications following sacral resections. These nerves innervate pelvic organs including the bowel, bladder and external genitalia. Bowel and bladder malfunction are common complications that are well documented. However, almost no information is available regarding the incidence, severity and characteristics of sexual problems patients experience following this rare surgery.

Methods Used: Patients who underwent sacral resections at the University of Washington Medical Center between January 2001 and September 2007 were identified using a retrospective chart review. Subjects were mailed surveys to assess orthopaedic function, sexual function, bowel and bladder function and pain before and after surgery. Orthopedic function was assessed using the self-administered questionnaire TESS (Toronto Extremity Salvage Score). Sexual function was measured using a self-administered questionnaire: IIEF (International Index for Erectile Function) or the FSFI (Female Sexual Function Index). One question regarding bowel and bladder control has been added to the end of the sexual function questionnaires. The Oswestry Disability Questionnaire and SF-36 further assess function. Survey responses were compared with information regarding surgeries performed, indications and complications pulled from the patient’s charts. Statistical analysis includes the Chi Square test.

Summary of Results: This study will quantify the incidence and degree of the complications for patients receiving a sacral resection at a major medical center. It will also report survival following this major operation.

Conclusions: Sacral resection is a serious surgery that is necessary cure cancers of the sacrum. It has serious complications that need to be quantified so that physicians and patients can make informed decisions about their care.

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EXTERNAL FIXATION OF PUBIC SYMPHYSIS DIASTASIS FROM POSTPARTUM TRAUMA, A CASE REPORT
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Purpose of Study: Pubic Symphysis Diastasis (PSD) is recognized as a possible complication of pregnancy. Past studies describe postpartum PSD may come from numerous changes during pregnancy- changes such as increased concentration of hormone relaxin in serum may cause widening and loss of stiffness within the pubic symphysis thus potential instability in the pubic joint. The instability of the pubic joint along with the mechanical burden during labor increase the risks of orthopaedic complication in pubic symphysis among pregnant women. PSD is conservatively treated with a pelvic girdle and rest with some success. However diastasis larger than 3 cm may require surgical intervention to preserve the integrity of the pubis symphysis joint. Most surgical procedures for reduction of PSD have been via internal fixation with plates and screws on superior pubic rami bilaterally. Although internal fixation provided structural support, postpartum PSD patient with significant pelvic organ damage is not suitable for internal fixation due to high possibility of soft tissue infection and osteomyelitis after the operation.

Methods Used: External fixation is an alternative pathway for diastasis reduction that has not been studied extensively on PSD patients caused by postpartum trauma. This study will present a postpartum trauma PSD patient with pelvic organ damage who was treated with external fixation and recovered from PSD with extensive return of function.
Summary of Results: One year following the pelvic external frame removal, the patient returned for a final followup visit that demonstrating a normal heel-toe gait without pain with ambulatory endurance up to one mile without pain. X-ray photo showed 1.7 cm gap in the pubic symphysis. The patient indicates complete restoration of function and full range of activity that she was participating in premorbidity. She reports normal sitting, standing, lying, and normal nonpainful sexual relations with her husband.

Conclusions: External fixation provided an excellent alternative pathway to reduce PSD with internal fixation. External fixation is highly recommended for PSD patient who also suffer significant pelvic organ damage and/or hemorrhage at the pubic ramus.

445 EFFICACY OF ANAL FISTULA PLUG & FIBRIN GLUE VERSUS CONVENTIONAL TREATMENT IN CLOSURE OF COMPLEX ANAL FISTULAS
W. Chung1, P. Kazemi1, and T. Phan1, 2

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Purpose of Study: Complex anal fistulas, unlike simple fistulas, are more difficult to treat because fistulotomy would predictably result in incontinence. The purpose of this study was to compare the use of novel surgical approaches, involving fibrin glue and the anal fistula plug, to that of conventional surgical approaches, involving an extensive fistulotomy and flap closure or seton drain insertion, in the management of patients with complex anal fistulas (high to mid-transsphincteric, horseshoe, supra-sphincteric, Crohn’s, recurring). In all approaches, care is taken to preserve anal continence.

Methods Used: This is a retrospective cohort study of all patients treated for complex anal fistulas by a single colorectal surgeon at the University of British Columbia, from 1997 to 2007. The primary outcome was full healing (external fistula opening closed with no drainage or infection) at 12 weeks postoperatively. Preservation of continence was a secondary outcome.

Summary of Results: There were 124 males and 52 females with median age of 44 (range 19–81). Fifty-eight patients were treated by flap closure and fistulotomy external to the sphincter, 85 underwent seton drain insertion and fistulotomy external to the sphincter, 17 were injected with fibrin glue, and 16 had insertion of a fistula plug. Full healing rates were 66% for flap closure, 19% for seton drainage, 29% for fibrin glue, and 38% for plug insertion. Continence was preserved in all patients.

Conclusions: Closure of the primary fistula opening using a biologic anal fistula plug provides an alternate simple method of treating complex anal fistulas. Despite this method being more reliable than fibrin glue closure, healing rates were highest using conventional flap closure. However, given the low morbidity and relative simplicity of the procedure, the anal fistula plug should be considered an alternative effective treatment for patients with complex anal fistulas.

446 OSTEOXYTOMY FOR DIAPHYSEAL MALUNION IN PATIENTS WITH END-STAGE OSTEOARTHRITIS OF THE KNEE

Purpose of Study: Diaphyseal malunion predisposes to osteoarthritis of the knee and poses a problem for knee reconstruction since malposition of protheses is a known cause of early failure. Limb realignment may prove to be beneficial prior to proceeding with arthroplasty. The purpose of this study was to evaluate the outcome and effect of shaft osteotomy prior to total knee arthroplasty (TKA).

Methods Used: A search of the trauma database at a Level I trauma center between 1987 and 2007 was conducted. 23 osteotomies were performed on 22 patients with advanced degenerative arthritis of the knee in association with a diaphyseal malunion, at the request of an orthopaedic reconstruction subspecialist. Time intervals between surgical procedures and Knee Society knee and function scores were calculated. Patients were surveyed regarding pain relief and functional improvement.

Summary of Results: 16 femoral and 7 tibial osteotomies were performed. Femoral osteotomy improved the mean Knee Society knee scores from 46 to 77 and function scores from 34 to 62. Tibial osteotomy improved the knee scores from 53 to 82 and function scores from 28 to 50. On survey, patients indicated that osteotomy improved pain and function for a mean of 54 months. Four osteotomies were complicated by nonunion and required further intervention. Ten patients went on to TKA. The mean interval between femoral and tibial osteotomy and TKA was 89 and 73 months, respectively. TKA after femoral osteotomy improved the mean Knee Society knee scores from 56 to 88 and function scores from 41 to 72. TKA after tibial osteotomy improved the mean knee scores from 65 to 85 and function scores from 25 to 57. One TKA was revised after 11 months due to valgus malalignment and was complicated by a wound infection. There were no other infections or wound complications.

Conclusions: Femoral and tibial shaft osteotomy effectively delays TKA, relieves pain and improves function in patients who present with malunion and end-stage knee arthritis, while facilitating the eventual TKA. The complication rate and clinical results of TKA following shaft osteotomy appear to be similar to primary TKA.

447 EARLY EFFECT OF BOTULINUM TOXIN TYPE A ON ANKLE RANGE OF MOTION IN CHILDREN DIAGNOSED WITH SEVERE IDIOPATHIC TOE WALKING
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Purpose of Study: The purpose of this study is to report on the outcomes of children with idiopathic toe walking (ITW) who received Botulinum A Toxin (BTX-A) as an adjunct treatment to manipulations and casting and a maintenance protocol.

Methods Used: ITW severity was classified using gait analysis and defined by an absence of a 1st ankle rocker, an early 2nd rocker, and an early and increased first ankle moment. Subjects who met the three severity criteria received a single BTX-A injection into their right and left gastrocsoleus muscles and placed immediately in below-knee casts. Casts were changed two weeks later for a total casting period of four weeks. Subjects then entered a maintenance protocol involving the use of night splints or articulated ankle-foot-orthotics. Outcome measures collected at all follow-up visits were ankle dorsiflexion with knee in 90° and 0° flexion (DFF) and extension (DFE). Outcome measures studied included ankle dorsiflexion range (DFF/DFE) at immediate post-injection visits. Mean DFF/DFE at follow-up intervals are summarized (Table 1).

Summary of Results: Thirteen subjects with severe bilateral ITW participated. Subjects were followed an average of 48 weeks (7–168 weeks) post BTX-A injection. Mean DFF/DFE at follow-up intervals are summarized (Table 1).

Conclusions: Subjects demonstrated an early response to BTX-A in terms of improved DFF/DFE measures at immediate post-injection visits. Maintenance of this response was evident at the last follow-up visit. This is the first study to show early outcomes following BTX-A injections in ITW and provides evidence for obtaining and maintaining correction from a single BTX-A injection over a short-term follow-up period.
448 DESIGN AND INTEGRATION OF ONLINE CASE BASED INTERACTIVE MULTIMEDIA PROBLEM ORIENTED LEARNING IN UROLOGY USING STANDARDISED SOFTWARE FOR USE BY BOTH JUNIOR MEDICAL STUDENTS DURING THE RENAL BASIC SCIENCE BLOCK AND SENIOR MEDICAL STUDENTS DURING CLINICAL CLERKSHIP


Purpose of Study: To develop an online resource of Urology Cases for undergraduate students that would accessible from all 3 sites of the University of British Columbia. To test the ease of use and accessibility of interactivity.

Methods Used: Common office and emergency problems were selected and assigned to a medical student, urology resident and UBC Urology faculty member. Using commercially available interactive software the cases were developed on a collaborative basis to reflect current standards of practice. Each case contained an Introduction, pre-test and post-test questions, images, bibliography, and a link to UBC Urology Faculty member. Keywords were selected to allow for search engine function.

Summary of Results: Library of 38 interactive developed at the three regional campuses. Password protection allows remote access by students on rural rotation. Development for students by students said regional campuses. Password protection allows remote access by students on rural rotation. Development for students by students said regional campuses.

449 SYNTHESIS AND BIOCOMPATIBILITY OF A NOVEL SCAFFOLD FROM APATITE-COATED MICROSPHERES

H.E. Davis, R. Rao, and J.K. Leach. University of California, Davis, CA.

Purpose of Study: Increasing amounts of hydroxyapatite in composite scaffolds for bone regeneration have proven to increase implant osteoconduction and osseointegration. By coating microspheres with biomineral before scaffold formation (premineralization), we hypothesized that beneficial cell-ceramic interactions could be maximized and greater amounts of hydroxyapatite would be incorporated than conventional approaches (postmineralization).

Methods Used: Poly(lactide-co-glycolide) microspheres were formed using a standard double emulsion technique. Microspheres to be mineralized were placed in modified simulated body fluid (mSBF) at 37°C for 7 d. Sodium chloride and microspheres, both mineralized and nonmineralized, were combined prior to loading in a die and compressed at 1500 psi for 1 min to yield solid disks. The samples were exposed to CO2 gas for 16 h resulting in the growth of CO2 pores within the polymer matrices. The NaCl particles were leached from the polymer matrices in DI H2O for 24 h. Postmineralized scaffolds subsequently underwent incubation in mSBF for 7 d. Substrates were imaged using a scanning electron microscope. Scaffold deformity was measured during mSBF incubation. Biomineral deposition on microspheres incubated in mSBF was determined by mass increases and calcium quantification. The presence of apatite was confirmed with FTIR. Human mesenchymal stem cells were seeded on scaffolds and cell-secreted calcium and DNA-content were quantified in vitro at 0, 7, 14, 21, and 28 d.

Summary of Results: Microspheres incubated in mSBF for 7 d demonstrated a mass and calcium gain. The presence of carbonate apatite was confirmed by FTIR which displayed carbonate and phosphate peaks. Premineralized scaffolds exhibited irregular inner pore structures compared to postmineralized and nonmineralized scaffolds. Furthermore, premineralized scaffolds exhibited less deformation than postmineralized substrates after mineral deposition. Premineralized scaffolds possessed significantly more calcium at day 0 and induced greater cell-secreted calcium at days 14 and 28 compared to postmineralized scaffolds. Increased DNA content was detected in premineralized scaffolds at day 14.

Conclusions: Premineralized scaffolds possessed increased mineral deposition, less deformity, and enhanced osteoconductive potential over postmineralized constructs.

450 RISK FACTORS FOR DELIRIUM FOLLOWING MAJOR TRAUMA


Purpose of Study: Delirium is a common consequence of the physiologic stress of trauma. Delirium in post-injury patients has not been thoroughly studied. Delirium has been found to be a clinically relevant finding in hospitalized patients because it results in worse outcomes including increased morbidity and mortality, and increased length of stay. The objective of this study is to identify risk factors that predict the development of delirium in trauma patients and to compare outcome measures such as complication rate and length of stay in patients with and without delirium.

Methods Used: A prospective observational study was performed on patients admitted to the trauma ICU of a major urban level I trauma center. Patients were evaluated daily for arousal and delirium using validated tools including the Richmond Agitation Sedation Scale (RASS) and the Confusion Assessment Method in the ICU (CAM-ICU). Univariate and multivariate statistical analyses were performed.

Summary of Results: Delirium occurred in 57% (40) of subjects. Delirium presented most frequently on post-injury day 2 and lasted for an average of 4.7 ± 0.7 days. See Table 1 for results of univariate analysis. Multivariate analysis found injury severity score, Glasgow coma score and transfusion requirement to be the strongest predictors for developing delirium. In terms of outcome measures, subjects with
Conclusions: Delirium occurs in over half of the patients in the trauma ICU following major injury. Injury severity score, admission Glasgow coma score and transfusion requirement are the strongest predictors of delirium following major injury. Subjects with delirium have worse outcomes including increased complications, longer ICU stays and longer hospital stays.

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**THE EFFECT OF HYPERBARIC OXYGEN INDUCED IL-6 ON TNFα, IL-1 AND NEUTROPHIL POLARIZATION AND ADHESION**

M.R. Kraininock, D.A. Scott, L.L. Stephenson, W.Z. Wang, K.T. Khiabani, and W.A. Zamboni. University of Nevada School of Medicine, Las Vegas, NV.

**Purpose of Study:** Hyperbaric Oxygen Therapy (HBO) has been shown to decrease the damage caused by ischemia-reperfusion (IR) injury in skeletal muscle by inhibiting neutrophil polarization and adhesion. Previous work has implicated IL-6 as a possible mediator in the effect of HBO. Our work investigated the effect of HBO induced IL-6 on the pro-inflammatory cytokines TNFα and IL-1, as well as neutrophil polarization and adhesion.

**Methods Used:** The gracilis muscle flap was raised in male Sprague-Dawley rats (n = 5–7/group). Three groups were evaluated: (1) SHAM, (2) ischemia only (I), and (3) ischemia/reperfusion (IR). I and IR groups received 2 hours of global ischemia, and the IR group received 2 hours of reperfusion following ischemia. The gracilis muscle was harvested and approximately 1 x 10⁶ cells were isolated from each sample. Cells were stained with propidium iodide (PI) or Annexin V-FITC in order to detect necrosis or apoptosis, respectively. Twenty thousand cells from each sample were analyzed via flow cytometry.

**Summary of Results:** Ischemia is responsible for approximately 66% of cell death, and reperfusion significantly increases necrosis above the level measured following ischemia (I vs IR, 14.9 + 1.7% vs 22.0 + 1.3%, p < 0.01). Apoptosis is the major mechanism of cell death during muscle ischemia (apoptosis vs necrosis, 14.9 + 1.7% vs 30.9 + 3.6%, p < 0.01) and following reperfusion (apoptosis vs necrosis, 22.0 + 1.3% vs 35.7 + 3.9%, p < 0.01).

**Conclusions:** Ischemia is responsible for the majority of cell death in cells isolated from skeletal muscle. However, reperfusion significantly increases necrosis above levels measured following the ischemic insult. Apoptosis is the major mechanism of cell death in cells isolated from skeletal muscle following 2 h ischemia as well as 2 h ischemia/2 h reperfusion. A therapy aimed at blocking apoptosis could reduce IR injury; a common postoperative complication following muscular trauma.

Western Student Medical Research Forum
Student Scientific Session IX
8:30 AM
Saturday, February 2, 2008

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**NECROSIS AND APOPTOSIS IN CELLS ISOLATED FROM RAT SKELETAL MUSCLE FOLLOWING ISCHEMIA AND REPERFUSION**

J.L. Cartinella¹, X. Fang², L.L. Stephenson², K.T. Khiabani², W.A. Zamboni², and W.Z. Wang². ¹University of Nevada School of Medicine, Reno, NV and ²University of Nevada School of Medicine, Las Vegas, NV.

**Purpose of Study:** The major mechanism of ischemia/reperfusion (IR)- induced cell death in skeletal muscle is unclear. Furthermore, studies involving other tissues (e.g., heart, lung, and brain) have shown that reperfusion can be responsible for tissue injury and cell death above and beyond that incurred during the initial ischemic insult. The purpose of this study was to investigate necrosis and apoptosis in cells isolated from rat skeletal muscle and determine the extent to which ischemia and reperfusion contribute to cell death.

**Methods Used:** The gracilis muscle flap was raised in male Sprague-Dawley rats (n = 5–7/group). Three groups were evaluated: (1) SHAM, (2) ischemia only (I), and (3) ischemia/reperfusion (IR). I and IR groups received 2 hours of global ischemia, and the IR group received 2 hours of reperfusion following ischemia. The gracilis muscle was harvested and approximately 1 x 10⁶ cells were isolated from each sample. Cells were stained with propidium iodide (PI) or Annexin V-FITC in order to detect necrosis or apoptosis, respectively. Twenty thousand cells from each sample were analyzed via flow cytometry.

**Summary of Results:** Ischemia is responsible for approximately 66% of cell death, and reperfusion significantly increases necrosis above the level measured following ischemia (I vs IR, 14.9 + 1.7% vs 22.0 + 1.3%, p < 0.01). Apoptosis is the major mechanism of cell death during muscle ischemia (apoptosis vs necrosis, 14.9 + 1.7% vs 30.9 + 3.6%, p < 0.01) and following reperfusion (apoptosis vs necrosis, 22.0 + 1.3% vs 35.7 + 3.9%, p < 0.01).

**Conclusions:** Ischemia is responsible for the majority of cell death in cells isolated from skeletal muscle. However, reperfusion significantly increases necrosis above levels measured following the ischemic insult. Apoptosis is the major mechanism of cell death in cells isolated from skeletal muscle following 2 h ischemia as well as 2 h ischemia/2 h reperfusion. A therapy aimed at blocking apoptosis could reduce IR injury; a common postoperative complication following muscular trauma.

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**METHODS TO REGULATE ADRENERGIC NEUROVASCULAR TONE TO REDUCE SYMPTOM LEVELS**

J. Hwang, R. Barndt, S. Jagtap, and A. Cho. Bethel Public Service Clinic, Downey, CA.

**Purpose of Study:** Our pilot studies (PS) show that adrenergic neurovascular tone (ANVT) causes symptom levels (SL) in migraine, chronic fatigue, fibromyalgia and related anxiety. Our prospective studies (ProS) show rises in ANVT (systolic time interval changes, STI = PEP/LVET x 100%) predict significant (Sig) increase in SL (P < 0.01). Our PS show these disorders are inter-related by the hemodynamic effects of ANVT.
ADRENERGIC NEUROVASCULAR TONE: A CORONARY RISK FACTOR IN NORMOTENSIVE PEOPLE

J. Hwang, R. Barndt, S. Jagtap, and A. Cho. *Bethel Public Service Clinic, Downey, CA.*

**Purpose of Study:** Our pilot studies (PS) show that BP, CO & SVR are predicted by adrenergic neurovascular tone (ANVT) measured by systolic time intervals (STI = PEP/LVET X100%). Blood pressure is determined by cardiac output (CO, L/min) and systemic vascular resistance (SVR = [mean BP-6 mmHg] X 80/CO). Normal BP by our PS was 110 ± 10/80 ± 5, with SVR <1600 and CO >4, all in standard units (SU), and had no coronary collagen stenosis (CCS). Ultrasonic measurements (UM) were made of CCS & were found predictive of angiographic stenosis in parallel by PS methods previously reported by our clinic (R = 0.97, P < 0.001).

**Methods Used:** In this study, prospective studies (ProS) were done in a random sample of the general population (GP) with a full distribution of ANVT (10 sets with a continuous STI array 20–58%, 380 cases). Cases were selected by age 55 yrs., female/male 1/1, BP 110 ± 10/80 ± 5, LDL < 130, normal C reactive protein, triglycerides <150, HgbA1c < 6.0, Hgb > 13, all non-smokers, all Caucasians. Measurements were made of BP, PP (pulse pressure), RPP (rise in PP during isometric handgrip @5psi/3 min.) & symptom levels (SL = 0–19 and causes CCS in 15% of the GP at this age. Thus, ANVT should be lowered to reduce vascular risk in normotensive people.

**Summary of Results:** The G mean BP for the normotensive GP was found to be 110 ± 10/80 ± 5 over the full range of ANVT as shown in Table 1. G1-3. G1 & 2 had no CCS with CO 4-7 & SVR <1600. G2 had mild CF with Sig* higher SVR & lower CO versus G1. G3 was found to have abnormal CCS, SVR >1600 & low CO <4 with moderate CF, all Sig > G2 (P < 0.02) & Sig* > G1 (P < 0.01). In ProS RPP predicted STI (R = 0.97, P < 0.001). STI predicted SBP, CO & SVR at R = 0.97, P < 0.001. STI explains 94% of the variability of the BP, CO & SVR relationships in normotensive people since R² = 0.94.

**Conclusions:** STI < 29 is predicted by RPP > 19 and causes CCS in 15% of the GP at this age. Thus, ANVT should be lowered to reduce vascular risk in normotensive people.

<table>
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<th>G #</th>
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<th>RPP/2</th>
<th>PEP/2</th>
<th>CO/2</th>
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<td>21/42</td>
<td>93/108</td>
<td>25/14/14</td>
<td>90/95</td>
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R = Range (±), * = Significantly (Sig*) different from G1 controls at P<0.01 (all G P-values by T-Test).

**Summary of Results:** The G mean BP for the normotensive GP was found to be 110 ± 10/80 ± 5 over the full range of ANVT as shown in Table 1. G1-3. G1 & 2 had no CCS with CO 4-7 & SVR <1600. G2 had mild CF with Sig* higher SVR & lower CO versus G1. G3 was found to have abnormal CCS, SVR >1600 & low CO <4 with moderate CF, all Sig > G2 (P < 0.02) & Sig* > G1 (P < 0.01). In ProS RPP predicted STI (R = 0.97, P < 0.001). STI predicted SBP, CO & SVR at R = 0.97, P < 0.001. STI explains 94% of the variability of the BP, CO & SVR relationships in normotensive people since R² = 0.94.

**Conclusions:** STI < 29 is predicted by RPP > 19 and causes CCS in 15% of the GP at this age. Thus, ANVT should be lowered to reduce vascular risk in normotensive people.
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EMPLOYMENT AFTER HEART TRANSPLANTATION AND THE EFFECTS OF GOVERNMENTAL HEALTH INSURANCE POLICIES

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Purpose of Study: Employment post-transplant is considered a marker of improved clinical status and quality of life. But many US patients risk losing health insurance benefits if they are working post-transplant, discouraging post-transplant employment. However, this would not be the case in countries with national health insurance plans. We sought to compare the rates of post-transplant employment between countries with and without national health insurance plans.

Methods Used: We reviewed the literature for studies of post-transplant employment between 1 and 5 years after heart transplant. There were 3 studies in Europe (n = 126) and 5 studies from the US (n = 534). The European studies were from Canada and the United Kingdom, countries with national health insurance plans. We performed statistical analysis to compare the demographics and percentage of patients who were employed after heart transplantation.

Summary of Results: There were no significant differences in demographics, including age and gender, between the European and US studies. In a subgroup of studies, where data was available, pre-transplant employment was not significantly different between Europe and the US. However, more patients in the European studies had post-transplant employment compared to the US studies (53% vs 25%, p < 0.001).

Conclusions: While many factors can influence the decision for post-transplant employment, the presence of national health insurance plans may encourage post-transplant patients in other countries to seek employment, while the lack of such plans in the US provides a disincentive to post-transplant employment. Since the ability to work post-transplant may contribute to improved quality of life, national health care plans or health care government policies that would prevent loss of medical insurance upon employment should be pursued for heart transplant patients.

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CLINICAL AND MORPHOLOGICAL CHARACTERISTICS OF ACCELERATED TRANSPLANT CORONARY ARTERY DISEASE (TCAD) IN HUMAN GRAFTS

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Purpose of Study: Transplant coronary artery disease (TCAD) has been found in approximately 50% of patients who have undergone orthotopic cardiac transplantation and is a predominant cause of late mortality. Characterization of lesions has not been extensively described in the literature.

Methods Used: Explanted hearts were examined from 64 patients (48 males and 16 females) undergoing re-do transplantation for graft failure due to TCAD. Mean patient age was 41.9 years (range 3–70 yrs). Mean duration of implanted heart until re-transplant was 83.8 months (2–216 mos). Mean heart weight was 342.5 grams (110–1240 g). We reviewed surgical pathology reports of cases, retrieved microscopic slides from the UCLA Department of Pathology archives, reviewed all slides, then graded and recorded any pathologies present: coronary artery stenosis (%), intimal fibromuscular hyperplasia, intimal atheroma and intimal calcification on a grade of 0–3. Assessment of acute rejection was also recorded. This study was approved by the UCLA Institutional Review Board (IRB # G03-12-039/04).

Summary of Results: Varying degrees of stenosis, intimal fibromuscular hyperplasia, intimal atheroma and intimal calcification were seen in each of the four major coronary arteries. Forty-six patients (73%) experienced some form of acute rejection.

Conclusions: Grading of the various characteristics of TCAD revealed that all of the four main coronary arteries (LAD, Cx, RCA and LM) are prone to pathological changes. Ultimately, cardiac function is compromised by ischemia due to accelerated stenosis of these major vessels, leading to infarction and myocardial death. Such stenosis is generally caused by intimal fibromuscular hyperplasia, intimal atheroma or both.

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SURGICAL OUTCOMES IN ADULTS WITH EBSTEIN’S ANOMALY OF THE TRICUSPID VALVE

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Purpose of Study: Ebstein’s anomaly (EA) is a congenital heart disorder characterized by a malformed tricuspid valve (TV). Patients with EA have tricuspid regurgitation (TR) and may require TV surgery. An echocardiographic grading system was devised by Celermayer (CG) for predicting survival in neonates. Our aim was to review surgical outcomes of adults at UCLA with EA who had TV surgery from 1983–2007.

Methods Used: Several pre-operative clinical variables were examined, including age, NYHA functional class, oxygen saturation, and presence of interatrial communication. Pre-operative echocardiographic data included RVEF, LVEF, degree of tricuspid regurgitation (TR), and Celermayer echocardiographic grade. Post-operative complications, residual TR and length of hospitalization were assessed.

Summary of Results: Fifty-four adults (22 males, 32 females) with EA were identified, with mean age 37+/-15 years. 36 patients (67%) had TV surgery, 20 repair and 16 replacement. NYHA class was 2.5+/-0.7, O2 sat 96% +/- 5%, and TR 2.25 +/- 0.7 (0–3 scale). Twenty-eight patients (78%) had ASD/PFO. Twenty-Five patients (69%) had supraventricular arrhythmias (SVT), 10 with accessory pathway. Hospitalization was 8.7 +/- 5.8 days, during which congestive heart failure occurred in 3 patients (8%), pacemaker placement in 6 patients (17%). SVT in 7 patients (19 %), and death in 1 patient (3%). In 27 patients with post-op echocardiograms, TR was 2, at 42 months post-repair, and 0.8 at 45 months post-replacement. The Celermayer severity grade could be calculated in 8 patients, the mean grade was 2.3. The Celermayer grade significantly correlated with pre-operative NYHA class (R = 0.98, p < 0.001). Moreover, the only patient that died in the perioperative period had both the highest Celermayer grade and NYHA functional class.
Conclusions: Tricuspid valve surgery in adults with Ebstein’s anomaly carries a low risk of perioperative mortality and morbidity. Post-operative tricuspid regurgitation is more common following valve repair as opposed to replacement. The Celemajer severity grade may predict patients at highest risk of perioperative mortality.

**POST TRAUMATIC STRESS DISORDER, PSYCHIATRIC DISORDERS AND CARDIOVASCULAR RISKS IN A VA OUTPATIENT POPULATION**

E. Park¹, V. Paith ², G. Twitchell ², R. Mallios ³, and J. Huang ², ³.

¹Western University Health Science, Pomona, CA; ²VA Medical Center, Fresno, CA and ³UCSF Fresno Medical Education Program, Fresno, CA.

**Purpose of Study:** Post Traumatic Stress Disorder (PTSD) following exposure to intense psychological and physical trauma may result in significant adverse health consequences. It is associated with other co-morbid psychiatric disorders and implicated in increased risk for cardiovascular disease. PTSD is more prevalent among veterans due to the stressors of combat experience. The purpose of this study was to determine multiple co-morbid psychiatric disorders and cardiovascular risk factors and their potential association with PTSD in a veteran outpatient population.

**Methods Used:** Demographics, vital signs, diagnoses, and lab results, were retrospectively retrieved from medical records of 16,571 VA outpatients with complete data. Chi-square, t-test, and multivariate logistic regression statistics were used.

**Summary of Results:** The prevalence of PTSD was 11% with significantly more cases in age groups of < 25 and 55–64 years Among PTSD patients, 61% of trauma event was combat-related and the mean global assessment function (GAF) score was 55/100. The co-occurring diagnoses were hypertension 67%, dyslipidemia 61%, diabetes 28%, coronary artery disease 14%, depression 6%, mood disorder 2%, and other anxiety disorder 1.7%. There were higher lipid levels and diastolic blood pressure in PTSD patients compared to those without PTSD, but the statistical significance disappeared when BMI was taken into consideration. However, there was significantly more depression, mood and other anxiety disorders in PTSD patients even after adjustment for both age and BMI in logistic regression analysis.

**Conclusions:** PTSD was more prevalent in two age groups among this veteran population with combat experience in Iraq/Afghanistan and Vietnam, respectively. A significant proportion of patients with PTSD had other psychiatric disorders with impaired psychosocial function as judged by their low GAF scores. Certain co-morbid conditions that carry high cardiovascular risks were also highly prevalent. Our results suggest the need of treating concomitant cardiovascular risks as well as other psychiatric disorders in the long-term management of PTSD. Prospective studies are warranted to determine the clinical cardiovascular outcomes.

**THE EFFECT OF WEIGHT LOSS ON CONVENTIONAL AND ADVANCED LIPID MARKERS**

A.S. Young and N. Doherty. WUHS/COMP, Claremont, CA.

**Purpose of Study:** Conventional lipid markers-total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL) and triglyceride (TG)-are markers of cardiac disease risk. LDL and HDL cholesterol are comprised of subparticles, which are also indicators of cardiac risk. These include the Î1a + Î1b LDL subparticle (LDLÎ1a + Î1b), and the 2b HDL subparticle (HDL2b). The purpose of this study is to investigate what effect intentional weight loss has on these markers of cardiac risk.

**Methods Used:** Patients were selected from a cohort of 69 patients who had visited a dietician and had two sets of lipid panels including both the conventional lipid markers and the subparticles of the LDL and HDL. The LDLÎ1a + Î1b is measured both as a percentage of total LDL, as well as quantified (Q-LDLÎ1a + Î1b) using gradient gel electrophoresis. The HDL2b is measured as a percentage of the total HDL. Patient weights were obtained at their first meeting with the dietician and after a period of follow up.

**Summary of Results:** The patients in the cohort were classified into two groups: those with weight loss more than 10 lbs, and those that remained within 5 lbs of their first weight. The two groups of patients were compared using a paired one-sided t-test. The results are summarized in the table.

**Conclusions:** In this initial analysis of the effectiveness of weight loss in the management of lipid levels, the results are mixed. Our analysis demonstrated a statistically significant (p < 0.05) effect of weight loss both the LDLÎ1a + Î1b concentrations and the HDL concentration. The study showed no impact on the total cholesterol, LDL, TG or HDL2b levels. Our study is limited by the small size of our cohort. In addition, we did not control for pharmacologic or genetic factors. A larger, prospective study would be needed to further clarify the effect of weight loss on the new advanced lipid markers.
Conclusions: Our results demonstrate that Gankyrin is over-expressed in breast cancer cell lines. We suggest that Gankyrin expression may serve as a molecular tool in the study of breast cancer and further work can help assess whether Gankyrin expression can be used as a diagnostic or prognostic marker in breast cancer.

Purpose of Study: This project evaluates changes in pulmonary function in patients receiving combinations of chemoradiation for the treatment of non-small cell lung cancer. Pulmonary function parameters included forced expiratory volume in 1 second (FEV1), forced vital capacity (FVC), and diffusion capacity for carbon monoxide (DLCO). These parameters were used to determine the threshold doses of radiation that caused pulmonary toxicity and mortality. This study aims to define the levels of pulmonary function testing that minimize the risk of toxicity and mortality in patients receiving definitive treatment for lung cancer.

Methods Used: We identified 836 patients as being diagnosed with lung cancer between January 1994 and March 2007 and having received treatment at the University of Washington or the Puget Sound Veterans Administration. Patients who were treated with definitive doses of radiation therapy were analyzed with regard to radiation dosage given and pulmonary function preceding and following radiation treatment. We analyzed patients for morbidity and mortality.

Summary of Results: Anticipated results pending data analysis will include the percentage of patients who developed significant acute and late morbidities including Grade 3 or greater radiation pneumonitis, and the percentage of patients who died within 1 month of definitive radiation treatment. Based on these rates of toxicity and mortality, we can determine the minimum levels of pulmonary function prior to treatment, as well as the post-treatment effect of radiation on pulmonary function.

Conclusions: We found that it is safe to administer definitive radiation therapy to patients with low FEV1 and DLCO levels. Should patients have borderline pulmonary function tests, at our institution this is correlated with a quantitative ventilation and perfusion scan as well as the clinical picture.

Conclusions: Increased density of mast cells in stroma surrounding prostate cancer supports previous studies that have demonstrated similar findings in malignancies. The monoclonal antibody V1b directed against the α1 subunit of collagen XI appears to be a new specific marker for mast cells.

Purpose of Study: The major NPC histologic subtypes have been shown to exhibit different ethnic distributions with variable survival characteristics. Tobacco smoking is known to increase the risk of nasopharyngeal carcinoma (NPC). However, the effect of smoking on survival of NPC cases is not described. This study investigated whether tobacco history influenced survival of NPC cases by race.

Methods Used: 355 incident NPC cases from 1988–2003 were analyzed from the California Cancer Registry, with follow-up through 2005. Smoking history was obtained by manually reviewing abstract text fields, and cases were classified as ever smokers (ES) vs. never smokers (NS). One year and five year overall survival (OS) rate estimates were calculated using the Kaplan Meier method, and compared with the Log-Rank test. Cox proportional hazard ratios were used to identify independent prognostic factors for survival.

Summary of Results: Identified among the NPC cases were 140 (39%) ES, 83 (23%) NS, and 132 (37%) with unknown smoking status. Among those with known smoking status, a greater proportion of ES were observed among WHO Type I NPC cases (n = 87; 74%) compared with WHO Types 2 (n=15; 44%) and 3 (n = 38; 53%) (p = 0.0006). Smoking was more common among Caucasians (75 (102, or 73%) compared to Asians (49 of 91, or 54%) (p = 0.013). 5-year OS rates for all NPC subtypes were not significantly different between smokers and nonsmokers (61% vs 51%; p = 0.17). Among Caucasians, 5-year OS improvements were observed for NS NPC cases (OS = 70%; n = 27) compared to ES (OS = 44%; n = 75) that were borderline significant (p = 0.051). No 5-year OS differences were observed for Asian NPC cases that were NS (OS=59%; n=42) vs. ES (OS=63%; n = 49) (p = 0.66). Subset
Western Regional Meeting Abstracts

465 RELATIVE ULNA LENGTH AND ESTROGEN EXPOSURE: POTENTIAL BIOMARKER OF BREAST CANCER?
J.C. Chang1, T. Hayes2, and J.T. Martin1. 1Western University of Health Sciences, Pomona, CA and 2Downey Regional Medical Center, Downey, CA.

Purpose of Study: Estrogen has been implicated in positively and negatively regulating bone growth in the extremities of individuals during adolescence. Previous bone length studies have shown that African American (AA) women have relatively longer ulnas compared to other ethnicities, and epidemiological studies have shown that AA women have a lower incidence of breast cancer (BC) than White women (W). Therefore, we hypothesized that individuals with BC would have altered long bone growth in comparison to controls.

Methods Used: In order to test this hypothesis, we examined anthropometric measures of bone length in BC and control subjects. A total of 186 subjects (N(BC)=133; N(Ctrl)=53) were recruited to participate in the study from hospitals and clinics in the Los Angeles, CA area. Measurements of the ulna length were made in triplicate by placing the elbow against the end of the osteometric board and marking the position just inferior to the styloid process of the ulna. Averages of the left and right ulna of an individual were calculated and the ulna:stature ratio (U:S) was determined by dividing by the individual’s height. Age at diagnosis, height, and other demographic and family history information were obtained through a questionnaire.

Summary of Results: Subjects were arbitrarily divided into those <45 years old and those ≥45 years old as a proxy for pre- and post-menopause since causes of BC occurring before and after menopause may differ and because age of menopause was not available for most controls. Only the ≥45 data (N(BC)=78; N(Ctrl)=53) are analyzed here. In a 2-way ANOVA with ethnicity and illness as factors, U:S varied significantly among ethnicities (F(2,124)=23.99; p<.0001) and between BC and controls (F(1,124)=16.83; p<.0001) with the U:S being higher in BC subjects than in controls and higher in AA than in other ethnic groups. However, certain variables, i.e. age and BMI, could have contributed to our finding. Regression analysis of these variables on U:S produced no significant relationships suggesting that an elevated U:S may well be a biomarker for post-menopausal BC.

Conclusions: More subjects should be measured and analyzed in order to determine whether U:S or other anthropometric measures can be useful as a biomarker of breast cancer.

466 LINKING THE RELATIONSHIP BETWEEN BIRTH ORDER AND BREAST CANCER
B.J. Teran1, J.T. Martin1, and V. Agarwal2. 1Western University of Health Sciences, Pomona, CA and 2New Hope Cancer and Research Institute, Pomona, CA.

Purpose of Study: The association between breast cancer (BC) and preceding levels of maternal estrogen exposure is not completely understood. The relationship between BC and early circulating estrogen levels is relatively strong despite the time difference that exists before the diagnosis of BC is made. Evidence indicates maternal estrogen levels may vary with birth order. At least one earlier study indicates that premenopausal BC subjects are less likely to be second in birth order compared to first. However, this study apparently did not evaluate the incidence according to sibship size. Therefore, we attempted to replicate the earlier findings by examining BC incidence in relation to birth order in each sibship size independently.

Methods Used: We identified birth order and other family history variables in a sample of 133 women with BC and 240 control women via questionnaire. Seven anthropometric measures were made of hand and arm traits that have been shown to reflect sex hormone levels during childhood and adolescent development. The incidence of BC in each sibship sample was arrived at by dividing the number of BC cases by the total number of cases in that sibship size category. We hypothesized that sibship size, birth order and relative ulna length would differ between women with BC and controls.

Summary of Results: We found that ulna length normalized to stature was not significantly related to birth order in either the BC group (r2=.316, p=.245) or controls (r2=.056, p=.65). The mean incidence of BC over sibship sizes did not significantly change with birth order (r2=.209, p=.30); However the lowest incidence of the first three sibship positions was in birth order position 2. Comparisons of larger sibships were problematic due to low sample sizes.

Conclusions: These results support earlier findings of a potential effect of birth order on risk for BC. Moreover, the results were obtained using an additional level of control (sibship size relationship) in comparison to previous studies. These conclusions lead to the possibility of an effect albeit not a strong one because of the magnitude of the significant difference obtained in our study. Future studies would need to include a larger sample size in order to determine the size of the effect between birth order and BC.

467 POSSIBLE ROLE OF 2ND TO 4TH DIGIT RATIOS (2D:4D) AS A MARKER FOR BREAST CANCER RISK
I.S. Chhabra1, R. Toussi1, J. Martin1, R. Rico1, and V. Davis1,2. 1College of Osteopathic Medicine, Western University of Health Sciences, Pomona, CA and 2Arrowhead Regional Medical Center, Colton, CA.

Purpose of Study: The burden of breast cancer is the highest among women in the western world. The etiology of breast cancer is unclear, but many factors, e.g., estrogen exposure, have been found which increase breast cancer risk. Evidence suggests that variation in the ratio of the 2nd and the 4th digit length (2D:4D) is associated with differences in sex steroid levels during development. Some investigators have suggested that the 2D:4D ratio may be positively associated with both fetal prenatal estradiol levels and with breast cancer risk. The purpose of our current study is to attempt to confirm the earlier work and to assess whether there is a lateralization of the relationship between the 2D:4D and breast cancer development.

Methods Used: We conducted a retrospective study recruiting breast cancer patients (n=104) and control subjects (n=142) from five hospitals, only women were included in the analysis. Bone measurements (wrist width, 2nd digit, 4th digit, hand, wrist, and ulna lengths) and questionnaires were administered on all subjects. Measurements were made in triplicates with a digital caliper measuring to 0.01 mm. Digit length was measured from the most proximal crease of the digit to the tip of the digit on the ventral surface of the hand.

Summary of Results: We performed an ANOVA analysis and found breast cancer patients demonstrated a larger ratio of 2D:4D then controls.
and Sciences: College of Osteopathic Medicine of the Pacific, Pomona, CA

Conclusions: Women with higher 2D:4D ratios demonstrate an increased risk for breast cancer, which confirms earlier work. If these results are proven to be strong then additional research needs to determine whether 2D:4D ratio is a better predictor of risk for breast cancer than other well-known risk factors. Ultimately, the 2D:4D ratio may make the identification of women who need early screening more correct.

468 FLUCTUATING ASYMMETRY OF THE UPPER EXTREMITY IN WOMEN WITH BREAST CANCER

A. Chang1, J. Zweig2, and J.T. Martin1. 1Western University of Health Sciences: College of Osteopathic Medicine of the Pacific, Pomona, CA and 2Pacific Hospital of Long Beach, Long Beach, CA.

Purpose of Study: Fluctuating asymmetry (FA) is the deviation of bilateral congruence between the right and left side of the body and is thought to be an index of early developmental instability. Generally speaking, it is thought that fluctuating asymmetry is inversely related to a person’s overall health. A previous study had shown that women with breast cancer (BC) had higher FA than women who did not have BC. However, it was not clear whether this asymmetry might have been caused by the tissue’s response to the tumor. In our study we hypothesize that women with BC would also have a higher FA than women without BC in a series of upper extremity measurements.

Methods Used: Patients were recruited from various hospitals and clinics in Southern California; 138 women with breast cancer and 137 female controls were obtained. However, the 138 patients with BC included 34 patients with BC plus other chronic diseases, therefore these 34 patients were not included in this analysis. Each patient had 3 sets of bilateral measurements taken of the length of the lower arm, ulna, hand and the width of the wrist and hand. FA was calculated as FA = O/(R-L)/(R-L)*2/N where R = the mean of the 3 replicates on the right and L = the mean of the 3 replicates on the left.

Summary of Results: The results indicated that women with breast cancer (BC) had a higher FA in 3 of the 5 traits: ulna length (p=0.004), lower arm length (p=0.0004) and wrist width (p=0.0321).

Conclusions: These results suggest that women that later develop breast cancer may already have abnormal regulation of long bone growth during adolescence and/or childhood since growth in these traits mainly occur before the end of the adolescent years. Estrogen is known to regulate bone growth during adolescence and may play a direct role in FA and the likelihood of developing BC later in life.

469 SELF-REPORTED CHILDHOOD AGGRESSION AND ITS RELATIONSHIP TO BREAST CANCER

R. Toussi1, J. Chhabra1, R. Rico2, J.T. Martin1, and V. Davis1-2. 1Western University of Health Sciences, COMP, Pomona, CA and 2Arrowhead Regional Medical Center, Colton, CA.

Purpose of Study: There was expected to be over 200,000 new breast cancers, and over 40,000 deaths due to malignant breast cancer among U.S women in 2006, making it the second leading cause of cancer death. Although, there have been several breast cancer genetic markers identified that may help in prevention, the majority of breast cancers are idiopathic in nature. Screening factors that can help identify high-risk patients can help in the prevention and early detection of breast cancer. Several studies have identified relationships between hormonal abnormalities during childhood and breast cancer. Currently, under investigation is the role estrogen exposure has on breast cancer.

Methods Used: A retrospective study was conducted on a cohort of breast cancer patients identified through a hospital registry. The patients were invited to participate in the study, in which data was collected on demographics, breast cancer status, family history, and hormone-related variables (bone length and childhood aggression habits). A pre-validated aggression scale was adopted and modified in order to decrease the utilization of participant time.

Summary of Results: Initially, it was important to determine that the modified aggression scale was able to identify difference between genders. There was a significant difference between men (n=179) and women (n= 460), with mean aggression score of 8.9 (SD= 5.2) and 6.8 (SD = 4.5); (t (637) = −5.1; p < .001), respectively. Aggression scores were correlated to the other hormone-related variable (ulna-to-stature-ratio); r=.129; p < .05. Further, analysis was conducted on differences between breast cancer patients and non-breast cancer controls. Breast cancer patients reported significantly lower aggression scores (M= 6.0; SD = 4.0) compared to controls (M=7.2; SD= 4.4), (t (368) = 2.6; p < .05).

Conclusions: There appears to be a relationship between self-reported childhood aggressiveness and breast cancer. Low childhood aggressiveness in females may be an indicator of hormonal abnormalities, which may later increase risk of breast cancer. The relationship between these variables requires further investigation, and the causal factors should be identified.

Western Student Medical Research Forum
Student Scientific Session XI
8:30 AM Saturday, February 2, 2008

470 TIME-DEPENDENT EFFECTS OF CHROMIUM MESOPORPHYRIN ON THE INHIBITION OF HEME OXYGENASE ACTIVITY IN NEWBORN MICE

H.H. Xiao, T. Morisawa, R.J. Wong, and D.K. Stevenson. Stanford University, Stanford, CA.

Purpose of Study: Heme oxygenase (HO) is the rate-limiting enzyme that catalyzes the degradation of heme to produce bilirubin. Since excess bilirubin leads to jaundice, HO-inhibiting drugs, such as metalloporphyrins (Mps), are being studied as compounds for preventing neonatal hyperbilirubinemia. We have previously reported that tin mesoporphyrin (SnMP) potently inhibits HO, but is photoreactive and induces HO-1 hyperbilirubinemia. That catalyzes the degradation of heme to produce bilirubin. Since excess bilirubin leads to jaundice, HO-inhibiting drugs, such as metalloporphyrins (Mps), are being studied as compounds for preventing neonatal hyperbilirubinemia. We have previously reported that tin mesoporphyrin (SnMP) potently inhibits HO, but is photoreactive and induces HO-1 gene expression, properties that negate its clinical utility. The objective of this study was to determine the efficacy of an alternativeMp, chromium mesoporphyrin (CrMP) on the inhibition of HO activity in newborn mice at various times after administration.

Methods Used: CrMP (30 μmol/kg) was administered via oral gavage to 7d-old (newborn) mice. At various times after administration, animals were sacrificed. Liver, spleen, and brain were harvested and then sonicated in phosphate buffer. HO activity was then quantified by gas chromatography, expressed as pmoles of carbon monoxide (CO) produced/h/mg fresh weight, and then calculated as % inhibition (mean±SD) compared to time-matched control values as shown in the Table below.

Summary of Results: Liver HO activity was significantly inhibited 24h after CrMP administration, which progressively decreased, but persisted

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up to 5 wks. Spleen HO activity was affected only at 24h post-treatment. In contrast, brain HO activity was not inhibited at any time.

**Conclusions:** These findings show that CrMP is orally absorbed by the newborn mouse, exerts long-term inhibition of liver HO activity, but has only short-acting inhibition of spleen HO activity. In addition, CrMP does not appear to cross the blood/brain barrier. Thus, we conclude that CrMP may be an attractive alternative compound for use in the treatment of neonatal jaundice.

<table>
<thead>
<tr>
<th>Time</th>
<th>Liver</th>
<th>Spleen</th>
<th>Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>3h</td>
<td>16141%</td>
<td>9125%</td>
<td>449%</td>
</tr>
<tr>
<td>24h</td>
<td>6313%</td>
<td>26114%</td>
<td>-101%</td>
</tr>
<tr>
<td>48h</td>
<td>6611%</td>
<td>9351%</td>
<td>9111%</td>
</tr>
<tr>
<td>72h</td>
<td>6417%</td>
<td>15112%</td>
<td>-817%</td>
</tr>
<tr>
<td>2 wk</td>
<td>5113%</td>
<td>-312%</td>
<td>-817%</td>
</tr>
<tr>
<td>3 wk</td>
<td>2071%</td>
<td>666%</td>
<td>-815%</td>
</tr>
<tr>
<td>5 wk</td>
<td>1481%</td>
<td>444%</td>
<td>8110%</td>
</tr>
</tbody>
</table>

**471 INFASURF TREATMENT OF EXPERIMENTAL MECONIUM ASPIRATION IN RATS**

Z. Sacks, J.L. Iwanicki, W. Taeusch, and K. Lu. *UCSF School of Medicine, San Francisco, CA.*

**Purpose of Study:** In pilot experiments using Infasurf to treat meconium aspiration in rats, we observed that good responses were found with Infasurf at much lower doses than we had used in previous experiments with Survanta (Lu, Biol Neonate, 88:46, 2005). To check the validity of this observation, we carried out a series of experiments with Infasurf treatment ranging from 25 mg/kg to 50 mg/kg.

**Methods Used:** Rats were anesthetized, paralyzed and ventilated with 100% O2 at a tidal volume of 8 ml/kg. One hour after tracheal instillation of meconium, they were treated with Infasurf or Survanta.

**Summary of Results:** At all doses, good responses were found with Infasurf. Illustrative data for PaO2 and peak inspiratory pressure three hours after treatment are shown in the table compared with historical data (50 mg/kg Survanta) and a concurrent group of animals treated with 25 mg/kg Survanta.

**Conclusions:** Infasurf has some of the highest concentrations of hydrophobic surfactant proteins among the exogenous surfactants, and these have been associated with a reduction in surfactant inactivation. We conclude that Infasurf may be more effective than some other surfactants in treating meconium aspiration pneumonia and other lung injuries associated with surfactant inactivation.

**Summary of Results:**

<table>
<thead>
<tr>
<th>Age of Infant</th>
<th>Cyanosis</th>
<th>Apnea</th>
<th>Choking/Vomiting</th>
<th>Change in muscle tone</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 4 weeks</td>
<td>79% (19/24)</td>
<td>42% (10/24)</td>
<td>25% (6/24)</td>
<td>25% (6/24)</td>
</tr>
<tr>
<td>&gt; 4 weeks</td>
<td>44% (4/9)</td>
<td>56% (5/9)</td>
<td>11% (1/9)</td>
<td>22% (2/9)</td>
</tr>
</tbody>
</table>

**472 EARLY APPARENT LIFE THREATENING EVENTS ADMITTED TO AN NICU**

S.K. Sandhu1, J.E. Hodgman1, and L. Barton1.1 *Los Angeles County Women and Children’s Hospital, Los Angeles, CA* and 2 *USC Keck School of Medicine, Los Angeles, CA.*

**Purpose of Study:** To examine a group of early ALTEs and compare their risk factors with later ALTE and SIDS infants. In our experience the infants of ALTE and SIDS are both common and different. Our goal is to relate known risk factors in both ALTE and SIDS with a specific population of infants that presented with early ALTEs to our NICU. While most ALTEs occur in infants between the ages of one to two months, we studied a unique population of infants that presented predominately in the first month of life, with the majority under two weeks of age.

**Methods Used:** Data was collected from thirty-three infants who were admitted to our neonatal ICU during the years of 2000 to 2005 after experiencing a combination of apnea, color change, choking or vomiting, or alteration in muscle tone. Of the thirty-three infants, 2 infants were in the nursery at the time of the episode and 31 infants were admitted from the emergency room.

**Summary of Results:** Compared with older ALTEs, cyanosis (65%) was a more prominent symptom than apnea (48%). In the early ALTE episodes choking or vomiting (30%) was prominent as well. Sixty-four percent of these episodes occurred following feeding and 14% occurred during feeding. Prematurity was common with 30% of the infants less than 37 weeks.

**Conclusions:** Early ALTEs appear to be different compared to later ALTEs occurring after one month of age. More studies of these differences need to be done as well as follow up to identify the risk of later SIDS.

**Age of Infant | cyanosis | Apnea | Choking/Vomiting | Change in muscle tone**
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</tbody>
</table>

**473 INTRALOBAR PULMONARY SEQUESTRATION WITH ANOMALOUS SYSTEMIC ARTERIAL SUPPLY ILLUSTRATED BY ANGIOGRAPHY: A CASE REPORT AND REVIEW OF THE LITERATURE**

J. Rose, A. Barleben, and A. Abolhoda. *UC Irvine School of Medicine, Irvine, CA.*

**Purpose of Study:** Bronchopulmonary sequestration is a developmental abnormality accounting for fewer than 6.4 percent of all congenital pulmonary malformations. There are two anatomical variations; an intralobar sequestration (ILS) lies within the normal visceral pleura investment of the lung, while an extralobar sequestration possesses its own visceral pleura. In the setting of symptomatic ILS, surgical resection is recommended after visualization of the arterial supply.

**Methods Used:** A 31 year-old healthy male presented with two separate episodes of mild hemoptysis unresponsive to antibiotic therapy. Computed tomography of the chest revealed left lower lobe bronchietatic changes with suggestion of an anomalous arterial supply to the basilar segments. Thoracic aortogram revealed a large arterial feeding branch from the distal descending thoracic aorta at the level of T-11 vertebral body. Venous drainage was via two tributaries of the left inferior pulmonary vein into the left atrium. The patient was diagnosed with intralobar pulmonary sequestration. He underwent a left posterolateral thoracotomy and left lower lobectomy. Intraoperatively, we noted heavily neo-vascularized basilar segments with characteristic bogy appearance consistent with chronic inflammation. Localization of the systemic arterial supply inferior to the left inferior pulmonary ligament allowed safe ligation prior to mediastinal mobilization of the lower lobe. Lobectomy was conducted with minimal blood loss.

**Summary of Results:** The patient was diagnosed with intralobar pulmonary sequestration. He underwent a left posterolateral thoracotomy and left lower lobectomy. Intraoperatively, we noted heavily neo-vascularized basilar segments with characteristic bogy appearance consistent with chronic inflammation. Localization of the systemic arterial supply inferior to the left inferior pulmonary ligament allowed safe ligation prior to mediastinal mobilization of the lower lobe. Lobectomy was conducted with minimal blood loss.

**Conclusions:** This case demonstrates that while computed tomography remains the initial diagnostic test for ILS, delineation of exact origin and course of the anomalous arterial supply is best achieved by aortic angiography to prevent potentially fatal intraoperative hemorrhage. High resolution CT angiography with coronal reconstructions and contrast
enhanced three-dimensional MR angiography should be considered competing imaging modalities.

475
VENTILATION MODE ALTERS EXPRESSION OF AN IGF-1 TRANSCRIPT IN PRETERM LAMB LUNG


Purpose of Study: Mechanical ventilation (MV) of preterm neonates often induces bronchopulmonary dysplasia (BPD). Alveolar simplification is a primary characteristic of BPD and, in preterm lambs, is improved by using nasal CPAP as an alternative mode of ventilation, as well as by administering the histone deacetylase inhibitors, valproic acid (VPA) or trichostatin A (TSA) during MV. Those findings suggest that ventilation mode affects epigenetic regulation of gene expression in the lung. Insulin growth factor-1 (IGF-1) is involved in lung development and exhibits high levels of epigenetic regulation, often involving histone acetylation, which is greater in lung tissue from preterm lambs managed by nasal CPAP compared to MV. Therefore, we hypothesized that ventilation mode will alter the expression of IGF-1 variants in the lung of chronically ventilated preterm lambs.

Methods Used: Ovine sequences were cloned to design probes for IGF-1 variants A, B, Ea, and Eb. Variants A and B differ by the presence of exon 1 vs exon 2, respectively. Variants Ea and Eb differ by the presence or absence of exon 5, respectively. Real-time RT-PCR was used to quantify mRNA levels of those transcript variants in preterm lambs managed by MV, nasal CPAP, MV+VPA, or MV+TSA.

Summary of Results: Ovine clones for IGF-1A, B, Ea, and Eb were 68, 66, 172, and 51 base pairs, and demonstrated 75%, 70%, 89%, and 76% homology, to rat nucleotide sequence, respectively. Relative to the internal control GAPDH, the quantity of variant A mRNA was significantly lower in lung tissue of preterm lambs managed by nasal CPAP compared to MV. Therefore, we hypothesized that ventilation mode will alter the expression of IGF-1 variants in the lung of chronically ventilated preterm lambs.

Conclusions: We conclude that ventilation mode affects the epigenetic regulation of IGF-1 variants in the preterm lung. The recovery of IGF-1A mRNA levels in MV-lambs treated with VPA or TSA support the concept that histone acetylation contributes to the maintenance of pulmonary paracrine IGF-1A levels, as has been found in other models of perinatal stress in other species, and thus suggest that this mechanism of epigenetic regulation is conserved for IGF-1. (HL62875, HL56401, HD41075, CHRC).

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VENTILATION MODE ALTERS HISTONE ACETYLATION IN PRETERM LAMB LUNG


Purpose of Study: Prolonged mechanical ventilation (MV) of preterm neonates often induces bronchopulmonary dysplasia (BPD). Alveolar simplification is a primary characteristic of BPD. In premature lambs, alveolar formation is improved by using nasal CPAP as an alternative mode of ventilation, as well as by administering the histone deacetylase inhibitors, valproic acid (VPA) or trichostatin A (TSA), during MV. Those findings suggest that ventilation mode affects epigenetic regulation of gene expression in the lung. We hypothesized that histone acetylation in the lung will be affected by ventilation mode in chronically ventilated preterm lambs.

Methods Used: Preterm lambs (~132d gestation; term~148d), treated with antenatal steroids and postnatal surfactant, were managed by MV, nasal CPAP, MV+VPA, or MV+TSA (n=4 each). At the end of 3d, frozen lung tissue was analyzed by immunoblot for acetylated H3K9 and acetylated H3K14. Real-time RT-PCR was used to quantify mRNA levels of histone deacetylase 1 (HDAC1) in each of the preterm lamb groups.

Summary of Results: Normalized immunoblot results indicated that acetyl H3K9 and acetyl H3K14 protein abundance was statistically lowest in the MV group (table; p<0.05 by ANOVA and FPLSD). Relative to the internal control GAPDH, the quantity of HDAC1 mRNA was significantly greater in MV.

Conclusions: We conclude that ventilation mode affects acetylation of histones (histone modifications) and HDAC1 mRNA expression in the preterm lamb lung. Somewhat surprisingly, the TSA+MV group had the greatest expression of HDAC1 mRNA, suggesting that the specific HDAC inhibitor’s effect resulted in compensatory upregulation of HDAC1 mRNA. We speculate that specific subsets of genes are expressed in the lung, depending on mode of ventilation. (HL62875, HL56401, HD41075, CHRC).

<table>
<thead>
<tr>
<th>Group</th>
<th>Acetyl H3K9</th>
<th>Acetyl H3K14</th>
<th>HDAC1 mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>MV</td>
<td>0.45±0.11</td>
<td>5.39±0.63</td>
<td>5.41±0.58</td>
</tr>
<tr>
<td>Nasal CPAP</td>
<td>0.79±0.27</td>
<td>7.00±0.63*</td>
<td>2.28±0.11*</td>
</tr>
<tr>
<td>VPA+MV</td>
<td>1.07±0.23*</td>
<td>8.42±0.49*</td>
<td>4.74±0.39*</td>
</tr>
<tr>
<td>TSA+MV</td>
<td>0.85±0.17*</td>
<td>5.56±0.82</td>
<td>6.08±0.35</td>
</tr>
</tbody>
</table>

477
JAK1 EXPRESSION IS RESTORED IN THE LUNG FOLLOWING RETINOID TREATMENT OF CHRONICALLY VENTILATED PRETERM LAMBS


Purpose of Study: Chronic lung disease (CLD) of prematurity associated with mechanical ventilation (MV) is characterized in part by persistently cellular, thick mesenchyme, suggesting that cell proliferation may be dysregulated. Our recent studies showed that abundance of PCNA and STAT3 proteins was less in preterm lambs managed by vitamin A during MV (vitA+MV) or nasal CPAP compared to MV. Conversely, SOCS3 (provides negative feedback on STAT3) protein abundance was greater in the vitA+MV and nasal CPAP groups compared to the MV group. The upstream mediator of STAT signaling is JAK (Janus kinase). Whether JAK protein also is affected by retinoid treatment is unknown.

We hypothesized that JAK mRNA and protein abundance will be restored in lung tissue of preterm lambs treated retinoids during MV.

Methods Used: Preterm lambs (~132d gestation; term~148d), treated with antenatal steroids and postnatal surfactant, were managed by MV, vitA+MV, RARα agonist+MV (AM580), nasal CPAP (positive outcome control), or RARα antagonist+nasal CPAP (BMS 199453). At the end of 3d, frozen lung tissue was analyzed by real time RT-PCR for JAK1 mRNA expression and immunoblot for JAK1 protein abundance.

Summary of Results: JAK1 mRNA expression was greater (p<0.05) in the vitA+MV (14.4±2.6; normalized for GADPH expression; mean±SD; n = 4) and RARα agonist+MV (14.8±0.8) groups, equivalent to the nasal CPAP (16.2±0.1) and RARα antagonist+nasal CPAP (BMS 199453). The end of 3d, frozen lung tissue was analyzed by real time RT-PCR for JAK1 mRNA expression and immunoblot for JAK1 protein abundance.

Conclusions: We conclude that JAK mRNA expression and immunoblot for JAK1 protein abundance were restored in lung tissue of preterm lambs treated retinoids during MV.
(2.06 ± 0.87), and RAx antagonist-nasal CPAP (1.74 ± 0.62) groups, compared to the MV group (2.97 ± 0.73).

Conclusions: We conclude that retinoid treatment restored JAK1 expression in the lung of chronically ventilated preterm lambs. We propose that the JAK/STAT signaling pathway is involved in alveolar formation and that retinoid treatment restores the signaling that is dysregulated by MV. (HL62875, HL56401, HD41075, CHRC).

PHOSPHORYLATION OF VASODILATOR STIMULATED PHOSPHOPROTEIN (VASP) IN THE LAMB MODEL OF BPD

Purpose of Study: Ventilator-induced mechanical stretch contributes to the pathogenesis of bronchopulmonary dysplasia (BPD), although the mechanisms are unknown. We recently identified VASP as a unique molecule that rapidly responds to mechanical stretch, regulates remodeling of the actin cytoskeleton, and adapts to mechanical stretch by changing its phosphorylation status. We hypothesized that VASP senses stretch associated with mechanical ventilation in the premature lamb lung, reflected by changing its phosphorylation status.

Methods Used: To evaluate developmental changes in sheep, we compared 4 developmental age groups: fetus 125d gestation [fetal start; FS], fetus 131d gestation [fetal end; FE], term newborn 1d [TN1d], and term newborn 3d [TN3d] (term=147d). To evaluate responses to ventilation mode, we compared 6 groups of ventilated lambs: preterm, mechanical ventilation for 1d [P-MV1d] and 3d [P-MV3d] or nasal CPAP for 1d [P-CPAP1d] and 3d [P-CPAP3d], as well as term lambs for 1d [T-MV1d] and 3d [T-MV3d] (n=4/group). Lung tissue was analyzed by immunoblot and immunohistochemistry. We quantitated VASP phosphorylation by densitometry of each doublet band to calculate phospho-VASP to total-VASP (pVASP/tVASP) ratio. Immunohistochemistry localized tVASP and pVASP.

Summary of Results: The FE group had greater pVASP/tVASP ratio (0.21±0.01; X±SEM) compared to the FS, TN1d, and TN3d groups (0.15±0.02; p<0.01), indicating that VASP is developmentally regulated. Interestingly, the ratio for the P-CPAP1d group was the same as the FE group. In contrast, the P-MV1d and 3d groups had reduced pVASP/tVASP ratio (p=0.05). Surprisingly, the P-CPAP3d group also had reduced pVASP/tVASP ratio (p<0.05). The T-MV1d and 3d groups did not have altered pVASP/tVASP ratio. VASP was localized to airway epithelial cells, vascular smooth muscle cells, and lung parenchymal cells. At the FE stage, pVASP localized mostly in lung parenchymal cells.

Conclusions: Transient phosphorylation of VASP at the late fetal stage indicates active cell remodeling by fetal breathing movement and lung expansion in utero. MV appears to suppress cell remodeling. (HL62875, HL56401, HD41075, CHRC).

BRONCHOPULMONARY MALFORMATIONS: THE UTILITY OF COMPUTED TOMOGRAPHY IMAGING IN DELINEATING PATHOLOGY AND PLANNING SURGICAL RESECTION
R. Taylor1, M. See2, D. Jamieson3, and G.K. Blair1. 1University of British Columbia Faculty of Medicine, Vancouver, BC, Canada; 2University of British Columbia Faculty of Medicine, Vancouver, BC, Canada and 3University of British Columbia Faculty of Medicine, Vancouver, BC, Canada.

Purpose of Study: The purpose of this study was to assess the utility of CT imaging in delineating pathology and planning surgical resections in pediatric patients with various types of bronchopulmonary malformations.

Methods Used: This was a retrospective chart and x-ray study of patients at British Columbia Children’s Hospital who had been diagnosed with a bronchopulmonary malformation. The patients’ initial diagnosis, the nature and analysis of imaging studies, the operative details, and the pathology were tabulated and compared.

Summary of Results: CT imaging accurately delineates anomalous vasculature in pediatric patients with bronchopulmonary malformations.

Conclusions: CT imaging is an important part of the diagnostic workup of bronchopulmonary malformations and essential in the planning of surgery in a pediatric patient. Its role and timing in the pre-operative work up of patients diagnosed with bronchopulmonary malformations will be discussed.

Residents Forum
8:30 AM Saturday, February 2, 2008

CORONARY SPASM AFTER HEART TRANSPLANTATION
B. Itagaki, M. Kawano, M. Hamilton, and J. Kobashigawa. David Geffen School of Medicine at UCLA, Los Angeles, CA.

Purpose of Study: Coronary spasm has been infrequently reported during coronary angiography in heart transplant patients. It is believed that coronary spasm represents coronary endothelial cell dysfunction and may be a marker for the development of cardiac allograft vasculopathy (CAV) and less long-term survival. There are patients who present with a clinical syndrome suggestive of coronary spasm which include angina (re-enervation) and EKG changes. These patients can present without significant angiographic coronary artery lesions.

Methods Used: Between 1991 and 2006 we reviewed 701 heart transplant patients and 2,701 angiograms. Of these we found 41 patients whose angiogram revealed coronary artery spasm. 4 additional patients had normal coronary arteries by angiography but presented with angina and EKG changes consistent with coronary spasm. These patients were treated with nitrates and some with cyproheptadine (4 mg po tid) to prevent recurrent coronary spasm. Patients with CAV were categorized into groups defined as any vessel with mild (>30%), moderate (>50%), or severe (>70%) stenoses.

Summary of Results: There was a total of 45 patients with coronary spasm. The average time from transplant of the documented coronary spasm was 3.8 years. Conditional 5 year survival from the time of documented coronary spasm was 84.4%, which is comparable to historical controls in our program (5 year survival conditional on being alive at 4 years is 87%). 24/45 (53.3%) patients with coronary spasm had concomitant CAV. Of these, 6 patients had mild CAV, 7 moderate CAV, and 11 severe CAV. The 11 patients with severe CAV had a lower 5 year conditional survival compared to historical controls (45.5% vs. 61.1%).

Conclusions: Coronary spasm without concomitant cardiac allograft vasculopathy does not portend poor outcome. However, coronary spasm in the presence of severe cardiac allograft vasculopathy is a marker for lower long-term survival.

THROMBOPHAGOCYTOSIS AND PLATELET SATELLITISM IN RHABDOMYOLYSIS, AN UNUSUAL CASE OF THROMBOCYTOPENIA
D.L. Collins and J. Huang. UCSF Fresno, Fresno, CA.
**Purpose of Study:** Thrombocytopenia is a frequent clinical encounter. One of its common causes is pseud thrombocytopenia from platelet clumping, inadequate sampling, platelet satellitism (PS), or neutrophilic thrombophagocytosis (TP). PS or TP is an unusual phenomenon of unknown etiology reported in septicemia, lymphoma, vasculitis, and healthy individuals. PS was also observed in an EDTA-dependent manner. Although the underlying mechanism remains undefined, it was thought to be associated with platelet conformational changes due to autoantibodies, other non-immunologic mediators, or chelation of calcium ions. Hypocalcemia is commonly observed in early stages of rhabdomyolysis. Similar conformational changes may occur in platelets in hypocalcemia secondary to rhabdomyolysis. We present a case of rhabdomyolysis, hypocalcemia, and thrombocytopenia with PS and TP.

**Methods Used:** A 34 year-old alcoholic man was hospitalized for seizures and rhabdomyolysis. His outpatient medicine was Carbamaze pine. His baseline platelets and total calcium were 188,000/ul, and 9.5mg/dl, respectively. Admission lab values were: CPK 108,000U/L, platelets 66,000/ul, and corrected total calcium 8.1mg/dl. There was no evidence of infection or addition of new medications. During the evaluation of thrombocytopenia, a peripheral blood smear demonstrated TP and PS. By day 6, his platelets were 129,000/ul, corrected calcium was 9.6mg/dl, and CPK was 5900U/L. Repeat peripheral smear was normal. Anti-platelet antibodies were negative. The patient did not experience any bleeding or clotting complications.

**Summary of Results:** N/A.

**Conclusions:** Our patient had no evidence of sepsis, malignancy, or autoimmune disease, and he was previously on carbamazepine with normal platelet counts. His TP and PS coincided with hypocalcemia from rhabdomyolysis. His thrombocytopenia along with TP and PS disappeared with resolution of rhabdomyolysis and normalization of calcium. EDTA was the anticoagulant for sampling in all tests. This unique case underscores the importance of peripheral blood smear in the evaluation of thrombocytopenia. Thrombocytopenia in the setting of hypocalcemia may be spurious, and should prompt the physician to order a blood smear looking for PS or TP. Positive identification will direct focus of therapy on underlying pathology and help avoid unnecessary further testing or treatment.

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**482 TUBEROUS SCLEROSIS COMPLEX IN A PATIENT WITH EPILEPSY, DEVELOPMENTAL DELAY AND NORMAL NEUROIMAGING**

D. Gano. UBC, Vancouver, BC, Canada.

**Purpose of Study:** Tuberous sclerosis complex (TSC) is a genetic neurocutaneous syndrome that affects multiple organ systems. Characteristic neuroimaging findings are found in those with significant neurological disease; this patient highlights an exception.

**Methods Used:** The methodology is a case report.

**Summary of Results:** The patient presented with intractable epilepsy in the first month of life. MRI and CT scan of the brain were normal. EEG demonstrated focality. At age one she developed hypopigmented macules, prompting evaluation for TSC. Echocardiography showed rhabdomyomas. Investigations were otherwise negative. Medically refractory epilepsy persisted. Her development was globally delayed. MRI scans of the brain obtained yearly from ages 1–4 were normal. Suspicion for TSC remained high. Genetic testing, once available, confirmed the diagnosis.

**Conclusions:** Abnormal neuroimaging in TSC patients with epilepsy and delay is paradigmatic. This case contributes to the TSC spectrum. If suspected when clinical criteria are not satisfied, genetic studies should ensue.

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**483 THE EXTREME SCREENING**

S. Kumat and L. Shah. University of Nevada School of Medicine, Las Vegas, NV.

**Purpose of Study:** Newborn screening is important to identify potentially treatable inborn errors of metabolism. We report a fatal case of a congenital metabolic disorder.

**Methods Used:** A 3 month old infant presented with a 1-week history of decreased feeding, irritability, diarrhea and abdominal distention. Despite changing to soy based formula, non bloody diarrhea persisted. His birth and past history was uneventful. He was afebrile, irritable but consolable with tachycardia and jaundice. His anterior fontanel was flat and soft. His abdomen was soft, distended non-tender with a palpable liver and presence of moderate ascites including scrotal swelling. He has strong peripheral pulses and the neurological exam was normal. The patient was admitted for potential GI or renal causes of his diarrhea and dehydration. CT of his abdomen confirmed large ascites. Serum chemistries showed hyperkalemia hypophloremic acidosis with liver function test of AST 150. ALT 49, alkaline phosphates 156, total protein 3.7, albumin 1.8, total bilirunin 2.68 with direct 1.08. Urine was positive for blood and protein. An initial blood gas confirmed metabolic acidosis with respiratory compensation. Within 24 hours he became hemodynamically unstable with hypotension, poor perfusion persistent tachycardia and severe metabolic acidosis. He received multiple blood products, albumin, and received dopamine and epinephrine. His stools were guaiac positive and a coagulation panel showed a PT 67.4, INR 2, PTT 197, fibrinogen 80 and D-dimer 1.3. Despite extensive resuscitative efforts, the patient continued to deteriorate. At the time of his death, he had a progressive lactic acidosis up to 27. Eventually his blood culture became negative and he was diagnosed with Tyrosinemia type I. Conclusions: The diagnosis of Tyrosinemia should be considered in patients with diarrhea, abdominal distension, ascites, hyperbilirubinemia, hyperbilirubinemia, coagulopathy and metabolic acidosis. Diagnostic evaluation including reviewing the results of newborn screening can aid in the diagnosis of patients and allow for appropriate intervention.

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**484 MYSTERIES OF SHOCK**

S. Kumat and L. Shah. University of Nevada School of Medicine, Las Vegas, NV.

**Purpose of Study:** Clinicians should be aware of the constellation of symptoms caused by Toxic Shock Syndrome (TSS). We present two cases of patients with TSS whose illness began with generalized symptoms before the classic signs and symptoms were evident.

**Methods Used:** Case 1: An 8 year old male had a 2-day history of high fever and dizziness. Vital signs showed temperature 106.4F, HR 118, respirations 32, BP 78/50. Physical exam revealed pharyngeal and conjunctival injection, dry mucous membranes, cervical lymphadenopathy, a fine-follicular hyperemic blanching rash on the trunk and extremities, and a small well healed scar and a tender fluctuant area around the left patella. CBC revealed WBC 15.6 with 73 PMNs, 23 Bands. Urinalysis was positive for blood and protein. Chemistry panel showed sodium 129, glucose 206, BUN 60, creatinine of 1.5, AST 265, ALT 124, total bilirubin 2.7. Despite IV hydration and antibiotics, he became hypotensive requiring pressor support.

Case 2: A 13 year Caucasian girl presented with abdominal pain, profuse vomiting, diarrhea, high fever, decreased urine output, dizziness and a new rash. Vital signs showed a temperature of 104F, HR 145, Respirations 28, and BP 82/43 mmHg. Physical exam revealed a nonpurulent conjunctival and oropharyngeal injection, cervical lymphadenopathy, and a truncal
macular erythematous rash. The last menstrual period was 3 weeks ago with no tampon use and she was not sexually active. Laboratory evaluation revealed: WBC 30,000 with 80% neutrophils and 16% bands, hematocrit 35, platelet 216, normal LFT’s, BUN 29 and Creatinine 3.6. Urinalysis and abdominal CT scan were normal.

Summary of Results: In Case 1, radiographs of the left knee revealed a foreign body at the patella. Culture and gram stain showed Methicillin Sensitive Staphylococcal Aureus. After foreign body removal, the patient immediately improved on IV antibiotics. In Case 2, blood, throat, urine and vaginal cultures were negative, but stool cultures grew Staphylococcal Aureus. She was treated with aggressive fluid replacement and IV antibiotics. Her symptoms resolved and she was discharged home in stable condition.

Conclusions: The diagnosis of Toxic Shock Syndrome should be suspected when faced with a febrile, hypotensive patient who has a diffuse erythematous rash. Foreign bodies should be thought of as a cause for Toxic Shock Syndrome, especially if the patient fails to improve with initial treatment.

LAPAROSCOPIC RETRIEVAL OF TRICHOBEZOAR: CASE REPORT AND REVIEW OF THE LITERATURE


Purpose of Study: Trichobezoars, the accumulation of ingested hair in the form of masses or concretions, are a rare cause of gastric outlet or small bowel obstruction typically presenting in children and adolescents. They are associated with trichotillomania, an impulse-control disorder characterized by hair pulling behavior and hair eating, trichophagia.

Methods Used: We illustrate the case of a 10 year-old female who presented to an outside hospital with a two day history of multiple episodes of emesis and abdominal pain. A CT of the abdomen showed two 2 x 6 cm foreign bodies resembling hairballs. The patient was transferred to our ED for further workup and management. The patient underwent an esophagogastroduodenoscopy (EGD) with removal of one bezoar. The other was too large for retrieval via EGD.

Summary of Results: Therefore the patient was taken to the operating room for diagnostic laparoscopy and laparoscopic retrieval. Access to the stomach contents was obtained via a small incision in the antrum, exposing the lumen of the stomach until the level of the pylorus. The remaining bezoar was removed and the rest of the stomach and proximal duodenum were explored. A follow-up upper GI series failed to reveal any further defects and the patient is currently recovering bowel function.

Conclusions: This case illustrates an uncommon presentation of a patient with obstructive symptoms. Traditional management includes endoscopic and open surgical techniques. When endoscopic techniques have been exhausted and a foreign body remains, laparoscopy should be performed, as it is both effective and less invasive than traditional open exploration.

SEROSTATUS OF INFANTS BORN TO HUMAN IMMUNODEFICIENCY VIRUS POSITIVE MOTHERS IN SOUTHERN NEVADA: OUTCOME OF AN ENHANCED MATERNAL-CHILD INTERVENTION PROGRAM 2005–2007

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Purpose of Study: In 2004, the Center for Disease Control and Prevention (CDC) identified Nevada as the only state west of Mississippi with an elevated number of women of childbearing age (15 – 44 years) diagnosed with HIV infection. Although the state has the nation’s 35th largest population, it ranks 14th in the nation for the rate of people living with HIV/AIDS. After years without documented perinatal HIV transmission, the state witnessed its first case in 2004. In 2005, an enhanced maternal-child HIV program was initiated to provide a comprehensive approach to eliminate perinatal HIV transmission. The objective of this study is to report on the outcome of an enhanced intervention through a comprehensive approach to eliminate perinatal HIV transmission.

Methods Used: This is a retrospective review of data collected from the enhanced maternal-child HIV program of births by HIV+ mothers from September 2005 through September 2007. All infants were followed to 24 months of age. HIV DNA PCR was performed at birth, 1 month, and 4 months on all infants. Infants were considered HIV negative if DNA PCR obtained at 1 month and 4 months were negative. Infants younger than 4 months of age with one negative DNA PCR are considered indeterminate.

Summary of Results: Rapid HIV tests were performed on 3181 pregnant women presenting in labor with unknown HIV serostatus and 0.13% was confirmed reactive. Thirty-four infants were born during the study period to HIV+ mothers. Final results of HIV infection status were available for 79% of infants. Fifty-nine percent were males and 41% were females. Fifty-five percent were African American/Black, and 37% were Hispanics. The status of 7 infants was indeterminate since they are less than 4 months of age. The overall infant infection rate for the two years was 7.4%. During the same period, 2 additional cases were diagnosed not previously identified in older children.

Conclusions: A comprehensive program provides enhanced communication between obstetric and pediatric providers and helps eliminate missed opportunities for prevention of perinatal HIV infection. It also provides an improved care for HIV+ pregnant women and outcomes for HIV exposed infants.
proportion of families that received comprehensive education on the specific disorder diagnosed in their children.

Summary of Results: Sixty three cases of primary immunodeficiency disorder were identified. Seventy-one percent (45/63) were among children over 2 years, 16% were children aged 13 – 24 months, and 11% (7/63) of affected children were less than 12 months. Sixty-four percent (40/63) were males and 38% (23/63) were females. Hypogammaglobulinemia was the most identified disorder occurring in 19% (12/63) of patients followed by DiGeorge Syndrome at 18% (11/63). Ninety-eight percent (62/63) of families received comprehensive education on the specific disorder. Fifty-nine percent of patients were seen at least twice annually following diagnosis.

Conclusions: Understanding the spectrum of immunodeficiency disorder and needs of families with these disorders in Southern Nevada will enable us to develop a comprehensive program that will meet the needs of these families.

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DELAYED DIAGNOSIS AND TREATMENT OF PERINATALLY ACQUIRED HUMAN IMMUNODEFICIENCY VIRUS INFECTION
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Purpose of Study: Children can acquire human immunodeficiency virus (HIV) from an infected mother during pregnancy, labor, and postnatally through breast milk. The technology currently available is capable of identifying infants exposed perinatally to HIV within 4 months of age. Early identification of infants who acquired HIV perinatally has been associated with reductions in morbidity and mortality. This study examined cases of perinatal HIV infections where delayed diagnosis occurred in our comprehensive maternal-child HIV program.

Methods Used: This is a retrospective review of data collected from an enhanced maternal-child HIV intervention program. All cases of birth to HIV+ mothers were reviewed in detail. The assessment of perinatally acquired HIV infection diagnosed from September 2005 through September 2007 was performed to identify reasons for delay in diagnosis.

Summary of Results: Delayed diagnosis occurred in 85.7% (6/7) of cases with perinatal HIV, which was confirmed during our review period. Fifty percent of these delays occurred due to unidentified risk at the time of birth, 17% of delays occurred due to a lack of follow up after a requested HIV test in 17% (1/6), and 33% (2/6) of delays were due to a lack of adequate follow up after birth. In 100% of cases where delay occurred, the mother had inadequate prenatal care. In 100% of cases where delay was caused by an unidentified risk at birth and lack of follow up test result, the hospital did not have a program that offered rapid HIV testing at point of delivery.

Conclusions: An enhanced communication between obstetric and pediatric providers within a comprehensive, multi-disciplinary program is necessary. This program will mandate that mandates documentation of maternal HIV serostatus at the time of delivery in order is needed to improve the outcome for HIV exposed infants. The availability of rapid HIV testing in labor and delivery will help to eliminate missed opportunities in identifying infants at risk and determines an early diagnosis.

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EVALUATION, TREATMENT, AND FOLLOW UP OF INFANTS WITH PRESUMED CONGENITAL SYPHILIS

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Purpose of Study: Untreated syphilis among pregnant women may result in spontaneous abortions, stillbirth or an infected infant. Congenital syphilis remains a sentinel public health event. Prevention relies on screening of pregnant women, adequate treatment of infected pregnant women, appropriate evaluation, treatment, and a follow up of infants born to mothers with untreated syphilis. The rate of syphilis among women in Nevada increased from 0.3% to 2.1% between 2003 and 2005. The objective of this study is to determine the adequacy of evaluation, treatment, and follow up of infants born to mothers diagnosed with syphilis in Las Vegas hospitals.

Methods Used: A retrospectively reviews of all deliveries from January 2000 through December 2006 was conducted in Las Vegas. We performed a detailed chart review of all infant-mother pairs where reactive maternal serological test for syphilis was documented. Data was abstracted from on demographics, adequacy of evaluation, treatment, and follow up.

Summary of Results: During the review period, 73,373 live births occurred. Reactive serological test for syphilis was documented in 0.09% (63/73733) pregnant women. Of these women, 60.9% had prenatal care. Adequate treatment of pregnant mothers was documented in 62.5%. The ethnicity of the mothers included: 33.3% of were African-American, 31.7% were Hispanic, 21.7% were Non Hispanic White, and 11.3% were documented as other. Less than a quarter of the mothers documented illicit drug-use (17.2%). In 2.1% of infants, RPR was not documented at birth. Long bone evaluation was done on 21.9% of infants. The data gave evidence for an adequate follow up.

Conclusions: Despite rising incidence of syphilis among women, infants born to pregnant women with reactive serological test for syphilis were inadequately evaluated, treated and followed up. We recommend an integrated perinatal prevention initiative that will integrate management of syphilis in pregnant women and their infants into an existing program for HIV and hepatitis B.

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FEASIBILITY AND ACCEPTABILITY OF ROUTINE HUMAN IMMUNODEFICIENCY VIRUS SCREENING: EXPERIENCE IN AN URBAN OUTPATIENT SETTING
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Purpose of Study: The introduction of highly active antiretroviral therapy in 1995 led to dramatic improved survival among individuals with HIV infection. Despite the maximal benefit of therapy that occurs when it is initiated early in the course of the disease, approximately 25% of those with HIV are unaware of their diagnosis. In 2006, the Center for Disease Control and Prevention recommended routine HIV screening in all health care setting for all patients aged 13–64 years. Healthcare providers were concerned about disruption of normal office flow with this recommendation. The purpose of our study was to determine the feasibility and acceptability of this recommendation in an urban outpatient setting.

Methods Used: From March to July 2007, patients receiving care at the LIED ambulatory care clinic were informed that HIV testing will be part of their annual health maintenance testing unless they opt out. The
participants blood sample were obtained, patient charts were reviewed by an investigator, and a clinic visit was conducted to abstract data on demographics and self reported risk factors.

**Summary of Results:** There were 742 patients who received care during the study period. The mean age was 49.9 years. There were 48% males and 52% females. The overall acceptance rate was 43%. No significant difference among males and females who accepted testing (41% vs 44%). No significant difference among race/ethnic groups who accepted testing (whites 41%, Blacks 41%, Asian/Pacific Islanders 53%, Non white Hispanic 47%). No significant difference in insurance type (44% insured versus 41.9% uninsured). Only 10% had self reported risk factor documented in the chart with the most common being heterosexual intravenous drug user.

**Conclusions:** Routine HIV screening is both feasible and acceptable in an urban clinic without interruption of the clinic flow. An on site phlebotomist improves the rate of completing the testing among those who accepted testing. Further understanding of the reason why people opted out is important to overcome the barriers to testing.
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