1 INTRAUTERINE GROWTH RESTRICTION BY UTEROPLACENTAL INSUFFICIENCY AND MATERNAL TOBACCO SMOKING EXPOSURE CAUSES GENDER-SPECIFIC CHANGES IN mRNA EXPRESSION OF HIPPOCAMPAL ESTROGEN RECEPTOR


Purpose of Study: 2 major causes for intrauterine growth restriction (IUGR) in the United States are uteroplacental insufficiency (UPI) and maternal tobacco smoking exposure (MTSE). Both UPI and MTSE impacts neurodevelopmental outcomes in offspring born with IUGR, particularly in males. A common characteristic of IUGR animal models is increased hippocampal apoptosis. Neuronal apoptosis is modulated by estrogen signaling - estrogen is anti-apoptotic in the hippocampus. Estrogen mediates its effects on hippocampal apoptosis through estrogen receptors (ER), which are encoded by the genes esr1 and esr2. Currently, little is known about the effects of IUGR by either UPI or MTSE on hippocampal expression of ERS.

Methods Used: IUGR was induced by 2 methods in 2 separate experimental groups: (1) uterine ligation in pregnant rats at day 19 of gestation to mimic UPI, and (2) exposure of pregnant rats to tobacco smoke from day 11 of gestation to term to mimic MTSE. Both groups underwent caesarean section at term. The pups were killed, hippocampi were dissected and RNA extracted. Reverse transcriptase real-time PCR was performed to assay expression of esr1 and esr2.

Summary of Results: UPI decreased esr1 (51% reduction, p=0.01) and esr2 (55% reduction, p=0.02) expression in male pups compared to controls. UPI did not alter esr1 or esr2 expression in female pups. In contrast, MTSE increased esr2 expression (123% increase, p=0.05) in female pups compared to controls, but no changes were seen in males. MTSE did not alter esr1 expression in either gender.

Conclusions: We conclude that the consequence of UPI and MTSE on hippocampal ER mRNA expression is different, despite both UPI and MTSE causing IUGR. The mechanisms behind MTSE induced IUGR is unknown, but the impact is likely through MTSE induced fetal exposure to UPI, nicotine, and/or some other compounds. Given the different observed effects of MTSE and UPI on hippocampal ER gene expression, we speculate that mechanisms through which MTSE affects ER expression occur through exposure to nicotine and/or some other compound.

2 MECHANICAL VENTILATION OF PRETERM LAMBS LEADS TO MORE APOPTOSIS AND LESS PROLIFERATION OF NEURONS AND GLIA

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Purpose of Study: Prolonged mechanical ventilation (MV) of the premature human neonate is associated with lung injury. Frequently, the brain also is injured. Our studies, using chronically ventilated preterm lambs, indicate that MV changes histone marks in the brain. These changes could affect regulation of cell processes, such as apoptosis and proliferation of neurons and glia. The present studies purpose was to test this possibility. We hypothesized that prolonged MV increases apoptosis and decreases proliferation among neurons and glia in the brain of preterm lambs.

Methods Used: Preterm (PT) lambs, treated with antenatal steroids and postnatal surfactant, were managed by MV or high-frequency nasal ventilation (HFNV; akin to bubble nasal CPAP) for either 3d or 21d. We use HFNV as the positive gold-standard for alveolar formation in the lung. At the end of 3d or 21d of ventilation, cortical brain tissue from the temporal lobe was fixed and analyzed by double immunofluorescence to colocalize cleaved caspase 3 or PCNA within neurons (neuronal marker-positive), immature oligodendrocytes (O1-positive), or reactive astrocytes (GFAP-positive).

Summary of Results: Neurons in gray matter had significantly (p<0.05) more colocalization of cleaved caspase 3 in the MV group compared to the HFNV group at 3d (22±3* vs 7±3/mm3) and at 21d (22±3* vs 11±4*). Conversely, neurons had significantly less colocalization of PCNA in the MV group compared to the HFNV group at 3d (17±3* vs 23±7) and at 21d (27±7* vs 16±1). Among glial cell types, the only colocalization difference was for immature oligodendrocytes with cleaved caspase 3 at 21d. Significantly more for immature oligodendrocytes were apoptotic in the MV group compared to the HFNV group (29±3* vs 16±1).

Conclusions: We conclude that MV for 3d or 21d is associated with a shift in balance between cell death and proliferation in the brain. The shift is toward more apoptosis and less proliferation of neurons and immature oligodendrocytes. These results are consistent with less brain-derived neurotrophic factor in the same MV preterm lambs compared to We speculate that imbalance results from epigenetic alterations in chromatin structure of genes that regulate apoptosis and proliferation. (HL62875, HL56401, HD41075)

3 IDENTIFICATION OF A NOVEL BIOMARKER THAT DISCRIMINATES HEART FAILURE ETIOLOGY


Purpose of Study: Despite improvements in the diagnosis and treatment of CHF, these patients remain at high risk of cardiac mortality and ongoing morbidity. Presently, the clinician’s ability to diagnose the etiology, determine prognosis or titrate therapy are limited. We sought to identify circulating biomarkers that might address these limitations.

Methods Used: We analyzed plasma samples collected from 60 CHF subjects through the Ahmanson-UCLA Cardiomyopathy Center and 44 normal subjects. Non-abundant plasma proteins were enriched by depletion of an antibody-affinity column. Masses of the eluted plasma proteins were determined by high-resolution MALDI-TOF mass spectroscopy.

Summary of Results: 794 proteins were detected within the plasma samples by MALDI TOF-MS. 34 proteins were significantly different between the CHF patients and normal patients, using independent t-tests with a P<0.05 and adjusted for multiple comparisons using the Bonferroni Correction. Significant proteins were then compared to a panel of clinical variables (etiology, New York Heart Association (NYHA) classification, left ventricular ejection fraction (LVEF), and outcome to identify potentially important biomarkers. This analysis demonstrated that the levels of 19 proteins correlated with etiology, 15 with LVEF, 2 with NYHA, and 2 with outcome. Three of the unknown proteins were tentatively identified based on accurate mass measurements (less than 0.5 Da error) as isoforms of APO C-III. To further study APO C-III, we performed ELISA assays on 72 independent subjects. The study population included 12 aged-matched normal subjects and 60 CHF subjects, 30 dilated and 30 ischemic, all of them male, to measure circulating apolipoprotein C-III levels in plasma. APO-C-III levels were elevated in subjects with dilated CHF compared to ischemic etiology and normals (P<0.00001). APO-C-III levels remained significantly different (P=0.00002) after a multivariate analysis was done to account for total cholesterol, total triglycerides and age.
We have used a novel, high through-put method to identify diet with rapamycin or placebo for 16 weeks. Rapamycin is a potent and specific inhibitor of mTOR, a downstream effector of the mammalian target of rapamycin (mTOR), which is a key regulator of cell growth and metabolism. In this study, we administered rapamycin in the diet of mice and observed changes in various parameters associated with metabolic health. Our results suggest that rapamycin and/or its analogs have the potential for development as anti-obesity therapies.

Methods Used:
C57BL/6J mice were placed on a high fat, high carbohydrate diet and fed rapamycin or placebo for 16 weeks. Rapa-mycin was administered in the diet at a constant dose. Body composition analysis revealed decreased body fat mass in the drug-treated group. Rapamycin-fed animals showed a significant decrease in body weight compared to placebo-fed animals. Plasma and hepatic triglyceride levels were reduced and morphologic analysis of the liver revealed decreased fat accumulation in hepatocytes. Gene expression analysis of adipose tissue revealed decreased expression of adipocyte-specific markers. These results suggest that rapamycin and/or its analogs have the potential for development as anti-obesity therapies.

Conclusions: These results suggest that rapamycin and/or its analogs have the potential for development as anti-obesity therapies. Further studies are needed to confirm these findings in larger study populations.

5 BODY MASS INDEX AND ACUTE INJURY RISK IN CHILDREN AGE 5-17 YEARS

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Purpose of Study: Obesity is often associated with insulin resistance, which may promote and increase the risk of cardiovascular disease. However, the exact molecular mechanisms by which obesity leads to insulin resistance are poorly understood. A hallmark of visceral obesity is the accumulation of adipose tissue macrophages which can contribute to insulin resistance. Aberrant, chronic activation of the intracellular nutrient sensing protein, mammalian target of rapamycin (mTOR), a downstream effector of the insulin signaling pathway, has been implicated in the pathogenesis of insulin resistance. We hypothesized that oral administration of rapamycin, a potent and specific inhibitor of mTOR, could reduce insulin resistance and improve adipose tissue macrophage accumulation.

Methods Used: C57BL/6J mice were placed on a high fat, high carbohydrate diet and ran on a wheel for 16 weeks. Rapa-mycin was administered in the diet at a constant dose. Body composition analysis revealed decreased body fat mass in the drug-treated group. Rapamycin-fed animals showed decreased body weight gain compared to placebo-fed animals. Plasma and hepatic triglyceride levels were reduced and morphologic analysis of the liver revealed decreased fat accumulation in hepatocytes. Gene expression analysis of adipose tissue revealed decreased expression of adipocyte-specific markers. These results suggest that rapamycin and/or its analogs have the potential for development as anti-obesity therapies.

Conclusions: These results suggest that rapamycin and/or its analogs have the potential for development as anti-obesity therapies. Further studies are needed to confirm these findings in larger study populations.

6 EXAMINATION OF VERBAL INTELLIGENCE AND PHONOLOGICAL PROCESSING IN CHILDREN WITH AUTISM SPECTRUM DISORDERS AND THEIR FAMILY MEMBERS

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Purpose of Study: Autism Spectrum Disorders (ASD) have a strong genetic influence, and a broader autism phenotype (BAP) has been observed in first-degree family members. It is well known that children with autism have an array of communication deficits, including phonology, semantics, syntax, and pragmatics. The overall aim of this project was to better understand verbal language use in individuals with ASD and their family members.

Methods Used: A total of 279 family members from families with 2 or more ASD children participated. Verbal intelligence quotients (VIQ), a measure of vocabulary and reasoning, were assessed with age-appropriate Wechsler scales. Non-word repetition (NWR), a measure of phonological processing, was assessed with the CTOPP.

Summary of Results: As expected, ASD children exhibited lower VIQ compared to mothers, fathers, and unaffected siblings (F(3,274) = 59.8, p < .01). To examine decreased VIQ in the context of overall cognitive function, discrepancy scores were calculated between verbal and non-verbal IQ. ASD children’s IQ discrepancy scores were significantly greater than relatives (F(3,274) = 19.8, p < .01). Both our higher-functioning (HF) and lower-functioning (LF) ASD groups exhibited a mean discrepancy pattern that suggested better non-verbal IQ compared to VIQ (p < .05). For phonological processing, mean performance for all groups was significantly below the norm on the NWR task, however siblings performed relatively better than ASD children and fathers (F(3,256) = 2.3, p < .05). Investigation of the relationship between VIQ and NWR revealed significantly higher ratio scores in ASD children and siblings compared to parents (F(3,253)=11.976, p < .01).

Conclusions: These results suggest that relatively poorer phonological processing may be an endophenotype of ASD and a part of the BAP. The ratio of the phonological processing to VIQ suggests that the relation between these two domains changes with age, but not affected status. Taken together, these data suggest that multiple aspects of language processing contribute to both autism and the broader phenotype and will be important quantitative traits for genetic analyses.

7 INCIDENCE, ETIOLOGY, AND SURVIVAL TRENDS FROM SUDDEN CARDIAC ARREST IN CHILDREN AND YOUNG ADULTS AGE 0-35 IN KING COUNTY, WASHINGTON: A 30-YEAR REVIEW

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Purpose of Study: Sudden cardiac arrest is a leading cause of death in children and young adults, although little is known regarding the epidemiology
of such events. This study determined the incidence, etiology, and outcomes of out-of-hospital cardiac arrest (OHCA) in individuals age 0–35.

Methods Used: This was a retrospective cohort study of OHCA in children and young adults from January 1980 through December 2009 in King County, Washington. Cases of OHCA were identified from the King County Public Health Division of Emergency Medical Services (EMS) Cardiac Arrest Database, an ongoing registry of all cases of OHCA involving an EMS response. Incidence rates were calculated using population census data, and the etiology of cardiac arrest determined by review of autopsy reports, death certificates, hospital and other available records.

Summary of Results: A total of 361 cases (26 cases age 0–2, 30 cases age 3–13, 60 cases age 14–24, and 245 cases age 25–35) of OHCA occurred, with an overall incidence of 2.28 per 100,000 person-years (2.1 in age 0–2, 0.61 in age 3–13, 1.44 in age 14–24, and 4.40 in age 25–35). The most common etiologies of OHCA were congenital abnormalities (84.0%) in age 0–2, hypertrophic cardiomyopathy (17.9%) in age 3–13, presumed primary arrhythmia (23.5%) in age 14–24, and coronary artery disease (42.9%) in age 25–35. The overall survival rate was 26.9% (3.8% in age 0–2, 40.0% in age 3–13, 36.7% in age 14–24, and 27.8% in age 25–35). Survival increased significantly throughout the study time period from 13.0% in 1980–1989 to 40.2% in 2000–2009 (p < 0.001).

Conclusions: The incidence of OHCA in children and young adults is substantially higher than previously reported. This study details the various cardiac disorders leading to OHCA in the young, allowing an increased focus on age related risk factors and development of effective screening models for prevention. This study demonstrates an important increase in survival, providing compelling support of contemporary resuscitation protocols for OHCA in the young.

8 PEDIATRIC MALARIA TREATMENT DELAYS ACROSS SOCIOECONOMIC LEVELS IN SUBURBAN ACCRA, GHANA

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Purpose of Study: The study reported here examines how a Ghanaian family’s socioeconomic status (SES) affects the delay-time before a child with Plasmodium falciparum malaria is brought to a government hospital. It was hypothesized that a delay of appropriate clinical treatment results from a family’s apprehension of inability to pay the hospital fee.

Methods Used: In this ethnographic study, parents of pediatric patients with blood smear-confirmed malaria (N=15) were interviewed through both quantitative questionnaires and narratives at the Legon Hospital Pediatrics Ward. The questionnaires ascertained the dependent variable (time lag during which the child languished at home), as well as the independent variable of SES, measured by: 1) monthly income range, 2) health insurance, and 3) years of household education. Over the course of four months, 170 hours of dialogue and observation supplemented the numerical data.

Summary of Results: There was actually no statistical association between delay-time and family financial standing, nor a correlation between delay-time and enrollment in Ghana’s recently-established National Health Insurance Scheme. Parents of all backgrounds postpone clinical treatment even after the onset of clear malaria symptoms in order to conserve their time; the waiting lines at hospitals are often several hours long and the illness is endemic in West Africa. Yet, this study does confirm that lower-SES households less effectively prevent malaria. Only half of families in the lowest income group utilized insecticide-treated mosquito nets, while all six families in the highest income group use the precaution.

Conclusions: In this township, the study suggests that low-SES families do not disproportionately delay clinical treatment. Especially when raising multiple children, parents of all SES groups delay at home and self-treat with herbal teas or street-bought (and often counterfeit) chloroquine and artesunate anti-malarials. This has its risks. Delayed clinical care and the rapid deterioration of originally-uncomplicated P. falciparum cases contribute to 20,000 pediatric malaria fatalities in Ghana annually.

9 TISSUE SPECIFIC ROLE OF DNA Methyltransferase 4 DURING ZEBRAFISH DEVELOPMENT

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Purpose of Study: DNA methylation is a heritable epigenetic marker. The mechanism by which DNA methylation and gene expression are regulated during embryonic development is an area of active research. The zebrafish presents a unique model which is well suited to investigate mechanisms of normal embryonic development because of its easily observed rapid embryonic development and availability of tools to manipulate gene expression. Recent research has started to establish tissue specific roles for the various DNA methyltransferases (DNMTs) during development. These studies have also shed some light on the basic mechanisms by which DNMTs regulate expression of targeted genes. DNMT3 is one of the three groups of DNMTs found in humans and it has six known zebrafish orthologs: Dnmt3/4/5/6/7/8.

The focus of this project was to investigate the role of zebrafish dnm4 in normal development. Whole mount in situ hybridization assays revealed that dnm4 is robustly expressed in hematopoietic compartments as well as the site of hematopoietic stem cell generation. We hypothesized that dnm4 controls zebrafish hematopoiesis by regulating the transcription of hematopoietic genes.

Methods Used: We took a candidate approach and investigated whether the knockdown of dnm4 caused transcriptional up regulation of aid, mbd4, and gadd45a. These targets were chosen based on recent findings both in published studies and our lab’s unpublished work that indicates they play a role in hematopoiesis. Antisense morpholino knockdown technology was used to create zebrafish embryos with substantially decreased dnm4 expression. RNA was isolated from whole fish samples and then cdNA was created. RT-PCR was conducted to test the relative expression normalized to 28s of the target genes.

Summary of Results: Expression of our target genes was found to be two to three times higher in dnm4 morphants than in controls.

Conclusions: Taken together these data provide evidence that dnm4 plays a role in hematopoietic differentiation and establishes a possible tissue specific role for dnm4 during embryonic development. Future studies will be aimed at further characterizing the hematopoietic phenotype of dnm4 morphants and verifying that changes in gene expression correlate with tissue specific changes in DNA methylation of target genes.

10 INTRADILI17β (E2) ATTENUATES THE GNRH MEDIATED INCREASE IN INTRACELLULAR Ca2+

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Purpose of Study: Controlling the ovulatory LH surge underlies fertility regulation. The LH surge is initiated by E2 actions at the hypothalamus and the pituitary to increase GnRH receptor expression on gonadotropes - the LH producing cells. Interestingly, however, E2 acutely but transiently suppresses GnRH mediated LH release thus increasing pituitary content of LH just prior to the surge. We hypothesized that E2 acutely attenuates the GnRH elicited increase in intracellular Ca2+ - the cellular event underlying GnRH mediated LH secretion.

Methods Used: We established cultures of pituitary cells from transgenic mice in which expression of a fluorescent protein (YFP) is confined to gonadotropes. The distinct excitation spectra of YFP and the Ca2+ indicator FURA allowed for selection of gonadotropes and subsequent imaging of GnRH induced changes in intracellular Ca2+. After 18 hr in culture, cells were “loaded” with FURA for 1 hr. Cells were then incubated in Ca2+ containing or Ca2+ free media and received 100mM E2 or vehicle followed by a 2nM GnRH pulse at 2 and 12 min post-E2. Increases in intracellular Ca2+ were determined by a change in FURA emission spectra. Both amplitude (peak height) and magnitude (area under curve) of the Ca2+ response in 136 cells was analyzed 1 min post-GnRH.

Summary of Results: Approximately 93% of YFP positive cells displayed a Ca2+ response following the initial GnRH pulse. Of these, 84% displayed a secondary Ca2+ response; however, in both Ca2+ and Ca2+ free conditions, the magnitude and amplitude of the secondary response was reduced (p<0.05) as compared to the initial response. E2 did not alter the percentage of cells responding to either GnRH pulse. However, in both Ca2+ and Ca2+ free conditions, E2 further reduced (p<0.05) the ratio of secondary versus primary response magnitude by 25% and 31%, respectively.

Conclusions: Consistent with our hypothesis, E2 attenuates the GnRH induced Ca2+ response in gonadotropes - a potential explanation for the acute block of GnRH mediated LH release by E2 prior to the ovulatory LH surge. The rapidity of the inhibitory effect of E2 suggests a non-nuclear mechanism.
of action. If correct, new generations of E2 agonists/antagonists may allow for fertility regulation without the myriad physiological effects resulting from nuclear actions of E2.

11 WILSON DISEASE: ABNORMAL METHIONINE METABOLISM IN PATHOGENESIS OF LIVER DAMAGE
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Purpose of Study: Wilson disease (WD) is a recessive disorder of copper accumulation with established genetic background but unclear pathogenesis. Aberrant hepatic methionine metabolism plays a role in the pathogenesis of liver injury by regulating homocysteine (Hcy) and S-adenosylhomocysteine (SAH), which induce endoplasmic reticulum (ER) stress and subsequent induction of lipogenic pathways. We hypothesized that abnormal methionine metabolism is involved in the pathogenesis of hepatic steatosis in WD.

Methods Used: Liver samples were collected from 195 WD patients and age-matched controls. Hepatic expression of the key enzymes involved in methionine metabolism, including SAHH, was analyzed by qRT-PCR.

Summary of Results: Reduction in SAHH expression was associated with increased hepatic triglyceride levels and higher cytoplasmic lipid droplets in hepatocytes compared to the controls. SAHH mRNA expression, protein levels, and activity were significantly lower in the WD samples than in the controls, which were significantly higher in the WD samples.

Conclusions: Reduced hepatic SAHH expression was associated with increased SAH and subsequently with increased plasma Hcy as well as the activation of ER stress, apoptosis, and lipogenesis markers.

12 VITAMIN D INHIBITS PROLIFERATION AND INDUCES DIFFERENTIATION OF C2C12 SKELETAL MYOBLAST CELLS BY MODULATING THE EXPRESSION OF PRO-MYOGENIC AND ANGIOGENIC GROWTH FACTORS
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Purpose of Study: Vitamin D (VD) is widely recognized for its regulation of calcium and phosphate homeostasis in relation to bone development and maintenance and for its synergistic effects on target organs such as intestines, kidneys and PTH glands. It has been shown to improve muscle performance and reduce falls in VD deficient older adults. It is known to have age-related loss of muscle mass and strength and an increased rate of falls.

Methods Used: C2C12 myoblasts were treated with or without 1,25D in a time course manner. VDR expression was analyzed by Immunofluorescence (IF), Real Time PCR (qRT-PCR) and Western Blots. Expression of muscle lineage and proliferation markers was assessed by immunocytochemistry (ICC) and western blots. Expression of pro-myogenic markers and angiogenic growth factors was analyzed by qRT-PCR arrays and confirmed by qRT-PCR.

Summary of Results: Addition of 1,25D to C2C12 myoblasts induces: a) decrease expression of MyoD and myogenin (early and intermediate myogenic markers respectively); b) increase expression of IGF-II (fibroblast growth factor 2; FST ( follistatin-alpha myostatin inhibitor)), IGF-1 binding site found on the mannose 6-phosphate receptor for heparan sulfate. Enzyme replacement therapy (ERT) has been used to treat lysosomal storage diseases but has not been successful for MPS IIIB. In particular, ERT for MPS IIIB has been limited by inadequate cellular uptake of recombinantly produced human NAGLU enzyme. In this study, we create, characterize, and test a novel enzyme: NAGLU fused to the insulin-like growth factor 2 (IGF-II) peptide (rhNAGLU-IGF-II). The IGF-2 motif was selected to enable recombinant NAGLU to enter cells via the insulin-like growth factor 2 binding site found on the mannose 6-phosphate receptor for high affinity delivery to lysosomes.
Methods Used: Following the molecular cloning of the recombinant NAGLU-IGF-II construct, rhNAGLU-IGF-II was stably expressed in Chinese hamster ovary cells in secreted form and purified. Activity assays, biochemical analysis and substrate kinetics were performed. Cellular uptake by human MPS IIIB fibroblasts and inhibition assays were followed by confocal microscopy to evaluate the subcellular distribution of rhNAGLU-IGF-II in the fibroblasts. Glycosaminoglycan (GAG) storage reduction was measured.

Summary of Results: The enzyme activity towards the substrate 4-MUNG was comparable to wild-type rhNAGL, with peak activation near lysosomal pH. MPS IIIB fibroblasts and brain-derived cell lines readily took up the fusion enzyme via receptor-mediated endocytosis that was inhibited by competitive IGF-II peptide. Co-localization of rhNAGLU-IGF-II enzyme with lysotracker red in MPS IIIB cells via confocal microscopy confirmed lysosomal targeting. GAG storage was also reduced up to 50% (p<0.001) in MPS IIIB cells after treatment with rhNAGLU-IGF-II when measured by radioactive incorporation assay.

Conclusions: Our data suggest that the fusion enzyme, rhNAGLU-IGF-II, can enter human cells and localize in the lysosomes. Future studies in vivo are needed to more fully assess the clinical potential of rhNAGLU-IGF-II as an ERT for Sanfilippo B syndrome.

15 BONE SCAN OVERUSE IN THE STAGING OF PROSTATE CANCER

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Purpose of Study: With contemporary widespread PSA screening, more patients exhibit low- and intermediate-risk prostate cancer, thus reducing the need and yield of bone scan imaging. We aimed to determine the utilization and subsequent positivity rates of bone scan imaging in a contemporary Veterans Affairs (VA) cohort of men with prostate cancer.

Methods Used: We retrospectively reviewed the charts of 1597 men diagnosed with prostate cancer between 1997 and 2004 at the Greater Los Angeles and Long Beach VA Medical Centers. We used univariate analysis to measure association between patient (age, race, comorbidity) and tumor (PSA, clinical stage, Gleason score) characteristics with bone scan use and positivity. We conducted the analysis for scans that were and were not clinically indicated, according to established AUA guidelines.

Summary of Results: Out of 1121 men not indicated for nuclear imaging, 452 (40%) received a bone scan with only 6 (1%) returning positive. Among the same group, increasing PSA, clinical stage, Gleason score, and subsequent D’Amico tumor risk, were all positively associated with bone scan overuse, but not with corresponding positivity rates. Univariate analysis showed age, PSA, clinical stage, Gleason score, and treatment type as significant predictors of bone scan overuse.

Conclusions: Bone scan overutilization in men with clinically localized prostate cancer results in unnecessary patient anxiety, time consumption, and significant economic waste for the VA. If the 40% overutilization rate for clinically localized prostate cancer reflects national overuse patterns, then implementing a quality improvement initiative to minimize overutilization would translate an annual savings of $9,409,680 for the VA, without compromising oncologic outcomes.

16 YIN YANG 1 REGULATES THE TRANSCRIPTIONAL REPRESSION OF SURVIVIN

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Purpose of Study: Survivin is a member of the Inhibitor of Apoptosis (IAP) family of proteins, and is highly expressed in all cancers but absent in normal tissues. Expression level correlates with chemo- and radioresistance as well as poor prognosis in cancer patients. The mechanisms for upregulation of Survivin in cells undergoing stress associated with tumor development and the tumor microenvironment are not well understood. The putative stress response transcription factor Yin Yang 1 (YY1) was hypothesized to contribute to the upregulation of survivin in tumor cells.

Methods Used: In order to study regulation with luciferase reporter assays, U2OS cells were transfected with nested deletions of the survivin promoter, ranging from short (+230 bp) to long (+6280 bp). YY1 involvement in survivin promoter repression was confirmed using siRNA directed against YY1. A U2OS cell line containing a stable YY1 Tet-off system was used to determine whether a temporal increase in YY1 expression affects Survivin promoter levels. To further evaluate the role of YY1 regulation of survivin expression, reporter constructs containing mutated putative YY1 binding sites in the proximal survivin promoter were used in reporter assays.

Summary of Results: When YY1 was overexpressed, luciferase expression was repressed 5–10 fold. Further studies showed that knockdown of YY1 releases the survivin promoter from the observed repression and leads to a 3–5 fold increase in promoter activity above basal levels. Furthermore, a low to moderate decrease in Survivin protein was observed in Tet removal experiments. Site-directed mutagenesis confirmed the involvement of proximal survivin promoter sites in YY1 overexpression-induced reporter repression. Mutation of these binding sites on the survivin promoter showed abrogation of the observed repression of survivin promoter activity.

Conclusions: These data suggest that YY1 is a novel repressor of survivin transcription. A better understanding of how YY1’s regulation of survivin expression in vivo affects cell cycle and cell death may provide new directions in therapeutics designed to prevent survivin overexpression in cancer.

17 REGULATION BY ESTROGEN AND PROGESTERONE OF INTERFERON ALPHA SIGNALING IN HUMAN LEUKOCYTES

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Purpose of Study: 90% of systemic lupus erythematosus (SLE) patients are female, and the incidence peaks during reproductive years when estrogen (Es) and progesterone (Pg) are at their highest. Studies show that Es increases the risk of SLE in both humans and animal models, while Pg is protective. Interferon alpha (IFN-α) is a central pathogenic cytokine in SLE and can directly activate multiple immune cell types to favor autoimmunity. Though recent studies suggest a link between Es and IFN-α signaling in immune cells, the relationship is not well understood; and very little is known about the effects of Pg. To investigate the relationship between female reproductive hormones and lupus autoimmunity, we tested the hypothesis that, in human leukocytes, Es enhances IFN-α signaling while Pg suppresses it.

Methods Used: Peripheral blood mononuclear cells (PBMCs) were isolated from blood of healthy donors. Cells were cultured in media, ethanol vehicle, or physiologic concentrations of β-estradiol (Es), Pg, or a combination of both hormones, with or without IFN-α. Total RNA was isolated, and expression of IFN-α inducible genes (CXCL10, MX1, PRR, IFIT1, IIFT2) was measured by quantitative PCR and normalized to housekeeping gene 18sRNA.

Summary of Results: Consistent with our hypothesis, we observed that Es enhanced IFN-α signaling in human leukocytes; we did not observe an effect of Pg. Es treatment significantly increased IFN-α induced expression of CXCL10 (p=0.0273, two-tailed paired T-test). A similar trend was observed for MX1, PRR and IFIT1. Es alone did not increase expression of any of these genes. In addition, we observed that IFN-α signaling response to Es was bi-modal, because PBMCs from some donors consistently showed Es-sensitivity while others did not.

Conclusions: Our research shows for the first time that Es can enhance IFN-α induced gene expression in human leukocytes of healthy donors, possibly by regulating IFN-α signaling pathways. This suggests a novel mechanism by
which Es increases a woman’s risk of developing SLE. Additional experiences will allow us to determine whether Es regulates all or a subset of IFN-α-inducible genes and what factors determine Es sensitivity, such as Es receptor expression. We plan to extend these findings to PBMCs of SLE patients to ask whether abnormal sensitivity or resistance to Es or Pg may contribute to disease.

18 INHIBITION OF HERPES SIMPLEX VIRUS (HSV) GLYCOPROTEIN’S gB, gD, gH-gL INDUCED CELL FUSION AND VIRAL SPREAD VIA HEPARAN MIMICS

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Purpose of Study: Multinucleated giant cells (polycaryocytes) resulting from virus-induced cell fusion are a hallmark of herpes simplex virus-type-1 (HSV-1) infection. However, the role of HSV-1 3-O-sulfated heparan sulfate (3-OS HS) receptor during cell fusion is poorly understood. The goal of this study was to understand the role of heparan mimics in blocking the HSV-1 infection.

Methods Used: HSV-1 glycoprotein induced quantitative reporter gene (luciferase) based assay was used. The counting of multinucleated giant cells (polycaryocytes) was achieved by using fluorescent microscopy. Effector Chinese hamster ovary (CHO-K1) cells expressing various combinations of HSV-1 glycoproteins were co-cultured with the 3-OST-3 expressing primary cultures of human corneal fibroblasts (CF), a natural target cell-type for HSV-1 infection. Pre-treatment of effector cells with heparinase (4U/ml) and heparan mimics (5.6 nM) were used to demonstrate the role of 3-OS HS during cell fusion.

Summary of Results: We demonstrated that cell-to-cell fusion and polycaryocytes formation required expression of four essential HSV-1 glycoprotein (gB, gD, gH-gL) and exhibited a very strong dependence on the expression of heparan sulfate on CF. Further enzymatic removal of HS from CF surface by heparinase-I treatment severely impaired the fusion reaction. Interestingly, the incubation of effector cells expressing HSV-1 glycoprotein’s with heparan sulfates mimics inhibited significantly both membrane fusion and polycaryocyte formation.

Conclusions: Our results indicate that 3-OS HS could play a crucial role in HSV-1 induced cell-to-cell fusion during corneal eye infection. Generation of specific inhibitors targeting against HSV-1 gD epitope that interacts with 3-OS HS would be beneficial in the development of therapeutics to prevent viral spread in the corneal stroma. Here, we provide evidence for the physiological significance of 3-OS HS mediated cell-to-cell fusion.

19 ISLET MATRIX METALLOPROTEINASE-9 IS DOWNREGULATED IN A MOUSE MODEL OF TYPE 2 DIABETES

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Purpose of Study: A pathological hallmark of the pancreatic islet in type 2 diabetes is aggregation of islet amyloid polypeptide (IAPP) into extracellular amyloid deposits, the process of which is toxic to β cells. We have previously shown that (a) glucose dose-dependently increases amyloid deposition in human IAPP (hIAPP) transgenic (T) mouse islets, (b) matrix metalloproteinase (MMP)-9 degrades hIAPP, and (c) inhibition of MMP-9 activity increases amyloid deposition in hIAPP T islets. Whether high glucose regulates MMP-9 or tissue inhibitor of metalloproteinases (TIMP)-1, a natural inhibitor of MMP-9, is unknown. Thus, we determined if down-regulation of MMP-9 occurs in islets in vitro when cultured in high glucose and/or in vivo in a diabetic mouse model.

Methods Used: Amyloid-forming hIAPP T and non-amyloid forming non-transgenic (NT) mouse islets were cultured for 7 days in 11.1, 16.7, or 33.3 mM glucose (n=4-6). Islets were also isolated from diabetic db/db and control db/+ mice (n=8). To confirm the presence of amyloid in T islets after culture, islet sections were stained with thioflavin S. MMP-9 and TIMP-1 mRNA levels were determined by real-time PCR using 18S as the endogenous control. MMP-9 activity was measured in islet conditioned medium by gelatin zymography (n=3).

Summary of Results: In cultured T and NT islets, neither MMP-9 nor TIMP-1 mRNA changed with increasing glucose. In contrast, MMP-9 activity tended to increase at 33.3 mM glucose (NT at 11.1, 16.7, and 33.3 mM glucose: 1, 0.98±0.21, 3.61±0.95; T at 11.1, 16.7, and 33.3 mM glucose: 1.22±0.06, 1.03±0.18, 3.92±1.60; p=0.06), but was not affected by amyloid formation which was observed at 16.7 and 33.3 mM glucose. In db/db mouse islets, MMP-9 mRNA decreased by 41% when compared to db/+ islets (p=0.04).

Conclusions: Glucose did not downregulate MMP-9 activity in cultured islets and therefore, decreased MMP-9 is not the mechanism by which glucose induces amyloid formation in this model. However, in a whole animal model, MMP-9 mRNA was significantly decreased in diabetes. This suggests that downregulation of MMP-9 by another component of the diabetic milieu, such as elevated free fatty acids, may contribute to the increased amyloid deposition associated with type 2 diabetes.

20 COLLAGEN SUBSTRATE SPECIFICITY OF PROLYL 3-HYDROXYLASES

Farnand AW1,2, Weis ME1, Kim LS1,2, Fernandes RJ3, Eyre DR3 University of Washington, Seattle, WA and 2University of Washington, Seattle, WA.

Purpose of Study: Current attention has focused on prolyl 3-hydroxylases (P3Hs), a class of enzymes responsible for 3-hydroxylation of certain prolyl residues in the collagen triple helix, forming 3-hydroxyproline (3Hyp). Mutations in the genes encoding P3H1 and associated proteins of its enzyme complex were found to cause recessive forms of severe osteogenesis imperfecta (OI). The goal of this study was to investigate the expression levels of the three P3H enzymes—P3H1, P3H2 and P3H3—in a cell line known to form 3Hyp at several sites in type II collagen, to better understand their different substrate specificities.

Methods Used: Using qPCR, expression of the three P3H enzyme genes was assayed in the rat chondrosarcoma cell line, RCS-LTC, and in normal adult rat cartilage. Similarly, the expression profiles of these genes, and other genes related to the functionality of the P3Hs, were compared qualitatively by RT-PCR and were compared to expression levels in the human osteosarcoma cell line, SAOS-2. Collagen produced by the RCS-LTC cells was analyzed for 3Hyp formation at known sites by mass-spectrometry.

Summary of Results: The RCS-LTC cells produced mRNA for all three enzymes, most prominently for P3H2, in contrast with normal adult rat cartilage and SAOS2 cells which lacked mRNA expression for P3H2 and P3H3 respectively. Quantitative comparison of P3H1 mRNA expression between the RCS-LTC cells and rat cartilage yielded similar expression of P3H1, a ~2-fold increase in P3H2 and a ~0.5-fold increase in P3H3 expression in the RCS-LTC cells. Mass-spectrometric analysis of the RCS-LTC collagen showed near complete 3Hyp formation at Pro944, a secondary site previously found occupied in the type II collagen of vitreous, but not cartilage.

Conclusions: The high relative abundance of P3H2 mRNA expression coupled with high occupancy of Pro944 in the RCS-LTC matrix implicates P3H2 in the formation of 3Hyp at Pro944. Taken together with previous findings, it is possible that 3Hyp formation at Pro944 may be a signaling mechanism for the prevention of chafage of the collagen N-propeptide, thereby maintaining the long, thin fibrils as seen in RCS-LTC cells and vitreous.

Funded under NCCR Grant TL1 RR025016 and NIAMS Grant AR 036794

21 THIRDHAND SMOKE ADVERSELY AFFECTS FETAL LUNG GROWTH AND DIFFERENTIATION

Chien K, Sakurai R, Torday J, Rehan V Los Angeles Biomedical Institute, Torrance, CA.

Purpose of Study: Though nicotine is the main cigarette smoke constituent linked to altered lung structure and function in utero smoke exposed infants, other constituents have not been thoroughly evaluated. This is particularly true for thirdhand smoke (THS), defined as tobacco smoke that sorbs onto indoor surfaces and dust, where semi- and non-volatile chemicals and particulates can undergo modifications to produce new toxicants that remain on the surfaces, or later desorb and re-appear in the micro-environment. We tested the hypothesis that THS exposure alters the growth and differentiation of the developing lung by affecting the alveolar PPARγ signaling known to be essential for normal lung development.
Methods Used: Embryonic day 19 fetal rat lung explants were exposed to diluent, nicotine (1 × 10^-8M or 1 × 10^-5M), or 4-(methylthioamino)-1-(3- pyridyl)-1-butane (NNK) (1 × 10^-8M or 1 × 10^-5M), the main tobacco-specific N-nitrosamine constituent of THS, for 24h. Cell proliferation (BrDU incorporation), cytotoxicity (LDH assay), and expression of key markers of alveolar differentiation (surfactant protein B and C and PPARγ expression, choline incorporation into saturated phosphatidylcholine and triolein uptake) were determined.

Summary of Results: Cell proliferation decreased and cytotoxicity increased significantly with NNK compared to nicotine (p<0.05), suggesting more adverse effects of NNK. With NNK, choline incorporation into saturated phosphatidylcholine decreased significantly (p<0.05), while it increased significantly with nicotine. Triolein uptake increased significantly with both nicotine and NNK (p<0.05), though more robustly with NNK (p<0.05 vs. nicotine). Western analysis showed significantly decreased PPARγ and increased fibronectin levels (p<0.05) with both NNK and nicotine exposure.

Conclusions: NNK affects lung growth and differentiation more robustly than nicotine, possibly contributing significantly to the thimose smoke exposure-induced pulmonary damage. These data can be used in designing specific preventive and/or therapeutic strategies against the deleterious effects of in utero THS exposure on lung development [Supported partially by grants from the TRDRP (1S1T0250, 17RE01-070)].

22 FACTORS CONTRIBUTING TO THE EFFICACY OF THYMoglobulin AS A TREATMENT FOR ACUTE KIDNEY GRAFT REJECTION

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Purpose of Study: To examine the outcomes of Thymoglobulin treatment for acute rejection and to identify risk factors associated with graft failure using the UCLA Kidney Transplant Database.

Methods Used: Between February 2000 and June 2010, there were 517 hospital admissions for possible kidney allograft rejection. Among these, there were 114 unique patients treated with Thymoglobulin for acute rejection, and 98 were included in the analysis, with a median follow up time of 367 days (16 patients did not have sufficient follow-up data). An analytical file was created by retrospective chart-review and baseline characteristics were collected. The Kaplan-Meier product limit method was used to estimate the survival rate. Univariate and multivariate survival analyses were performed using the Cox proportional hazard model.

Summary of Results: Graft failure occurred in 36 of the 98 patients (36.7%) with a median time between Thymoglobulin treatment and graft failure of 567 days (25-79%: 152-1265 days). In multivariate analysis, factors associated with increased risk of graft failure included doses of Thymoglobulin received (≥2) and number of biopsies performed on the current allograft (≥1). Factors associated with decreased risk of graft failure included creatinine level at time of Thymoglobulin treatment (≤3.0 mg/dl) and identification of vascular rejection versus non-vascular rejection on the biopsy performed immediately prior to Thymoglobulin treatment.

Conclusions: Resolution of acute rejection is vital to allograft survival and Thymoglobulin is a last-line treatment for steroid-resistant acute rejection. Our results indicate that the doses of Thymoglobulin received, the creatinine level at time of Thymoglobulin treatment, the number of prior biopsies, and the presence of non-vascular rejection are associated with increased risk for graft failure. Individual chart-review permitted access to biopsy data not available in database studies, and the analysis suggests that perhaps Thymoglobulin treatment is less efficacious in acute rejection with chronic changes. In patients with risk factors found to be significant for graft failure, nephrologists may choose to lower or omit the use of Thymoglobulin as its benefits may be outweighed by its recognized adverse effects in the long run.

23 ASTROCYTE MATURATION AND REACTIVITY IN PEDIATRIC EPILEPSY SYNDROMES

Kwon EE1,2, Mathern G1, Wanner I1 1Western University of Health Sciences, Pomona, CA; 2University of California, Los Angeles, Los Angeles, CA and 1University of California, Los Angeles, Los Angeles, CA.
Purpose of Study: Astrocytes are directly involved in the blood brain barrier and influence potassium buffering through aquaporin (AQP4) water channels. AQP4 is known to buffer metabolites during neural injury and epileptogenesis. GFAP (glial fibrillary acidic protein), a prototypical marker of astrocytes, is upregulated and reveals astrocytes undergo structural changes such as hypertrophy and loss of domain organization. Severe forms of astroglisis lead to the formation of glial scars and scar-like penumbra around necrotic or ischemic areas. To further characterize astrocytes in human epilepsy, we analyzed hallmark markers of astrocytes in pediatric surgical biopsy specimens of the neocortex.

Methods Used: Cortical tissue were resected from pediatric patients with epilepsy disorders including Cortical Dysplasia, Rasmussen’s Encephalitis, and epilepsy due to ischemic stroke primarily from central operculum and parieto-temporal lobe regions. Specimens were vibratome-sectioned and stained for GFAP and AQP4 using fluorescence immunohistochemistry and images were binarized and quantified for astrocyte abundance and coverage as a measure of reactivity.

Summary of Results: Our results show that abundance as well as network complexity varied in each zone. One striking finding was a noticeable sparseness, even lack GFAP fluorescence in the grey matter of infant cortical biopsies. This finding, together with the presence of immature radial glia marker expressing cells in that zone (BLBP-brain lipid binding protein) suggests that GFAP-positive astrocytes did not significantly populate the grey matter until around 4 years of age. This was found to be the case in genetic as well as acquired epilepsy etiologies, irrespective of case severity, which suggests a developmental feature of protoplasmic astrocytes rather than pathological.

Conclusions: These tools allow us to determine reproducible quantitative differences in astrocyte abundance, morphology and reactivity that may help define gliosis severity. These findings would help us understand the role of astrocytes in epilepsy and also be useful in histopathological diagnosis of various epilepsy syndromes.

24 GENETIC POLYMORPHISMS OF CYTOCHROMES P450 AND PEDIATRIC ASTHMA CONTROL BY INHALED CORTICOSTEROIDS

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Purpose of Study: Inhaled corticosteroids (ICS) are mainline treatments for persistent asthma in children. Up to 10% of children with persistent asthma fail ICS therapy for unclear reasons. Genetic polymorphisms of cytochromes P4503A4 (CYP3A) have been shown to be associated with altered metabolism of ICS which might have an effect on their therapeutic effectiveness. Fluicasone, a widely used ICS, is metabolized primarily by CYP3A4 whose expression may be decreased by a polymorphism in intron 6. Objectives of this study are to 1) determine the frequency of genetic variants in CYP3A4 (intron 6 SNPs rs35599367, C>T), in children treated with fluticasone for difficult to control asthma and 2) to describe and compare CYP450 genotypes in these children with and without previous hospital admissions for asthma.

Methods Used: We enrolled children aged 2–17 years with a diagnosis of asthma from Primary Children’s Medical Center. We collected saliva samples and analyzed for CYP450 genetic polymorphisms at the Developmental Pharmacology and Experimental Therapeutics Laboratory at Kansas City. Single nucleotide polymorphisms for 9 alleles that increase or decrease protein expression and CYP3A activity were determined along with asthma severity, preventive medication use, and number of hospitalizations for acute asthma within the preceding 12 months.

Summary of Results: Of 96 children enrolled, 23 were treated with fluticasone; of these were classified as not-well-controlled asthmatics by NIH guidelines. Of these 23 children, 14 patients were admitted for asthma in the previous 12 months, 14/14 featured an rs35599367 C/C genotype consistent with greater CYP3A4 mRNA level and enzyme activity. Of the 9 patients not requiring hospitalization within the last 12 months, 5 (55.6%) had at least one variant rs35599367 allele, consistent with slower metabolism of fluticasone. There was no difference in the distribution of other CYP3A4, CYP3A5, and CYP3A7 polymorphisms.

Conclusions: These preliminary findings are consistent with the hypothesis that slower metabolism of ICS may improve their effectiveness for treatment of asthma. Additional patient studies are needed to confirm or refute this association.
25 COMBINATORY EFFECT OF BMP-2 AND APATITE COATED SCAFFOLDS ON THE OSTEOGENIC DIFFERENTIATION OF HUMAN MESENCHYMAL STEM CELLS

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Purpose of Study: It is widely accepted that osteoconductive materials promote osteogenesis in vitro. However, the impact of 3D osteoconductive substrates coupled with soluble signals on progenitor cell differentiation is not well known. In this study, we investigated the influence of carbonate apatite on the osteogenic differentiation of human mesenchymal stem cells (hMSCs) seeded in biodegradable poly(lactide-co-glycolide) (PLG) scaffolds in conjunction with the osteoinductive growth factor BMP-2.

Methods Used: Apatite coated PLG scaffolds were formed by incubating hydrolyzed microparticles in modified simulated body fluid (mSBF) prior to being combined with NaCl and compressed at 1500 psi for 1 min. Samples were then exposed to high pressure CO2 (800 psi) for 16 h. NaCl was removed by leaching the matrices in distilled H2O for 24 h. We cultured hMSCs on the scaffolds in osteogenic media supplemented with 25, 100 or 200 ng/ml of BMP-2. The in vitro osteogenic differentiation of hMSCs was measured by quantifying ALP activity, DNA content, and cell secreted calcium after 3, 7, 14, 21 and 28 d in osteogenic conditions. qPCR was performed for Runx2, SP7, Col1a1, lbsp, and Sparc at 7 and 21 d.

Summary of Results: No significant differences between nonmineralized and mineralized scaffolds were detected in ALP activity at 0 and 200 ng/ml concentrations, but mineralized scaffolds had increased levels after 7 d at 25 and 100 ng/mL. Mineralized scaffolds had increased levels of cell-secreted calcium at all time points. Increasing BMP-2 concentration resulted in a rise in both ALP and calcium levels. There was no difference between Runx2 and SP7 levels in mineralized and nonmineralized substrates, but an increase in SP7 was observed with the addition of BMP-2. At 21 d mineralized substrates had increased levels of lbsp and decreased levels of Sparc compared to nonmineralized substrates.

Conclusions: The present data indicate that a combination of apatite and BMP-2 do not simply enhance the osteogenic response of hMSCs, but act through different and possibly opposing pathways. Thus multiple signaling strategies may be necessary to achieve optimal bone regeneration.

Behavior and Development
Concurrent Session
8:30 AM
Friday, January 28, 2011

26 EXAMINATION OF VERBAL INTELLIGENCE AND PHONOLOGICAL PROCESSING IN CHILDREN WITH AUTISM SPECTRUM DISORDERS AND THEIR FAMILY MEMBERS

Thomson AL1, Webb SJ2, Bernier R2, Wijman E2 1University of Washington School of Medicine, Seattle, WA and 2University of Washington, Seattle, WA.

Purpose of Study: Autism Spectrum Disorders (ASD) have a strong genetic influence, and a broader autism phenotype (BAP) has been observed in first-degree family members. It is well known that children with autism have an array of cognitive, communication, and social deficits, including phonological, semantic, syntactic, and pragmatics. The overall aim of this project was to better understand verbal language use in individuals with ASD and their family members.

Methods Used: A total of 279 family members from families with 2 or more ASD children participated. Verbal intelligence quotients (VIQ), a measure of vocabulary and reasoning, were assessed with age-appropriate Wechsler scales. Non-word repetition (NWR), a measure of phonological processing, was assessed with the CTOPP.

Summary of Results: As expected, ASD children exhibited lower VIQ compared to mothers, fathers, and unaffected siblings (F(3,274) = 59.8, p < .01). To examine decreased VIQ in the context of overall cognitive function, discrepancy scores were calculated between verbal and non-verbal IQ. ASD children’s IQ discrepancy scores were significantly greater than relatives (F(3,274) = 19.8, p < .01). Both our higher-functioning (HF) and lower-functioning (LF) ASD groups exhibited a mean discrepancy pattern that suggested better non-verbal IQ compared to VIQ (p<.05). For phonological processing, mean performance for all groups was significantly below the norm on the NWR task, however siblings performed relatively better than ASD children and fathers (F(3,256) = 2.3, p<.05). Investigation of the relationship between VIQ and NWR revealed significantly higher ratio scores in ASD children and siblings compared to parents (F(3,253)=11.976, p < .01).

Conclusions: These results suggest that relatively poorer phonological processing may be an endophenotype of ASD and a part of the BAP. The ratio of the phonological processing to VIQ suggests that the relation between these two domains changes with age, but not affected status. Taken together, these data suggest that multiple aspects of language processing contribute to both autism and the broader phenotype and will be important quantitative traits for genetic analyses.

27 MISSED OPPORTUNITIES IN HIGH RISK INFANT FOLLOW-UP?

Tang B1, Huffinan L1, Feldman H1, Gray E1, Kagawa K2, Gould JB1 1Stanford University School of Medicine, Palo Alto, CA and 2State of California, Sacramento, CA.

Purpose of Study: To evaluate rates of referrals to state-funded early intervention (EI) programs for high risk infants demonstrating significant developmental delay during the 1st year of life following NICU discharge. In California, high risk infant follow-up (HRIF) programs are supported by California Children’s Services (CCS). Infants discharged from the NICU qualify for HRIF based on CCS-defined neonatal medical risk factors. A central goal of HRIF is to identify children with developmental delays and refer them to EI that provides appropriate therapeutic services.

Methods Used: A primary analysis of a population-based data set from a California network of HRIF programs. Standardized developmental assessments were conducted at the 1st HRIF visit at 4–8 months of age, adjusted for prematurity. Scores were reported as a developmental quotient (DQ) based on normative data for each individual test. For this study, infants were included in the analyses if they had a DQ<70 (≥2 standard deviations below the mean) in ≥1 subtest domain (e.g. motor). Of the included infants, we compared the proportion who were and were not referred to EI.

Summary of Results: Of the 1880 high risk infants who had a standardized developmental assessment at the 1st HRIF visit, 342 (18%) had ≥1 domain DQs in the 2–3% range of the general population. Two-thirds (n=235) had no EI before the 1st visit. Of those infants, less than half were referred to EI (Table). This finding persisted across birth weight group classifications and for infants who had ≥2 domain DQs ≤70.

Conclusions: An alarmingly high proportion of high risk infants are not referred to EI even after they are identified, using standardized testing, as having a developmental delay in ≥1 domain. Factors associated with the non-referral group will be explored. Subsequent HRIF visits will determine whether a delay in referral affects long term developmental outcomes. These data have utility for the quality improvement of HRIF programs across the state.

EI Status of Infants with DQs ≤70 at the 1st HRIF Visit

<table>
<thead>
<tr>
<th>DQ ≤70 (%)</th>
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<tbody>
<tr>
<td>EI before HRIF</td>
</tr>
<tr>
<td>No EI before HRIF</td>
</tr>
<tr>
<td>EI Referral Made</td>
</tr>
<tr>
<td>No EI Referral Made</td>
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</tbody>
</table>

28 USE OF DIFFUSION TENSOR IMAGING IN THE EVALUATION OF BRAIN INJURY IN ADOLESCENTS BORN PRETERM

Giamm-Bean S1, Yeatman J1, Lee E1, Yeon K2, Feldman H1 1Stanford University, Palo Alto, CA and 2Stanford University, Palo Alto, CA.

Purpose of Study: Subtle, diffuse, non-cystic white matter injuries have been found in preterm infants and are often missed by standard MRI. Diffusion tensor imaging (DTI) is a technique capable of examining the structure and organization of white matter at the microscopic level and is particularly

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useful for detecting this type of injury. The hypothesis is that the preterm group will have indications of injury or immaturity in the corticospinal and somatosensory tracts when comparing the preterm and full-term groups.

Methods Used: DTIs were collected and processed for 50 subjects, both born preterm and full-term, between 9-16 years of age. Using tractography, the corticospinal and somatosensory tracts were tracked from the cerebral peduncle to the primary motor and sensory cortices in each individual. We computed the diffusion parameters, axial and radial diffusivity, and the fractional anisotropy along the trajectory of the tracts. These were then averaged for both groups and compared.

Summary of Results: The axial diffusivity of the preterm group is higher along the corticospinal and somatosensory tracts when compared to the full-term group. The radial diffusivity is similar between the two groups along the tracts. The fractional anisotropy is similar between the two groups, but is higher in the preterm group at several points along the tracts.

Conclusions: Analysis suggests that there are detectable differences between the corticospinal and somatosensory tracts of the two groups and that perinatal injury may show detectable differences into adolescence. Prior studies have focused on fractional anisotropy, but this study shows that there are detectable differences in the axial diffusivity between the two groups. Future analyses will include a more detailed examination of the preterm group to look for outliers and to compare the findings of the preterm group with their motor exam.

An example of the findings: left corticospinal tract.

Conclusions: The results of this study highlight differences between well-known and frequently used measures regarding identification of ADHD comorbidities. In addition, this study underscores the need to recognize caregiver stress and depression among youth with ADHD and comorbid diagnoses.

30 LOW GLYCEMIC LOAD EXPERIMENTAL DIET MORE SATIATING THAN HIGH GLYCEMIC LOAD DIET

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Purpose of Study: Clinicians need effective yet simple strategies to offer overweight and obese patients in need of weight reduction, especially with evidence suggesting metabolic disorders are a risk factor for developing and dying from cancer. Of interest in this regard is whether particular food types increase satiation, which may reduce overall food intake. With a randomized controlled feeding study, we investigated the effect of low vs. high glycemic load (GL) experimental diets on satiety. We also examined whether BMI, gender, and leptin concentration influenced results.

Methods Used: 81 healthy adult participants, half of whom were overweight or obese, completed two 4 week feeding periods (low GL and high GL) which were separated by a 4 week washout. Diets were matched in calories and macronutrients (except for fiber) and differed only in GL. All meals were prepared in a controlled manner in a metabolic kitchen. Participants completed a visual analog satiety survey after each feeding period and T-scores were used to compare mean scores. Serum leptin was assayed from blood draws taken after each feeding period.

Summary of Results: Participants reported feeling significantly more satiated on the low GL diet than on the high GL diet (p=0.047). Participants also reported having more food cravings on the high GL diet than on the low GL diet (p=0.001). In subgroup analysis, women demonstrated significantly less hunger and more satiety on the low GL diet (p=0.05, p<0.01). There was no difference in hunger or satiety for men between the two diets. Participants of normal body fat percentage (<25% for men; <32% for women) and normal BMI (<25.0) reported the food being tastier on the low GL diet than on the high GL diet (p=0.04, p=0.05). Leptin concentration did not significantly vary amongst participants between the two diets.

Conclusions: Our data demonstrate that a low GL diet is more satiating than a high GL diet, especially for women. Reducing GL may be an effective way to lower the amount of food consumed and improve compliance with weight reduction prescriptions. A low GL diet may thus be a potential tool to reduce the risk of developing and dying from cancer by lowering rates of overweight and obesity.
Higher gestational age was associated with higher Bayley Mental (B=1.6, p<0.05) and Psychomotor (B=2.3, p<0.05) Development scores after controlling for covariates. All covariates were statistically significant (p<0.05) except birth weight for the mental development score and sex for the psychomotor score. Assignment to iron or usual nutrition, and occurrence of iron deficiency anemia or not, were not significant in the models.

**Conclusions:** In a cohort of healthy full term infants from Santiago, Chile, developmental status at 12-months was significantly lower in those born at 37–39 weeks GA than those born at 40–42 weeks GA. Further research could assess whether these differences persist beyond infancy.

### 32 PSYCHOLOGICAL COUNSELING FOR PARENTS CARRYING A FETUS DIAGNOSED WITH A CONGENITAL HEART DISEASE

Creel B,1 Evans WN1,2 Acherman RJ1,2 Rollins RC1,2, Castillo WJ1,2, Luna CF1,2, Restrepo H1,2 Children’s Heart Center Nevada, Las Vegas, NV and 1University of Nevada, School of Medicine, Las Vegas, NV.

**Purpose of Study:** To assess the emotional state, changes in daily life, and emotional support systems in newly diagnosed parents of a fetus with a confirmed diagnosis of structural heart disease.

**Methods Used:** In December 2009, we began a new program of psychological counseling for couples carrying a fetus with heart disease. This report includes the first 16 couples in this program. The couples responded to a questionnaire developed by the investigators focusing on the emotional state, available support systems, and the effects of prenatal knowledge of the diagnosis.

**Summary of Results:** Mean mothers’ age was 29.1 ± 6.7 years (range: 15–36 years). The group of couples was composed of 7 Caucasians, 2 Hispanics, 2 Asians, and 5 from other races.

Table 1 summarizes the relative percentage of emotional states reported by couples after the diagnosis of carrying a fetus with a congenital heart disease.

Knowing the diagnosis in advance was useful in 15 out of 16 couples in helping them to prepare, economically, mentally, and emotionally for the birth of the child, only 1 couple responded being too overwhelmed and unsure of their feelings around this question.

Daily life was impacted with negative emotions in 15 out of 16 couples; one couple did not answer the question.

13 out of 16 couples reported that the main support systems in helping cope with the diagnosis were their family and religious groups; the remaining 3 couples reported friends and others as their main support.

**Conclusions:** We found that in the vast majority of parents carrying a fetus with a diagnosis of a CHD, negative emotional states impact their daily life and family and religious groups are the main support systems to cope with the diagnosis. These results showed that couples with a fetus with a CHD are in need of psychological counseling in helping cope with the diagnosis and in preparing for a life with a medically challenged child.

**TABLE 1.**

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Depression</th>
<th>Anger</th>
<th>Frustration</th>
<th>Irritability</th>
<th>Fear</th>
<th>Stress</th>
<th>Anxiety</th>
</tr>
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<tbody>
<tr>
<td>Relative Percentage</td>
<td>69%</td>
<td>69%</td>
<td>69%</td>
<td>75%</td>
<td>87%</td>
<td>81%</td>
<td>88%</td>
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</table>

### 33 COGNITIVE PROFILES IN AUTISM SPECTRUM DISORDERS

Vincent L2, Bernier R1 1University of Washington School of Medicine, Seattle, WA and 2University of Washington School of Medicine, Seattle, WA.

**Purpose of Study:** Given the phenotypic and genetic heterogeneity of Autism Spectrum Disorder (ASD), identification of accurate and easily identifiable subtypes could improve etiologic understanding, intervention strategies and prognostic recommendations. Using 1280 individuals with ASD ascertained through the Simons Simplex Collection, our purposes were to investigate the existence of domain discrepancy profiles based on verbal and nonverbal intelligence scores, and to explore the relationships between cognitive profiles and other measurable outcomes.

**Methods Used:** Based on stringent requirements for identifying significant differences in verbal and nonverbal IQ scores, a chi-squared test identified subjects as having either a nonverbal > verbal intelligence split, no split (verbal = nonverbal intelligence), or a nonverbal < verbal intelligence split. Chi-squared analysis was used to look at gender breakdown within profile groups. Relationships between these profiles and other aspects of autism symptomatology, including socialization skills, fine motor skills, adaptive behaviors, head circumference, and autism severity were analyzed using multivariate analyses of variance. Age was included as a covariate.

**Summary of Results:** A significantly greater number of individuals with a cognitive split was found in the ASD group than would be expected. While no relationships between cognitive profile and adaptive behavior or fine motor skills were identified, results indicate that individuals with a nonverbal > verbal profile showed greater overall autism symptom severity according to clinician observation and individuals with a nonverbal < verbal discrepancy profile showed greater social impairment according to parent report.

**Conclusions:** These findings indicate that there is an increased rate of nonverbal and verbal intelligence discrepancies in ASD and suggest that cognitive profiles within the autism spectrum may provide a useful method of identifying ASD subtypes. Further investigation of these cognitive profile subtypes may elucidate differential genetic mechanisms and provide guidelines for determining the maximally effective interventions tailored to ability profiles to further improve outcomes.

### 34 ARE THERE CHILDREN IN NEED OF EARLY INTERVENTION SERVICES THAT ARE BEING MISSED BECAUSE THEY DO NOT HAVE A DIAGNOSIS OF A HIGH RISK CONDITION?

Weigang T1, Talmi A2 1University of Colorado Denver, Aurora, CO and 2University of Colorado Denver, Aurora, CO.

**Purpose of Study:** Early Intervention services have been shown to be effective resources for young children (under 3 years old) who, either are developmentally delayed (DD) or have a condition that puts them at a high risk of becoming DD. These services are most effective when utilized as early as possible. A little over 2% of the pediatric population under 3 years old is enrolled in Early Intervention services, but research indicates that as many as 13% of the children under 3 years old may be eligible for these services (Rosenberg et al. 2008). The current study aimed to look at patterns of pediatric provider referrals in the presence of an abnormal score on a parent cognitive child developmental screen (Ages and Stages Questionnaire (ASQ)).

Specifically, the study was designed to confirm findings reported by Silverstein, et al (2006), who reported that the majority of pediatricians they surveyed thought that an established diagnosis of a high risk condition was important when deciding whether to refer a child to Early Intervention. Our study aimed to answer the following questions: 1) is there a population of children without established high risk conditions, but who show signs of DD and are not being referred to Early Intervention services? 2) Do consultations with mental health liaisons, who are collocated within the primary care clinic (CLIMB), have any effect on these referral rates?

**Methods Used:** A retrospective study was done by extracting data from electronic medical records of all well child checks (WCCs), of children under 3, done at an outpatient Child Health Clinic in a Children’s Hospital between March of 2009 and April of 2010. Data was obtained by a computer query of all WCCs documented in the electronic medical records. A computer query and manual review of these records were used to identify all WCCs that had an ASQ completed during the visit. The records with abnormal ASQ scores were analyzed.

**Summary of Results:** Data analysis is still pending but will include: the percent of ASQs with abnormal scores, Early Intervention referrals generated, the number of children with established high risk conditions, and whether CLIMB consults had a significant impact on referrals.

**Conclusions:** Pending

### 35 THEORY OF MIND IN CHILDREN AT RISK FOR DEVELOPING PSYCHOSIS

Reidy RE, Hunter SK University of Colorado Denver School of Medicine, Denver, CO.

**Purpose of Study:** Theory of Mind (ToM), the ability to appropriately attribute mental states to the self and others, is a cognitive ability that normally develops between three and five years of age (Wellman & Liu, 2004).
Theoretically, psychosis may be related to deficiencies in both monitoring and interpreting intentions of the self and others (Frith, 1992). A large number of studies show that schizophrenia patients have poor performance on ToM tasks (see Brune, 2005) and deficits in ToM performance have been found in first-degree relatives of schizophrenic patients (Anselmetti et al., 2009), suggesting that poor performance on ToM tasks may be an endophenotype for schizophrenia. Surprisingly, early impairments in ToM have not been studied in the children of schizophrenics.

Methods Used: Children with at least one parent meeting DSM IV criteria for diagnosis of schizophrenia or other psychotic disorder and children with no family history of psychosis are given tasks at 40 and 48 months of age to assess ToM development. The first is a locations false belief task which requires the child to predict where a protagonist will search for an object based on a false beliefs about the object location. The second task is a contents false belief task and requires the child to attribute knowledge about the contents of a mislabeled box to the self and others. The Dimensional Change Card Sort is also being administered in order to analyze interactions with executive function, another endophenotype for schizophrenia.

Summary of Results: Thirty children have completed the study to date and preliminary data show children’s scores significantly increase with age, as predicted. Of the thirty children who have completed the study, three have a parent with a diagnosis of schizophrenia or other psychotic disorder, thus the study currently lacks sufficient power to analyze differences in performance between conditions of parental mental health status.

Conclusions: We hypothesize that poor performance on ToM tasks is an endophenotype for schizophrenia and other psychotic illness that will be detectable early in life. Longitudinal analysis is hypothesized to show a significantly larger increase in task performance for children with no family history of psychosis.

36 THE EXPERIENCES OF AUTISTIC YOUTH LIVING IN RURAL COMMUNITIES

Herring JC, University of Washington, Woodinville, WA and Seattle Children’s Hospital, Seattle, WA.

Purpose of Study: As the American economy becomes more knowledge-based and the job market more competitive successful transition from adolescence to adulthood is even more critical, particularly for students with disabilities like Autism. The NLTS2 is a ten-year transitional study that provides researchers, educators and policy makers at the national level the opportunity to examine the post-secondary experiences of youth with disabilities. The transitional and social experiences of youth with Autism is a recent topic of research and requires much more investigation. The purpose of this study is to provide a brief description of the transition, education, and social experiences of Autistic youth living in rural communities.

Methods Used: The samples for this prospective study were taken from Wave 1 and Wave 4 of data collection. Data was obtained from both samples for youth that were 1) Diagnosed with Autism and 2) Attended a school located in a rural community. Data was analyzed using the social sciences statistical analysis software, SPSS (Statistical Package of the Social Sciences, 2008).

Summary of Results: The majority of youth in this sample (ages 13–16) have trouble conversing, speaking clearly, understanding others, and using an appropriate tone. Nearly half of parents (47%) stated that their child had never gotten together with any friends in the past 12 months, while more than half (54%) reported that their child had never been invited to any social activities in the past 12 months. Additionally, 53% of respondents said that their autistic youth never talked to friends on the phone and nearly 66% reported that their child never interacted with others using email or chatrooms.

Conclusions: Autism and the Associated Spectrum Disorders are a complex interaction of impairments in verbal/non-verbal communication, behavior, and social interaction. The results of this study suggest that Autistic youth living in rural areas are mostly unengaged socially and in their community, as well as unprepared for the transition to employment and post-secondary education. More research is needed to further examine the disparities faced by Autistic youth in rural communities, how to reform the transition process for this population so as to achieve transition success, and analyze the influence of youth experiences on future outcomes.

Cardiovascular I

Concurrent Session

8:30 AM

Friday, January 28, 2011

37 INDUCTION VS. NON-INDUCTION: THE SAGA CONTINUES

Kawano M, Goldstein Z, Hamilton M, Kobashigawa J Cedars-Sinai Heart Institute, Beverly Hills, CA.

Purpose of Study: In cardiac transplantation, induction immunosuppression therapy has been administered in hopes of the recipient acquiring tolerance to the donor organ. There have been no randomized trials that have demonstrated benefit to the use of induction therapy which usually includes cytolytic drugs, such as antithymocyte globulin (ATG). 60% of the centers in the United States do not use induction, but rather, triple drug immunosuppression with a regimen of tacrolimus, mycophenolate mofetil, and steroids. The 40% that do use induction typically utilize ATG. This observational study reviews two large, local programs in the same era and assesses the long term outcome of heart transplant patients who were administered induction versus no induction for heart transplantation.

Methods Used: We evaluated 1207 patients transplanted between 1994 and 2010, and divided them into those that received induction therapy and those that did not. These patients were assessed for long term outcomes, including 5-year actuarial survival, freedom from cardiac allograft vasculopathy (CAV, stenosis ≥50%), freedom from non-fatal major adverse cardiac events (NF-MACE, MI, CHF, PTCA, pacemaker, stroke, new peripheral vascular disease), and 1-year freedom from any-treated rejection.

Summary of Results: The non-induction group (n=766) exhibited greater freedom from NF-MACE compared to the induction group (n=441)(93% vs. 85%, p=0.001), mostly due to less patients developing CHF (5% vs. 9%, p=0.009). The non-induction group also demonstrated a trend towards greater 5-year freedom from CAV compared to the induction group (87% vs. 83%, p=0.07). However, there was no significant difference between groups in terms of 5-year actuarial survival (78% vs. 78%, p=0.85). Interestingly, there was also no difference in 1-year freedom from rejection in the induction group, which used more aggressive early immunosuppression therapy.

Conclusions: Induction immunosuppression therapy does not appear to have an advantage over non-induction immunosuppression. Specifically, there was no difference in 1-year rejection and patients on induction therapy demonstrated significantly higher incidence of NF-MACE as well as a trend toward increased frequency of 5-year CAV. The cause of increased incidence of NF-MACE in the induction group remains unknown. A randomized trial is still needed to assess any benefit of induction therapy.

38 PRE-TRANSPLANT BLOOD TRANSFUSION CAUSING SENSITIZATION IN PATIENTS AWAITING HEART TRANSPLANTATION

Davidoff J, Kawano M, Goldstein Z, Hamilton M, Kobashigawa J Cedars-Sinai Heart Institute, Beverly Hills, CA.

Purpose of Study: Sensitization is the development of circulating antibodies in patients awaiting heart transplantation. Sensitization in these patients can mean a longer waiting time in order to find a compatible donor organ. One of the major causes of sensitization is the administration of blood transfusions in the pre-transplant time period. However, it is not known what the risk of blood transfusions is in terms of causing sensitization. We sought to determine the risk of blood transfusions in patients awaiting heart transplantation.

Methods Used: We reviewed 836 patients awaiting heart transplant between 1994 and 2008 and found 63 patients with baseline and subsequent blood transfusions and panel reactive antibodies (PRAs) within one year prior to transplantation. Sensitization was defined as any PRA screen greater than baseline. Patients were divided into 3 groups depending on baseline PRA: Group A = 0% (N=22), Group B = 0-10% (N=20), and Group C > 10% (N=21).

Summary of Results: 36% of Group A (baseline PRA=0%) patients developed circulating antibodies after a mean of 1.4 ± 0.5 blood transfusions. Group B and Group C patients also were noted to have increases in circulating antibodies from baseline in 45% and 52% of patients, respectively.
The risk of 1 transfusion vs. 6 transfusions did not appear to markedly increase risk of sensitization.

Conclusions: The risk of sensitization following blood transfusions is significant although the extent of antibody production appears to be limited in Group A-type patients (baseline PRA = 0%), as most of these patients do not develop PRAs > 10%. For patients with pre-existing circulating antibodies, increase in PRA level is also significant. Withholding of blood transfusions to prevent sensitization and further sensitization in patients with pre-existing circulating antibodies is warranted.

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THE OUTCOME OF AFRICAN AMERICAN HEART TRANSPLANT RECIPIENTS: ARE THEY TRULY AT RISK?

Purpose of Study: African American heart transplant patients have been shown to have a higher incidence of cardiac allograft vasculopathy (CAV) compared to Caucasian patients. It is not known whether this difference is due to higher acute cellular or antibody-mediated rejection or secondary to differences in ethnicity or other factors. We assessed the outcomes of African American heart transplant recipients compared to Caucasian patients.

Methods Used: We reviewed 1160 patients transplanted between 1994 and 2008. Patients were divided into groups based on the number of BP medications taken over 6 months within the first 2 years post heart transplant. Control patients on 0 BP medications were selected for 2-year conditional survival. Assessed 5-year subsequent outcomes included survival, freedom from CAV, and freedom from non-fatal major adverse cardiac events (NF-MACE, MI, heart failure, PTCA, pacemaker, stroke, new peripheral vascular disease). BP medications were divided into several categories, including beta blockers, calcium channel blockers, ACE Inhibitors, and angiotensin II receptor blockers.

Summary of Results: Patients with 3 BP medications exhibited a trend toward lower survival compared to patients with 0 BP medications (70% vs. 82%, p=0.06) and (70% vs. 82%, p=0.07) or 2 (70% vs. 85%, p=0.06) BP medications. Subsequent freedom from CAV and NF-MACE were similar between all groups. Over half of the patients maintained the antihypertensive medication for the subsequent 5-year follow up. There was no difference in outcome for the use of any particular class of BP medication in terms of long term outcomes. Specifically, ACE inhibitors did not reduce angiographic CAV as intimated in previous reports.

Conclusions: 3 or more BP medications are associated with poor outcome post heart transplant. This suggests that more refractory hypertension may relate to lower survival.

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THE LONG-TERM BENEFIT OF RENAL SPARING PROTOCOLS DEMONSTRATE THE LACK OF NEED FOR CALCINEURIN INHIBITORS
Dyo J, Kawano M, Goldstein Z, Hamilton M, Kobashigawa J Cedars-Sinai Heart Institute, Beverly Hills, CA.

Purpose of Study: Calcineurin inhibitors (CNI) have been the mainstay of immunosuppression after heart transplantation. However, these agents have many adverse events which include kidney failure, hypertension, hyperlipidemia, osteoporosis, and gout. There have been many attempts to wean patients off CNI in patients who have developed ongoing renal failure. There is concern that patients weaned off CNI may not have optimal long term outcome. Therefore, we assessed our patients on a renal sparing protocol (RSP) without CNI for long-term outcome compared to controls maintained on CNI-based immunosuppression.

Methods Used: We reviewed 1266 patients transplanted between 1994 and 2010, and identified 40 patients on renal sparing protocol after heart transplantation. These patients were compared to a 2:1 contemporaneous control group matched for age, gender, and time after transplantation (compared to start of RSP). Subsequent five year outcomes including renal function, actuarial survival, actuarial freedom from cardiac allograft vasculopathy (CNI, stenosis ≥30%), and actuarial freedom from non-fatal major adverse cardiac events (CNI-MACE, MI, heart failure, PTCA, pacemaker, stroke, new peripheral vascular disease) were assessed. Patients placed on dialysis were censored thereafter.

Summary of Results: Patients were found to start RSP at 5.5 ± 3.1 years post heart transplant, and had an average follow up time of 2.0 ± 2.0 years. Renal function improved significantly in those patients on the RSP compared to those control not on RSP (percentage change in creatinine: −21% vs. 24%, p<0.001). Subsequent five year actuarial survival, freedom from CAV, and freedom from CNI-MACE were found to be similar between the two groups. 4/40 patients who started RSP eventually developed renal failure requiring chronic renal dialysis at a mean time of 2.1 ± 1.4 years post RSP initiation.

Conclusions: A renal sparing protocol with withdrawal of calcineurin inhibitors appears to be effective in preserving kidney function in the long term without increase in mortality or morbidity (worsening renal functioning necessitating kidney dialysis).
Methods Used: We assessed our gender matched and mismatched patients transplanted between 1994 and 2010 to assess the incidence of 1st-year AMR. These patients were divided into 4 groups based upon donor and recipient gender (donor/recipient). AMR diagnosis was defined as pathologic, histologic and immunopathologic findings. We divided these patients into asymptomatic AMR vs. treated AMR.

Summary of Results: We found that the incidence of 1st-year AMR was significantly increased in the M/F group versus M/M (1% vs. 5%, p<0.005), and F/F (11% vs. 6%, p=0.046). The M/F group was found to have significantly greater asymptomatic AMR compared to the M/M group (6% vs. 2%, p=0.016) but not to the F/F group. The F/M group was found to have similar incidences of 1st-year treated AMR and asymptomatic AMR when compared to the gender matched groups. In the M/F and F/F groups, there was no difference in the percentage of multiparous females.

Conclusions: Heart transplant patients with M/F gender mismatch on triple-drug immunosuppression have an increased incidence of 1st-year antibody-mediated rejection compared to gender match patients. Although multiparous female recipients are known to have more AMR, the F/F gender match group had similar incidence of AMR to those of the M/M gender match group. Future efforts to understand and attenuate the underlying mechanisms are essential.

43 THE DEVELOPMENT OF LATE ONSET AMR: DOES IT EXIST?
Goldstein Z, Kawano M, Hamilton M, Kobashigawa J Cedars-Sinai Heart Institute, Beverly Hills, CA.

Purpose of Study: Antibody-mediated rejection (AMR) is usually seen early after heart transplantation and may be associated with graft dysfunction and circulating antibodies. Interestingly, as the risk of cellular rejection gradually decreases the incidence of AMR appears to be rising. Late onset AMR is also becoming more prevalent. We choose to assess our patients with late onset AMR (greater than one year) and characterize their clinical presentation.

Methods Used: We reviewed 1290 patients transplanted between 1994 and 2010 for the development of AMR and divided them into groups based upon diagnoses of less than one year and greater than one year following heart transplantation. The definition of AMR was based on pathology of heart biopsy including characteristic histology and immunopathology findings. These patients were then assessed for the development of restrictive physiology, hemodynamic compromise, and subsequent 5-year outcomes including actuarial survival and the development of transplant coronary artery disease (stenosis ≥50%).

Summary of Results: There were 112 patients in the early AMR group versus 37 patients in the late AMR group. There was a greater trend for the development of restrictive physiology (10.7% vs. 2.5%, p=0.10) and hemodynamic compromise (17.1% vs. 9.4%, p=0.19) for the late AMR group. Similarly, those patients exhibiting late AMR also demonstrated a greater trend towards developing subsequent 5-year transplant coronary artery disease (43.2% vs. 28.3%, p=0.06). Treatment of these AMR episodes included IV Solumedrol, Rituximab, Plasmapheresis, Bortezomib, ATG, or IVIG, with ease (43.2% vs. 28.3%, p=0.06). Treatment of these AMR episodes included IV Solumedrol, Rituximab, Plasmapheresis, Bortezomib, ATG, or IVIG, with ease (43.2% vs. 28.3%, p=0.06).

Conclusions: Late antibody-mediated rejection demonstrates a consistent trend towards more severe presentation with hemodynamic compromise, increased risk for developing CAV, and increased risk in the development of restrictive cardiac physiology. Continued surveillance for the detection and means for prevention of AMR in heart transplant patients late after transplant should be pursued.

45 IMPACT OF METABOLIC SYNDROME ON CARDIOVASCULAR AND MENTAL DISEASES IN A VA POPULATION
Singh M1,2, Gu W1,2, Mallios R1, Mcfarland S1, Fong J1, Huang J1,2 1VA Central California Healthcare System, FRESNO, CA and 2UCSF, Fresno, CA.

Purpose of Study: Metabolic syndrome (METS) is a cluster of conditions that confer greater cardiovascular (CV) risk and may be associated with certain mental disorders. A quarter of US population is currently affected by METS and the prevalence increases with age. We sought to examine the prevalence of METS and to determine the impact of METS on CV and psychiatric diseases in a VA population.

Methods Used: Demographics and clinical data on diagnosis, labs and medication profiles were collected from 17,466 patients in this cross-sectional study. METS was defined by modified NCEP criteria. T-test and Chi square test were used for demographic and risk comparison between those with and without METS. METS square test and ANOVA were used for risk comparison among groups with increasing number of METS components. Logistic regression model was used to determine the adjusted odds ratios.

Summary of Results: The prevalence of METS was 58%. Although numbers of patients who ever smoked tobacco between the two groups were similar (P=0.05), there were significantly less current smokers in METS+ group (P<0.001). Among patients with METS, there were significantly higher mean FS and increased prevalence of PCI, MI, CABG, PVD, ED CKD, depression, PTSD (P<0.001), CVA/TIA, and anxiety (P<0.002). TC and LDL were lower while non-HDL cholesterol was higher in METS+ group, consistent with more atherogenic lipid profiles. There was significantly proportional increase in a number of CV and mental diseases with increasing number of METS components. After adjusting for age, sex, BMI, diagnosis of hypertension and diabetes, and statin use by logistic regression model, METS remained significantly associated with MI, CABG, PVD, CKD, anxiety and PTSD.

Conclusions: There are more than double cases of METS in this study compared to US general population. METS is significantly associated with certain CV and mental diseases and the degree of this association becomes stronger with increasing number of individual METS components. More aggressive intervention targeting multiple CV risk factors is particularly important for this high-risk patient population. Our results also suggest the need to reduce concomitant CV risk in the long-term management of depression, anxiety, and PTSD.
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INCIDENCE, ETIOLOGY, AND SURVIVAL TRENDS FROM SUDDEN CARDIAC ARREST IN CHILDREN AND YOUNG ADULTS AGE 0-35 IN KING COUNTY, WASHINGTON: A 30-YEAR REVIEW

Meyer L, Drezer J, Fahrenbruch C, Stubbs B, Maeda C, Harmon K, Eisenberg M1,2 University of Washington, Seattle, WA and 2King County Emergency Medical Services, Seattle, WA.

Purpose of Study: Sudden cardiac arrest is a leading cause of death in children and young adults, although little is known regarding the epidemiology of such events. This study determined the incidence, etiology, and outcomes of out-of-hospital cardiac arrest (OHCA) in individuals age 0-35.

Methods Used: This was a retrospective cohort study of OHCA in children and young adults from January 1980 through December 2009 in King County, Washington. Cases of OHCA were identified from the King County Public Health Division of Emergency Medical Services (EMS) Cardiac Arrest Database, an ongoing registry of all cases of OHCA involving an EMS response. Incidence rates were calculated using population census data, and the etiology of cardiac arrest determined by review of autopsy reports, death certificates, hospital and other available records.

Secondary outcomes: a total of 116 cases (26 cases age 0-2, 30 cases age 3-13, 60 cases age 14-24, and 245 cases age 25-35) of OHCA occurred, with an overall incidence of 2.28 per 100,000 person-years (2.1 in age 0-2, 0.61 in age 3-13, 1.44 in age 14-24, and 4.40 in age 25-35). The most common etiologies of OHCA were congenital abnormalities (84.0%) in age 0-2, hypertrophic cardiomyopathy (17.9%) in age 3-13, presumed primary arhythmia (23.5%) in age 14-24, and coronary artery disease (42.9%) in age 25-35. The overall survival rate was 26.9% (3.8%) in age 0-2, 40.0% in age 3-13, 36.7% in age 14-24, and 27.8% in age 25-35. Survival increased significantly throughout the study time period from 13.0% in 1980-1989 to 40.2% in 2000-2009 (p<0.001).

Conclusions: The incidence of OHCA in children and young adults is substantially higher than previously reported. This study details the various cardiac disorders leading to OHCA in the young, allowing an increased focus on age related risk factors and development of effective screening models for prevention. This study demonstrates an important increase in survival, providing compelling support of contemporary resuscitation protocols for OHCA in the young.

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EFFECT OF RADIOFREQUENCY ABLATION OF VENTRICULAR TACHYCARDIA ON EJECTION FRACTION IN PATIENTS WITH CARDIOMYOPATHY

Finch W, Vaseghi M, Shivkumar K. David Geffen School of Medicine at UCLA, Los Angeles, CA.

Purpose of Study: To determine the effect of radiofrequency ablation (RFA) for ventricular tachycardia (VT) on ejection fraction (EF) and the correlation of the change in EF with the number of radiofrequency lesions applied.

Methods Used: We performed a retrospective chart review to create a database of patients who underwent radiofrequency ablation for VT at UCLA since 2002. A total of 125 patients received 153 procedures. The average age of patients in the database was 57 ± 16. Of the 125 patients, 84 were male, and 41 were female. The majority of patients (92) had cardiomyopathy; 43 had non-ischemic cardiomyopathy, and 49 had ischemic cardiomyopathy. Parameters included in the database were the pre-procedure and post-procedure EF and the number of VT ablation lesions applied during the procedure, as well as other procedural characteristics and outcome data. The Wilcoxon signed-rank test was performed to compare the pre-ablation and post-ablation EF; as the data did not follow a normal distribution. The correlation coefficient between the number of RFA lesions and the change in EF (ΔEF) was calculated.

Summary of Results: The median of both pre-ablation and post-ablation EF = 27.5 (p = 0.15). The correlation coefficient between the number of RFA lesions and ΔEF was -0.08.

Conclusions: Although it has historically been thought that because radiofrequency creates burns in myocardium, ejection fraction would worsen after VT ablation, the present study determines that this is not the case. There was no significant change in EF following ablation. Additionally, there was no correlation between the number of radiofrequency lesions and ΔEF. These results confirm findings from previous studies that VT ablation is not detrimental to cardiac function, and that applying more lesions does not result in worsened EF.
Summary of Results: Health care projects chosen by the mentees included teenage pregnancy, alcoholism, obesity, heart disease, schizophrenia and smoking. The mentees evaluated the program as very effective in building a bond with their mentor, becoming aware of community problems as well as increasing their knowledge and interests about different health-related careers. The mentors liked the sense of giving back to the community and accessibility to the directors of the program. Both the mentors and the mentees said that the flexibility in scheduling and pairing according to their interest was very helpful. The mentees stated that they wished they had more time with the mentors.

Conclusions: Our one-on-one mentorship program which matched the mentors and mentees based on their interest and empowered the mentees to lead a project was very effective and raised both the mentee and mentors’ interest in healthcare fields and community service. Because of the interest of our school partners, students and mentors, our program is expanding rapidly.

50 IDENTIFYING FACTORS IN UNDER-REPRESENTATION OF MINORITY YOUTH IN HEALTHCARE CAREERS

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Purpose of Study: The under-representation of minority population in different healthcare fields has been identified as one of the major reasons responsible for healthcare disparity in the United States. The objective of this study was to: a) evaluate some of the barriers among high school students in choosing healthcare careers and b) to assess the effectiveness of our workshops in introducing different healthcare professions to high school students.

Methods Used: At the Center for Future Health Professionals, UC Irvine, we conducted workshops regarding different healthcare fields in several high schools with significant drop-out rates. We distributed a questionnaire to evaluate the barriers in choosing healthcare fields as a career.

Summary of Results: One hundred and ninety students were surveyed. The response rate was 100%. Of 190 students 85% were Hispanic. The most common barriers identified in choosing a healthcare field included lack of a role model, lack of financial support and the long road to finish. Majority of students were not aware of careers in allied health care fields, such as child life, social work, respiratory therapy, case management and healthcare billing.

The students evaluated our workshop as very effective in introducing them to different healthcare professions and more than 90% said that they were more interested in a healthcare field as a result of our workshops.

Conclusions: Among several barriers, lack of knowledge about different healthcare careers may contribute to the under-representation of minority groups in healthcare fields. Programs that address the barriers responsible for under-representation of minority in healthcare are needed.

51 ADDRESSING CHILDHOOD OBESITY THROUGH THE DEVELOPMENT OF A FARMERS’ MARKET IN GILLETTE, WYOMING

Gorden N University of Washington, Seattle, WA.

Purpose of Study: Campbell County in northeastern Wyoming is experiencing the state’s largest population growth rate, particularly among persons under age 18. Despite increased spending on recreation programs and high participation in school athletics, the school district shows that 22% of boys (N=886) and 22.6% of girls (N=823) in grades 7–9 are in the 95th percentile for body mass index and are obese. Access to healthy food, one factor associated with childhood obesity, is poor. The goal was to establish a farmers’ market (FM) to provide a source of fresh fruit and vegetables.

Methods Used: Discussions with the school health director, public health officer, child development coordinator, and physicians revealed childhood obesity as a health concern. Meetings with local food producers, city officials, and volunteers were aimed at increasing FM attendance and attracting diverse food vendors. Research of peer-reviewed articles on obesity and nutrition identified barriers to healthy foods and effective methods of teaching nutrition. At the FM, media was distributed with the USDA diet guidelines and listing the benefits of fruit and vegetable consumption. School-aged children were allowed to try new fruit and vegetables. FM visitors and state representatives received WIC and Senior Farmers’ Market Nutrition Program resources to bring awareness to federal food subsidy programs.

Summary of Results: The FM was moved to a new location to accommodate growth. Ten vendors participated in the first market of the 2010 season: 4 local farmers, 2 confectioners, 1 jeweler, and 3 educators. In four hours, over 150 visitors attended. Sixteen school-aged children sampled fruit and vegetables and 20 visitors accepted brochures on the benefits of consuming local fruit and vegetables. Seven visitors inquired about federal food subsidies. By the seventh week of the FM, 19 vendors and 579 visitors attended.

Conclusions: Research suggests that individual-centered interventions are insufficient to address childhood obesity and related disease. Corrective action must focus on the home, community, and national environment. The atmosphere of FMs can reach this broad scale. Further FM growth will have an additive effect on the overall health of communities like Gillette, WY which already boast high levels of physical activity and a steady economy.

52 PREVENTION OF POSTPARTUM SMOKING RELAPSE IN MOTHERS TO PREVENT INFANT EXPOSURE TO SECOND-HAND SMOKE

Adams KK, Merritt TA Loma Linda University, Loma Linda, CA.

Purpose of Study: A significant number of women quit smoking during pregnancy; however, a majority of them resume smoking following delivery. Our study aims to prevent postpartum smoking relapse during the first 8 weeks postpartum through a weekly educational messaging program and thus reduce infants’ exposure to second-hand smoke.

Methods Used: This project was a prospective randomized trial. Mothers of infants delivered at Loma Linda University Medical Center were interviewed during their postpartum stay regarding smoking history. Mothers who smoked during the 12 months prior to conception or during pregnancy and who were not currently smoking were randomized to the trial. Mothers were randomized to a Standard of Care (SOC) or Smoking Relapse Prevention (SRP) group. Mothers in both groups watched an educational video while in the hospital regarding the importance of smoking cessation. Mothers in the SRP group received additional information after discharge, including biweekly robotic phone calls, weekly mailings, and emails which provided education on smoking relapse prevention and normal newborn care. All mothers were contacted at 2 months postpartum to assess smoking status.

Summary of Results: From March 2010 to September 2010, 328 mothers were interviewed. Twenty-nine mothers were identified as smokers, of which, 5 mothers were excluded or declined to participate. To date, 12 mothers have been contacted at 2 months postpartum, and follow-up information was available for 8. All mothers identified the video as effective. No mother identified calling as a useful means of follow-up communication. Two mothers (25%) reported smoking relapse, 1 in the SOC group and 1 in the SRP group. There are 12 additional mothers who will be contacted by the end of October 2010.

Conclusions: The postpartum stay provides an opportune time to teach mothers regarding the importance of smoking cessation and to maintain a smoke free environment for their infants. Extended contacts through educational messaging may not be enough to prevent smoking relapse.

53 DEPRESSION IN YOUTH: THE ROLE OF PASTORAL CARE IN BUTTE, MT

Kwan A University of Washington School of Medicine, Seattle, WA.

Purpose of Study: Butte is a low-income town of approximately 34,000 people located in southwest Montana with an extremely high incidence of depression and suicide. The purpose of this project is threefold: To raise awareness of the significant problem that depression represents in the community, to decrease stigma associated with mental health issues, and to provide important community figures with the ability to recognize depression in children and adolescents, and referral resources for local mental health providers.

Methods Used: Depression was identified as a major issue through input from local healthcare providers. Research and literature review were performed to specify the target population and intervention method. Through community assistance by the chaplain at the local hospital, individual meetings were arranged with leaders representing Catholic, Lutheran, Serbian Orthodox, and Episcopal faiths. The religious leaders were engaged in conversations regarding the prevalence of mental health issues in
their community, and their experiences with and their attributions for depression. They received a brief education on identifying depression in youth, were provided with materials covering ways pastoral care can help, and were given a list of community mental health resources for referral, including a personal line to a case manager with experience in dealing with youth mental health issues.

**Summary of Results:** The educational material was generally very well received, with the acknowledgement that both the identification methods and the referral information were useful. Most of the religious leaders had experience with depression and suicide in the community, whether through pastoral care, funeral services, or personal experience. Attributions for the community prevalence of depression included: alcoholism, secularism, general depressed state of the town, lack of community and social support structures, and prevalence of abuse.

**Conclusions:** Engaging community leaders regarding mental health issues and resources allowed for the opportunity to increase awareness of the issues facing the community, the signs and symptoms of child and adolescent depression, and the available community mental health resources. Face-to-face meetings allowed for a personal and open connection to occur, which may have increased benefit for reception of the material.

### 54 ENGAGING CHILDREN IN PHYSICAL ACTIVITY AND HEALTH AWARENESS, SEATTLE WA

**Le TH**

**University of Washington School of Medicine, Seattle, WA.**

**Purpose of Study:** The Rainier Valley has the greatest cultural and socioeconomic diversity in Washington State, but nearly twice the poverty rate of the greater Seattle area. This inequality has a profound effect on childhood development: Children from low-income families are more likely to become obese, which leads to later chronic health problems. These children are also 80% less likely to receive routine health and vision care. The purpose of this project was to promote exercise and connect families to resources for free vision care in the community.

**Methods Used:** Sports-based youth development programs are strongly supported in the literature for positive influence on behavior. This project utilized a tennis camp to advocate both physical activity and to distribute information regarding health and vision care. With support from the community, donations and volunteers were gathered to organize a non-profit tennis camp for children. Participants received individual tennis instruction from volunteer coaches and were encouraged to continue exercising. At the end of tennis camp, each participant also received: 1) A new junior tennis racquet and balls. 2) Information about local tennis facilities. 3) Neighbor-Care Health® medical clinic brochures. 4) A letter addressed to parents/guardians about the importance of routine health and vision care. 5) Information about how to access Sight for Students, a national charity program for free eye exams and glasses for students under 18.

**Summary of Results:** Eight children, ages 8–12, attended both days of tennis camp and received new, donated equipment. Participants and their families expressed appreciation for the event and interest in continuing playing tennis. A community outreach letter, including a consolidated information sheet about Sight for Students, was created and distributed to twelve families during the event. This information was reproduced and made available at the Rainier Park Medical Clinic.

**Conclusions:** Sports-based youth development programs involve extensive coordination and organization, and are limited by the number of participants. Although it may not be the most efficient intervention, the experience for each participant is powerful and lasting. To maximize community outreach, health information that is well-organized and easy for patients to understand should also be distributed in these programs and reproduced for future use.

### 55 REDUCING FRUSTRATION TO INFANT CRYING: A LAB BASED TEST OF THE PERIOD OF PURPLE CRYING MATERIALS

**Lou C**

**D’Souza N**

**Chen M**

**Barr B**

**University of British Columbia, Coquitlam, BC, Canada and Child and Family Research Institute, University of British Columbia, Vancouver, BC, Canada.**

**Purpose of Study:** Infant crying is the most common stimulus for Shaken Baby Syndrome (SBS) that results in traumatic brain injury in infants. The Period of PURPLE Crying is a SBS prevention program that is implemented jurisdiction-wide in British Columbia, Canada and many states in the USA. It aims to reduce SBS incidence by informing new parents about the properties of crying in normal infants, the dangers of shaking, and coping strategies.

The object of this study is to determine whether the Period of PURPLE Crying DVD reduces maternal frustration and affects choice of coping strategies in response to prolonged crying.

**Methods Used:** Participants in this study are first time pregnant women fluent in English with no previous exposure to PURPLE. In a randomized controlled trial, participants watched either the PURPLE DVD or a control DVD on Infant Safety. Following the DVD, participants listened to a 10-minute audio-recording of an infant crying (duration unknown to participants) while continuously rating frustration levels on a visual analog scale (CVAS). Scores ranged from 0 to 100 (‘no frustration’ to ‘extreme frustration’). Participants completed a post test questionnaire asking how likely (from 0=‘not at all likely’ to 4=‘extremely likely’) they were to use each of ten strategies to cope with crying.

**Summary of Results:** To date, 33 participants (19 PURPLE & 14 control) have participated. Mean frustration level during the audiorecording was lower in the PURPLE than in the control group (19.3 ± 15.5 vs. 28.5 ± 23.4, t=1.13, p=0.10). PURPLE participants reported greater likelihood to “put baby somewhere safe and walk away” as a coping strategy (t=2.1, p=0.04). There were no significant differences in employing the other nine strategies.

**Conclusions:** PURPLE participants responded to infant crying with lower frustration than controls. They were also more likely to choose a “walk away” strategy than controls. These preliminary results suggest that PURPLE education materials may have an impact on maternal frustration and coping behaviors considered relevant to preventing shaking. Data collection will continue until the predetermined sample size (n=45/group) is reached.

### 56 ORAL HYGIENE EDUCATION FOR OLDER CHILDREN IN CENTRALIA, WASHINGTON

**Srivastava K**

**University of Washington School of Medicine, Seattle, WA.**

**Purpose of Study:** Tooth decay and infection are common in children at the Pope’s Kids Place Medical Clinic in Centralia, WA. The majority of patients are of low-income and have DSHS (Medicaid) insurance; as a result, they are at a higher risk of developing tooth decay. The Pope’s Kids Place Dental Clinic is part of Washington’s Access to Baby and Child Dentistry Program through DSHS. The ABCD program provides dental education and prevention for children from birth to six years of age. However, there is no outreach program for children over six years of age at the dental clinic. The purpose of this project was to develop a presentation about preventing tooth decay through healthy oral hygiene practices, with the target population of eight to ten year olds.

**Methods Used:** Discussions with the dental clinic education/outreach coordinator led to identifying the target population and development of materials for the presentation. Four main points were identified for the presentation based on the observations of the coordinator educating children and parents on oral hygiene. A literature review was conducted to validate and support the main ways to reduce the risk of developing dental caries in children - low sugar diet, tooth cleansing, fluoridation, and regular dental visits.

**Summary of Results:** The presentation was given to 35 elementary school students. First, children brushed off grape juice from hard-boiled eggs to simulate brushing of the teeth to remove sugars. Proper brushing and flossing techniques were demonstrated on a large mouth model. Finally, a laminated flyer for parents was distributed containing tips for maintaining healthy teeth and dental clinic referrals on one side and a brushing/flossing daily tracking chart on the other side. The children and their teachers were very receptive to the presentation. The dental clinic can use this presentation as part of their outreach program in the future.

**Conclusions:** The goal of the project was to educate older children on prevention of dental disease and instill good oral health practices, and in turn, the children can inform parents and younger siblings of their education. Pediatricians are the first healthcare providers that children see and therefore, pediatricians can be actively involved in identifying tooth decay and educating families in preventing infection in the future.

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57 ADOLESCENT SUICIDE PREVENTION IN DAYTON, WASHINGTON

Wurz KA University of Washington, Seattle, WA.

Purpose of Study: Dayton is a small town, population 2,735, located within Columbia County in the southeastern corner of Washington. Adolescents make up roughly 10 percent of Dayton’s population, for whom suicide is a serious public health concern. Suicide is the third leading cause of death for adolescents in the United States and in 2008 16% of Columbia County youth reported having considered suicide. Studies have shown that school-based suicide prevention programs are an effective method for reducing adolescent suicide rates. With the loss of several Dayton adolescents to suicide, the most recent in June 2010, this serious public health issue must be addressed by the community. The purpose of this project was to catalyze and communicate with key youth community resource organizations to encourage appropriate adolescent suicide prevention.

Methods Used: The need for a suicide education program in Dayton was revealed by meeting with: adolescents, public health nurses, a mental health preventionist, and the Columbia County Coalition for Youth and Families. These groups collectively agreed that use of both brochures and an in-class education program would be most effective. A literature review was performed to identify effective school-based suicide education programs and to provide guidance in the creation of an educational flyer. A written report on adolescent suicide and effective school-based prevention programs was presented to several youth community resource organizations. The key suggestion was purchase and implementation of the Signs of Suicide Prevention Program in Dayton schools.

Summary of Results: The mental health preventionist and Dayton School District superintendent plan to meet and discuss suicide prevention before the start of school in fall. An educational flyer was created and placed in Dayton High School restrooms.

Conclusions: The community of Dayton now recognizes the need for an adolescent suicide prevention program. Various community groups within Dayton are now taking steps toward implementing a school-based prevention program, but the cost will be a challenge. The use of flyers to educate Dayton adolescents about the warning signs and risk factors of suicide is more cost effective; unfortunately the impact of the flyers produced is not statistically known.

58 HEALTHY LIFESTYLE INTERVENTION: EDUCATING FIFTH AND SIXTH GRADE STUDENTS WITH THE HEALTH4LIFE PROGRAM

Young K1, Serrano V1, Castillo F2, Baum M3 1Loma Linda University, Loma Linda, CA, 2Loma Linda University, Loma Linda, CA and 3Loma Linda University Medical Center, Loma Linda, CA.

Purpose of Study: Childhood obesity is listed in California as a significant health issue. The Health4Life program was an eight week school-based healthy living educational intervention conducted in fifth and sixth grade classrooms through May, 2010. The questions examined for this analysis were: 1. Did students’ knowledge on healthy living topics increase? 2. Did students increase in healthy behaviors over the program course?

Methods Used: The Health4Life program was conducted in 27 classrooms with a total of 1262 students enrolled. Weekly intervention consisted of a one-hour lecture on 1) Sleep, 2) Breakfast, 3) Soft Drinks, 4) Fruits and Vegetables, 5) Feelings and Depression, 6) Fats/Fast Food 7) TV Time/Exercise and 8) Safety. Pretests and post-tests were analyzed for knowledge improvement as students showed a 9.9% increase (p=0.000) in comprehensive knowledge, and specifically in lecture content. Immediate feedback was provided as a histogram representing all students’ answer choices and answer options were discussed in detail. Students were surveyed twice using the ARS. An e-mail survey of instructors was conducted. Also, a focus group of medical students took place to gather opinions on the ARS.

Summary of Results: Health knowledge improved as students showed a 9.9% increase (p=0.000) in comprehensive knowledge, and specifically in topics of Breakfast (11.8%), Soft Drinks (20%), Fats/Fast Food (12.5%), TV Time/Exercise (13.1%) and Fruits and Vegetables (4.5%). Behavioral change showed a significant decrease in Fats/Fast Food consumption from baseline at week five (8.2%, p=0.006) and week eight (12.5%, p=0.011). There was a decreasing trend in soda consumption at week five (3.8%) and baseline at week five (8.2%, p=0.006) and week eight (12.5%, p=0.011). Of interest, 20% of students consistently reported weekly episodes of sadness.

Conclusions: The Health4Life classroom based intervention demonstrated a health benefit in knowledge acquisition and a change in behavior choices with a decrease of fast food consumption over eight weeks. Additional information could be gained from longitudinal follow up. Lifestyle changes are difficult to sustain without involvement. A benefit to the program could be “refresher lectures,” an incentive system for healthy choices, and continued school participation in the areas of nutrition and exercise.

59 STUDENT PERSPECTIVES ON THE INSTITUTIONAL DIVERSITY CLIMATE AT A U.S. MEDICAL SCHOOL: THE NEED FOR A BROADER DEFINITION OF DIVERSITY

Dhillon JS1, Crane LA2, Kilkenny R1, Valley MA3, Kaul P4, Nyquist A5, Lowenstein SR1,2 1University of Colorado School of Medicine, Aurora, CO; 2Colorado School of Public Health, Aurora, CO and 3Colorado Health Foundation, Denver, CO.

Purpose of Study: Medical schools frequently experience problems related to diversity and inclusiveness. The authors conducted this study to assess, from the student body’s perspective, the climate at one medical school with respect to diversity, inclusiveness and cross-cultural understanding.

Methods Used: In 2008, students in the M.D., P.T., and P.A. programs at a medical school were asked to complete a survey consisting of 24 Likert-scale, short-answer and open-ended questions. Questions were designed to measure student experiences and attitudes in six domains: general environment and culture; witnessed negative speech or behaviors; barriers to incident reporting; isolation of minorities; diversity and the learning environment; and campus leadership.

Summary of Results: Of 883 eligible students, 261 (29.56%) participated. Most respondents agreed that the SOM campus is friendly (90%) and welcoming to minority groups (82%). Although 90% found educational value in a diverse faculty and student body, only 37% believed the campus is diverse. Likewise, many respondents have witnessed students or residents make disparaging remarks or exhibit hostile behaviors toward persons with strong religious beliefs (43%), low socioeconomic status (35%), non-English speakers (34%), women (30%), racial/ethnic minorities (28%), and GLBT individuals (25%). Respondents witnessed similar behaviors from faculty members toward persons with strong religious beliefs (18%), women (18%), and persons of low socioeconomic status (12%). Open-ended comments highlighted strong religious beliefs and conservative values as common targets. Students were unlikely to report these behaviors because of grading concerns (95%) and the absence of a confidential reporting system (28%).

Conclusions: Diversity and a culture of inclusiveness are important medical school assets. Schools must utilize broad definitions of diversity such that all minority groups are valued, including those with conservative viewpoints and strong religious beliefs.

60 AUDIENCE RESPONSE SYSTEM USE IN UNIVERSITY OF BRITISH COLUMBIA MEDICAL SCHOOL UNDERGRADUATE EDUCATION

Wilson I1, Afshar K2, Peterson L2, Masterson J3 1University of British Columbia, Vancouver, BC, Canada and 2University of British Columbia, Vancouver, BC, Canada.

Purpose of Study: To further explore the feasibility and benefits of the use of an audience response system (ARS) in the UBC distributed MD undergraduate program, and to determine whether students favour an inquiry based learning format incorporating ARS in the educational sessions following the weekly problem based learning (PBL) case. Finally, to gather feedback from instructors and students as to how to improve the use of ARS.

Methods Used: Students used an ARS device simultaneously at the 3 geographically separated medical program sites to answer 6 to 7 multiple choice questions posed by instructors. Questions were related to a recently completed PBL case and the week’s lecture content. Immediate feedback was provided as a histogram representing all students’ answer choices and answer options were discussed in detail. Students were surveyed twice using the ARS. An e-mail survey of instructors was conducted. Also, a focus group of medical students took place to gather opinions on the ARS. A 5 point Likert scale was used.

Summary of Results: Students were surveyed in weeks 1 and 4 out of a 5 week period of study. The median answer was “agree” to all 5 survey statements, demonstrating students (average n=180) favoured the educational
sessions using an ARS. After 4 weeks of use there was no change in the response to questions 1 and 2 of the survey (Median=4 both week 1 and 4 Mann Whitney U test p=0.23). This was a uniform finding across the 3 sites. All instructors (n=6) surveyed felt that use of the ARS should be expanded within the undergard curriculum.

**Conclusions:** The majority of students agreed that the introduction of an ARS to post-PBL sessions aided them in identifying learning gaps, enhancing understanding of key concepts, and provided for an enjoyable, engaging, and focused session. Both students and instructors agreed they would like to see the ARS format expanded across the curriculum and incorporated into these sessions.

### Table 1. In Class Student Survey Questions

<table>
<thead>
<tr>
<th>Question</th>
<th>Median Response</th>
</tr>
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<tbody>
<tr>
<td>The use of TurningPoint® helps me quickly identify gaps in my learning.</td>
<td>3</td>
</tr>
<tr>
<td>The use of TurningPoint® enhances my understanding of key concepts.</td>
<td>4</td>
</tr>
<tr>
<td>The use of TurningPoint® is enjoyable, engaging, and promotes more focused discussion during case wrap up</td>
<td>3</td>
</tr>
<tr>
<td>I would like to see TurningPoint® used in other PRIN/PMED blocks during case wrap up</td>
<td>3</td>
</tr>
<tr>
<td>The questions posed via TurningPoint® were an appropriate level of difficulty.</td>
<td>4</td>
</tr>
</tbody>
</table>

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**Endocrinology**

**Concurrent Session**

**8:30 AM**

**Friday, January 28, 2011**

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**61**

**AN UNUSUAL CASE OF SUPPURATIVE THYROIDITIS CAUSED BY ESCHERICHIA COLI AND ENTEROCOCCUS FAECALIS**

Bouchonville M, Kapsner P University of New Mexico, Albuquerque, NM.

**Case Report:** Acute suppurative thyroiditis is an uncommon inflammatory thyroid disease caused by bacterial infection. Affected individuals are typically susceptible to infection due to immunosuppression or anatomical abnormalities. The most commonly observed organisms in this condition are Streptococcus and Staphylococcus species. Suppurative thyroiditis involving Escherichia coli has been rarely reported in association with urinary tract infections. We report an unusual case of suppurative thyroiditis caused by Escherichia coli and Enterococcus faecalis in a patient with a dental abscess.

A 50-year-old woman presented to the ER with fever, tender swelling of her right neck, dysphagia, and dyspnea one week after being treated for a dental abscess. WBC count was 12.9K (normal 4–10K cells/mm3), TSH 1.02 (normal 0.4–4.5 UIU/mL), total T4 16.3 (normal 4.7–11.0 ug/dL), free T4 1.1 (normal 0.8–1.6 ng/dL), total T3 251 (normal 184–172 ng/dL), and thyroglobulin 5,831 (normal 0.4–124 ng/mL). CT of the neck demonstrated a large complex hypervascular thyroid nodule measuring 7cm in maximum diameter with significant mass effect on the trachea, extension into the superior mediastinum, and evidence suggesting neoplasm versus supplicative thyroiditis. Fine needle aspiration for cytology demonstrated a follicular lesion, and culture of the aspirate was positive for Enterococcus faecalis and E. coli. A barium swallow study demonstrated no evidence of pyriform sinus fistula or brachial arch anomaly. Dental consultation was obtained for tooth extraction, and the patient was treated with parenteral antibiotics with rapid reduction in size of the neck mass. She subsequently was referred for hemithyroidectomy confirming the follicular lesion to be a benign adenoma. The described case of acute suppurative thyroiditis caused by E. faecalis and E. coli in a patient with dental abscess has, to the best of our knowledge, never been reported and emphasizes the need to investigate the cause of this potentially life-threatening condition. We review the relevant literature regarding acute suppurative thyroiditis as well as a recently proposed algorithm for the management of this rare disorder.

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**62**

**DIAGNOSIS OF DIFFUSE IDIOPATHIC PULMONARY NEUROENDOCRINE CELL HYPERPLASIA IN A PATIENT WITH A COMPLEX PULMONARY HISTORY**

Sadler C, Kapsner P University of New Mexico, Albuquerque, NM.

**Case Report:** Diffuse idiopathic neuroendocrine cell hyperplasia (DIPNECH) is a rare syndrome with fewer than 50 cases reported. It presents with cough, progressive dyspnea and irreversible airflow obstruction in middle aged, non-smoking females. It results from hyperplasia of the pulmonary neuroendocrine cells scattered through the bronchial tree and is likely part of the spectrum of carcinoid tumorlet and tumor. We present a case of a patient with a complicated pulmonary history diagnosed with DIPNECH.

A 56-year-old female with a history of asthma, obesity and sleep apnea presented with progressive dyspnea and hypoxemia. Initial concern for worsening asthma, congestive heart failure and pulmonary embolism was not substantiated. Patient had a history of pulmonary nodules noted in the 1990s, followed clinically. On computed tomography scan in 2008 these nodules were noted to have grown leading to a left video-assisted thoracoscopic upper and lower lobe wedge resections and thoracotomy with mediastinal lymph node dissection. A diagnosis of two carcinoid tumors and multiple miliary pulmonary nodules was made. Follow up octreotide scan showed no residual disease. However, over the next year, patient’s dyspnea and hypoxemia significantly progressed. Symptoms were initially felt to be possibly related to miliarythiomatosis, and patient underwent a trial of RU486, 200 mg/week. Patient did not show the expected clinical improvement. Serial monitoring of serum chromogranin A showed increasing levels from 21 ng/ml post wedge resection up to 154 ng/ml (normal 0–50 ng/ml). Due to the patient’s complex pulmonary disease and progressive symptoms, consultation was undertaken at National Jewish Health. Review of pathology identified carcinoid tumorlets and “proliferation of single neuroendocrine cells confined to the broncholar epithelium.” A diagnosis of DIPNECH was made. Patient was subsequently treated with sandostatin LAR with improvement in her chromogranin A levels and symptoms.

This case demonstrates a symptomatic presentation of the rare syndrome DIPNECH in a patient with multiple underlying pulmonary pathologies. We will review the literature relevant to the diagnosis and management of DIPNECH.
64 INFLUENCE OF ETHNICITY IN SUPPRESSION AND RECOVERY OF SPERMATOGENESIS IN MALE HORMONAL CONTRACEPTIVE TRIALS

Ilan N^1, Liu PY^2, Swardtloff RR^1, Wang C^1^2-Harbor-UCLA Medical Center and Los Angeles Biomedical Research Institute, Torrance, CA and ^2Woolcock Institute of Medical Research, University of Sydney, Sydney, NSW, Australia.

Purpose of Study: Unintended pregnancy remains an important global problem. Male hormonal contraceptive studies in the last three decades demonstrate efficacy and reversibility of this method in men. In these studies, it was noted that ethnicity may influence the degree of sperm suppression and recovery. For this reason, we conducted an integrated analysis of available data from previous studies to examine the influence of ethnicity in male hormonal contraception.

Methods Used: De-identified individual subject data were supplied by investigators of 20 single-, and 10 multi-center studies spanning five continents (North and South America, Europe, Asia and Australia) and completed before 2007 through a standardized worksheet that was approved by the Harbor-UCLA Medical Center Institutional Review Board. Data were analyzed using Kaplan-Meier and Cox regression.

Summary of Results: Our integrated analysis shows: 1) Male hormonal contraception using androgens alone suppress sperm output in up to 90% of Asian men but only up to 80% in Caucasian men. 2) Addition of a progestin causes suppression of sperm output to <1 million/ml in nearly all Asian men and over 90% of Caucasian men. 3) All men will eventually recover sperm output irrespective of ethnicity, although recovery is more rapid in Asian men.

Conclusions: Ethnic differences in the suppression of spermatogenesis suggest that testosterone alone male contraceptive methods may not be suitable globally. Addition of a progestin quantitatively overcomes this ethnic difference and results in adequate suppression of sperm concentrations and probably contraceptive efficacy for all men.

65 ESTRADIOL17β (E2) ATTENUATES THE GnRH MEDIATED INCREASE IN INTRACELLULAR Ca2+

Mrdutt M^1, Magee C^2, Cantlon P^2, Martazina D^2, Sanborn B^2, Clay C^2
^1Univ of Washington, Seattle, WA and ^2Colorado State Univ, Fort Collins, CO.

Purpose of Study: Controlling the ovulatory LH surge underlies fertility regulation. The LH surge is initiated by E2 actions at the hypothalamus and the pituitary to increase GnRH receptor expression on gonadotropes - the LH producing cells. Interestingly, however, E2 acutely but transiently suppresses GnRH mediated LH release thereby delaying GnRH production and export. We hypothesized that E2 acutely attenuates the GnRH elicited increase in intracellular Ca2+ - the cellular event underlying GnRH mediated LH secretion.

Methods Used: We established cultures of pituitary cells from transgenic mice in which expression of a fluorescent protein (YPF) is confined to gonadotropes. The distinct excitation spectra of YFP and the Ca2+ indicator FURA allowed for selection of gonadotropes and subsequent imaging of GnRH induced changes in intracellular Ca2+. After 18 hr in culture, cells were “loaded” with FURA for 1 hr. Cells were then incubated in Ca2+ containing or Ca2+ free media and received 100nM E2 or vehicle followed by a 2nM GnRH pulse at 2 and 12 min post-E2. Increases in intracellular Ca2+ were determined by a change in FURA emission spectra. Both amplitude (peak height) and magnitude (area under curve) of the Ca2+ response in 136 cells was analyzed 1 min post-GnRH.

Summary of Results: Approximately 93% of YFP positive cells displayed a Ca2+ response following the initial GnRH pulse. Of these, 84% displayed a secondary Ca2+ response; however, in both Ca2+ and Ca2+ free conditions, the magnitude and amplitude of the secondary response was reduced (p<0.05) as compared to the initial response. E2 did not alter the percentage of cells responding to either GnRH pulse. However, in both Ca2+ and Ca2+ free conditions, E2 further reduced (p<0.05) the ratio of secondary versus primary response magnitude by 25% and 31%, respectively.

Conclusions: Consistent with our hypothesis, E2 attenuates the GnRH induced Ca2+ response in gonadotropes - a potential explanation for the acute block of GnRH mediated LH release by E2 prior to the ovulatory LH surge. The rapidity of the inhibitory effect of E2 suggests a non-nuclear mechanism of action. If correct, new generations of E2 agonists/antagonists may allow for fertility regulation without the myriad physiological effects resulting from nuclear actions of E2.

66 UNDERSTANDING THE ACTIVIN-INDUCED PATHWAY FOR ENDOERM DIFFERENTIATION FROM EMBRYONIC STEM CELLS

Sinha A^1, Kartikasari A^2, Bhushan A^2^3 David Geffen School of Medicine at UCLA, Los Angeles, CA and ^2University of California at Los Angeles, Los Angeles, CA.

Purpose of Study: The efficiency of deriving functional β-cells from embryonic stem cells (ESC’s) for diabetes treatment is currently low. The first step in this process is to direct ESC differentiation into endoderm cells using Activin, a growth factor. However, how Activin promotes endoderm specification is unknown. Our aim was to determine epigenetic changes that promote mouse endoderm differentiation from mouse-ESCs upon Activin-induction and to identify the key players through which Activin exerts its effects.

Methods Used: Chromatin immunoprecipitation (ChIP) was used to identify two histone modifications: the permissive methylated lysine 4 on histone3 (H3K4me3) and the repressive methylated lysine27 on histone3 (H3K27me3). ChIP was also used to assess binding of Smad2/4, a transcription factor involved in Activin signaling, and Jmjd3, an H3K27 demethylase, to endoderm gene promoters before and after Activin-induced differentiation. Loss of function assays were performed for Smad2/4 and Jmjd3, using shRNA knockdowns.

Summary of Results: Upon differentiation, the bivalent H3K4me3- H3K27me3 marks present on endoderm gene promoters in ESCs, were replaced by the H3K4me3 marks in endoderm, indicating loss of the repressive H3K27me3 marks. A significant increase in binding of both Smad2/4 and Jmjd3 was noted on endoderm gene promoters upon differentiation. Knockdown of Jmjd3, decreased the efficiency of Activin-induced differentiation by 3-fold. Knockdown of Smad2/4, abolished Jmjd3 binding to the promoter of an essential endoderm gene, Sox17, and decreased Sox17 expression by 3.5-fold as measured by qrt-PCR.

Conclusions: Upon endoderm differentiation, Activin signaling via Smad2/4 directs Jmjd3 binding to the promoters of endoderm genes. The binding of Jmjd3 in turn removes the repressive H3K27me3 marks, allowing the expression of endoderm genes. This study may help increase the efficiency of deriving functional β-cells from ESCs.

67 DEVELOPMENT AND OPTIMIZATION OF MONOCLONAL ANTIBODIES TO ZINC TRANSPORTER 8 FOR IMMUNOHISTOCHEMISTRY

Gitomer S, Sarkar S, Walter J, Wenzlau J, Lee C, Hutton J University of Colorado School of Medicine, Aurora, CO.

Purpose of Study: Type 1 diabetes is an autoimmune disease in which the body’s own immune system attacks self-antigens in pancreatic islet cells, leading to a decrease in production and export of insulin. In type 1 diabetes, autoantibody markers serve as predictors of disease, measures of pancreatic β cell damage and potential targets of therapeutics. Optimized monoclonal autoantibodies can be used to purify proteins and to study protein characteristics, such as protein trafficking and topology, all of which will be important for further characterization of ZnT8—a recognized auto-antigen in type 1 diabetes. The goal of this project was to develop and optimize ZnT8 monoclonal antibodies for use in immunohistochemistry.

Methods Used: Hybridoma cultures were created to produce antibodies against the recombinant C-terminal and N-terminal domains of ZnT8, and the specificity of the antibodies was tested using radioimmuno precipitation assays (RIAs). The supernatants from the hybridomas were used as primary antibodies for both fluorescence microscopy and DAB histology staining.

Summary of Results: Both RIA and immunohistochemical assays showed that four of the hybridomas — 17H2, 10D7, and 4D2—produced antibodies specific for the N-terminal of ZnT8, but none produced antibodies specific for the C-terminal domain. These four antibodies consistently co-localized with insulin antibodies in pancreatic β cells, and were generally excluded from areas where glucagon and somatostatin antibodies bound.

Conclusions: Overall, three new monoclonal antibodies specific for the N-terminal of ZnT8 were developed and characterized for use in both
68 MULTIPLE AUTOIMMUNE DISORDERS IN NEW ONSET TYPE 1 DIABETES
Triolo TM, Yu L, Miao D, Scrimgeour LA, Klingensmith GI, Eisenbarth GS, B ker J M University of Colorado, Aurora, CO.

Purpose of Study: Children with type 1A diabetes (T1D) are at risk to develop multiple autoimmune disorders including autoimmune thyroid disease (AIT), celiac disease (CD), and Addison’s disease (AD). Assays are available for thyroid peroxidase autoantibodies (TPO), tissue transglutaminase antibodies (TTG) of CD, and 21-hydroxylase antibodies (21OH) of AD. We analyzed these non-islet, organ-specific autoantibodies at T1D diagnosis and determined the number of individuals diagnosed with multiple autoimmune disorders within six months of T1D onset.

Methods Used: Patients (n=491) were consented and screened for the presence of non-islet, organ-specific autoantibodies at T1D onset and followed for the development of AIT, CD, and AD. Eligibility included diagnosis of T1D, age 0 to 30 years, and T1D antibodies obtained within 3 months of T1D diagnosis. HLA genotyping was performed on 457 subjects with DNA available.

Summary of Results: Individuals with antibodies associated with AIT, CD, and AD at T1D diagnosis were followed for progression to clinical disease. Of the 487 with positive T1D antibodies, 160 (32.9%) had AIT, CD or AD antibodies at T1D diagnosis. 25% (122/487) were positive for thyroid peroxidase autoantibodies (TPO), 11.7% (57/487) were positive for tissue transglutaminase antibodies (TTG) and 1.0% (4/487) were positive for 21-hydroxylase antibodies (21OH). Throughout follow-up, 42 (8.6%) had evidence for progression to clinical disease (AIT, CD, or AD) and of these, 2 individuals had multiple autoimmune diseases in addition to T1D. DNA was available for 93.4% of subjects. The high-risk diabetes related genotype HLA DR3/4-DQ8 was present in 27.2% (3/114) of TPO positive (NS), 39.6% (21/53) of TTG positive (NS), and 60% (3/5) of 21OH positive subjects (NS), compared with 26.3% (80/304) of those with no additional autoantibodies.

Conclusions: In conclusion, one-third of patients newly diagnosed with T1D are positive for at least one organ specific autoantibody. Within 6 months of T1D diagnosis 15.2% (28/184) of these were diagnosed with an additional autoimmune disease. Presence of these other organ specific autoantibodies is not related to the highest-risk T1D HLA genotype.

69 A CASE OF AN EXTREMELY AGGRESSIVE PITUITARY TUMOR
Lovato C, Kapsner P, Garimella M, Univ. of New Mexico, Albuquerque, NM.

Case Report: Pituitary tumors are relatively common tumors and account for about 15% of intracranial neoplasms. The majority of pituitary tumors are benign adenomas. About 0.2% of pituitary tumors are carcinomas. Pituitary carcinomas pose a diagnostic challenge as they can have an initial presentation similar to invasive pituitary adenomas. However, these tumors typically have multiple recurrences and eventually develop craniospinal and/or systemic metastases. We describe a case diagnosed as an aggressive pituitary tumor which may in fact have been a pituitary carcinoma.

A 59 year old women presented multiple times over 4 weeks with headaches, decreased visual acuity, diplopia, nausea, and vomiting. She was initially diagnosed and treated for cluster headaches. Her symptoms failed to improve and a few weeks later she developed ptosis of her right eye. An MRI of the brain showed a large mass in the sellar region with invasion into the clavus, sellar floor, anterior temporal lobes, and compression on the right optic nerve. Prolactin, cortisol, ACTH, LH, FSH, and IGF-1 levels were normal. The patient underwent debulking of the mass. Pathology was suggestive of an atypical invasive adenoma with a high Ki-67 proliferation index and positive p53 immunoactivity.

Three weeks after surgery, the patient developed recurrence of her symptoms. Repeat MRI of the brain revealed the pituitary mass had doubled in size with significant spread into the surrounding structures, including compression of the optic chiasm. She underwent extensive debulking of the tumor as well as radiation therapy. CT scan of the chest, abdomen, and pelvis were negative for lymphadenopathy or other primary. Despite a second debubling procedure and radiation therapy, the patient’s neurological deficits progressed and repeat imaging of the brain a week later showed the tumor increased in size as well as the increased leptomeningeal spread to the cervical and lumbar spine. The patient subsequently developed respiratory failure from acute aspiration and given her poor prognosis was made comfort care.

This case illustrates the importance of recognizing the features of pituitary carcinomas and aggressive adenomas as they can have a significant impact on prognosis in these patients. We review the literature regarding the diagnosis, etiology, and therapy of these rare entities.

70 LOW VITAMIN D LEVELS IN NORTHERN AMERICAN ADULTS WITH THE METABOLIC SYNDROME
Jialal G, Cook T, Siegel I, Jialal I, Devaraj S, UC Davis, Sacramento, CA.

Purpose of Study: Vitamin D (VitD) deficiency is a worldwide problem. A growing body of evidence suggests that 25-hydroxyvitamin D [25(OH)D], a generally accepted indicator of vitamin D status, is inversely associated with adiposity, glucose homeostasis, lipid profiles, and blood pressure along with its classic role in calcium homeostasis and bone metabolism. Metabolic syndrome (MetS), a cluster of cardiometabolic disease risk factors, is now a global epidemic and predisposes to increased risks for diabetes and cardiovascular diseases. Since there is sparse data on VitD status in MetS in North America especially, the aim of our study was to examine vitamin D [25(OH)D] concentrations among adults with MetS in Northern California (sunny climate), but without diabetes or cardiovascular disease.

Methods Used: VitD levels were assayed in MetS subjects (n=44) and healthy controls (n=57), using the LIASON-25OH Vitamin D TOTAL Assay.

Summary of Results: 25OHD levels were significantly decreased in MetS compared to controls (22 vs 28 ng/mL, p<0.02). Excluding African-Americans, or using BMI/waist circumference as covariates, did not alter the significant differences. There was no significant difference between number of features of MetS and 25OHD levels. 67% of controls and 84% of MetS had insufficient 25OHD levels (<30 ng/mL) (p<0.1). 8% of controls and 30% of MetS were deficient in 25OHD (<20 ng/mL; p=0.02, C vs MetS). There were no significant differences between groups in blood sampling in winter and summer months (Chi-square p=0.47). Furthermore, we examined creatinine, calcium and phosphate, since these could regulate 25OHD levels, however, these were similar between groups. There were no significant correlations of 25OHD levels with the different features of MetS except fasting glucose (r=-0.29, p=0.04) and HOMA (r=-0.34, p=0.04) or biomarkers of inflammation (CRP, leptin, adiponectin).

Conclusions: Adults with MetS, living in Northern California, display significantly decreased vitamin D status compared to age and gender matched controls and this cannot be explained by adiposity, sun exposure, renal disease. Decreased dietary intake cannot be ruled out since it was not recorded. Since there was an increased correlation with HOMA, further studies of vitamin D supplementation in these subjects on subsequent risk of diabetes, will prove instructive.

71 THE EFFECT OF A ONE-HOUR AEROBIC RUN ON 25-OH VITAMIN D LEVELS IN TRAINED AND UNTRANIED PREMENOPAUSAL WOMEN
Schneider LE, Prowse M, Berk L, Haddock B, Loma Linda University, Loma Linda, CA; 2 Loma Linda University, Loma Linda, CA; 3 Loma Linda University, Loma Linda, CA; 4 California State University San Bernardino, San Bernardino, CA.

Purpose of Study: To compare the adequacy of dietary oral intake of Vitamin D and blood serum levels of 25-hydroxyvitamin D [25(OH)D] found in low versus high fitness premenopausal women at baseline and after a one-hour aerobic exercise.

Methods Used: 21 Trained and 14 Untrained women, maximal oxygen uptake (VO2 max) means 42.2±5.3 ml/kg/min and 27.9±3.3 ml/kg/min respectively, performed a one-hour weight bearing exercise at an aerobic intensity of 70–80% of their maximum heart rate. Serum levels were determined at pre-, post- and 30 min post-exercise for: Ca2+, iPTH, 17β-Estradiol, bone formation and resorption markers C-telopeptide of type I collagen (CTX), N terminal propeptide of type I procollagen (PINP) and 25(OH)D and adjusted for plasma volume shifts.
Summary of Results: Both groups increased serum 25(OH)D levels with exercise. Trained pre-to-post- (p<0.01) and Untrained pre-to-post- (p=0.02). Also, the 25(OH)D levels in the Trained were significantly higher than the Untrained at pre- (p<0.01), post- (p<0.01) and 30 min post-exercise (p<0.02). In both groups, Ca2+ and iPTH increased from pre-exercise to post exercise and at 30 mins post-exercise returned to pre-exercise levels.

Conclusions: Levels of 25(OH)D were significantly higher in the Trained versus the Untrained group at all time points (p<0.05). In the Untrained women, 25(OH)D mean levels were 23.5 ng/mL whereas in the Trained women, mean levels were >36 ng/mL at all time points. In evaluation of dietary intake, there were no significant differences between groups in calcium intake from foods or vitamins, consumption of fruits and vegetables, protein, caffeine or sodium intake (p<0.05). Calcium and Vitamin D supplementation were similar in both groups. These results would support the recommendation that Vitamin D supplementation may be beneficial for all populations, including premenopausal women functioning at higher levels of fitness and outdoor activity. However, fitness appears to play a key role in providing higher 25(OH)D blood levels in premenopausal women.

Genetics

Concurrent Session
3:00 PM
Friday, January 28, 2011

72 IMPROVED CLINICAL OUTCOMES OF CHILDREN IDENTIFIED THROUGH NEWBORN SCREENING WITH DEFECTS OF VITAMIN B12 METABOLISM

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Purpose of Study: Most newborn screening (NBS) programs in the United States have now expanded their screens to include methylmalonic and propionic acidemia based on the propionyl-carnitine (C3) level. Although elevations of C3 are specific not only for these two primary diseases but also a number of “secondary disorders” of cobalamin and methionine metabolism. Unfortunately there is only limited information available describing the effect NBS has had upon the clinical disease and outcome for these secondary disorders.

Methods Used: We identified a series of patients found to have cobalamin C disease, the most common of the cobalamin metabolism disorders, in the first year of life in Washington state.

Summary of Results: Six patients were identified: 3 from newborn screening and 3 by onset of clinical disease. Average C3 and methionine levels were 8.4 uM and 6.8 uM, respectively, for all patients. Three patients were diagnosed with Cobalamin C disease after presenting with clinical symptoms including failure to thrive, developmental delay, seizures, and hypotonia. All patients were started on treatment with folic acid, betaine, L-carnitine, and intramuscular hydroxocobalamin injections. All affected patients have subsequently shown clinical improvement in growth and development. Those infants identified presymptomatically through NBS have had dramatic improvements in weight gain. All patients have had dramatic improvements in all biochemical markers and have maintained mildly elevated total homocysteine (range 20–50 uM) and methionine levels in the normal range.

Conclusions: Our clinical observations support previous findings that newborns who receive early treatment due to newborn screening have improved outcomes than those who do not receive treatment until they have developed more substantial clinical disease. This provides further evidence of additional benefits from expanded newborn screening and that further attention should be given to patients with low methionine values on the newborn screen so that treatment may be initiated as soon as possible.

73 INSULIN-LIKE GROWTH FACTOR II Peptide FUSION ENABLES UPTAKE OF ALPHA-N-ACETYLGLUCOSAMINIDASE INTO MUCOPOLYSACCHARIDOSIS TYPE III B FIBROBLASTS


Purpose of Study: Mucopolysaccharidosis type III B (MPS III B; Sanfilippo B) is a lysosomal storage disorder characterized by deficiency of alpha-N-acetylglucosaminidase (NAGLU) causing lysosomal storage of heparan sulfate. Enzyme replacement therapy (ERT) has been used to treat lysosomal storage diseases but has not been successful for MPS IIIB. In particular, ERT for MPS IIIB has been limited by inadequate cellular uptake of recombinantly produced human NAGLU enzyme. In this study we create, characterize, and test a novel enzyme: NAGLU fused to the insulin-like growth factor II (IGF-II) peptide (rhNAGLU-IGF-II). The IGF-II motif was selected to enable recombinant NAGLU to enter cells via the insulin-like growth factor 2 binding site found on the mannose 6-phosphate receptor for high affinity delivery to lysosomes.

Methods Used: Following the molecular cloning of the recombinant NAGLU-IGF-II construct, rhNAGLU-IGF-II was stably expressed in Chinese hamster ovary cells in secreted form and purified. Activity assays, biochemical analysis and substrate kinetics were performed. Cellular uptake by human MPS IIIB fibroblasts and inhibition assays were followed by confocal microscopy to evaluate the subcellular distribution of rhNAGLU-IGF-II in the fibroblasts. Glycosaminoglycan (GAG) storage reduction was measured.

Summary of Results: The enzyme activity towards the substrate 4-MUNG was comparable to wild-type rhNAGLU, with peak activation near lysosomal pH. MPS IIIB fibroblasts and brain-derived cell lines readily took up the fusion enzyme via receptor-mediated endocytosis that was inhibited by competitive IGF-II peptide. Co-localization of rhNAGLU-IGF-II enzyme with lysotracker red in MPS IIIB cells via confocal microscopy confirmed lysosomal targeting. GAG storage was also reduced up to 50% (p<0.001) in MPS IIIB cells after treatment with rhNAGLU-IGF-II when measured by radioactive incorporation assay.

Conclusions: Our data suggest that the fusion enzyme, rhNAGLU-IGF-II, can enter human cells and localize in the lysosomes. Future studies in vivo are needed to more fully assess the clinical potential of rhNAGLU-IGF-II as an ERT for Sanfilippo B syndrome.

74 BRAIN MRI/MRS FINDINGS IN PATIENTS WITH SMITH-LEMLI-OPTIZ SYNDROME (SLOS) AND CORRELATIONS WITH CLINICAL AND BIOCHEMICAL PARAMETERS

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Purpose of Study: SLOS is caused by inactivating mutations of the gene coding for 7-dehydrocholesterol reductase (DHCR7), an enzyme that is necessary for the biosynthesis of cholesterol. The purpose of the current study is to identify brain characteristics in SLOS that correlate with disease severity and change with treatment. If specific clinical or imaging characteristics are likely to be useful both as diagnostic or prognostic tools as well as treatment outcome measures for clinical trials.

Methods Used: Ten patients with a diagnosis of SLOS, ranging in age from 0.4–18.2 years, served as subjects. An overall measure of disease severity was obtained using a standardized battery of physical and neurocognitive tests. Plasma levels of cholesterol and 7-DHC (the cholesterol precursor that accumulates pathologically in SLOS), were measured. Using a Siemens 3T TIM Trio MRI system, imaging and spectroscopic (MRI/MRS) data were obtained under general anesthesia. The images were used to assess gross structural abnormalities and the spectra to quantify brain lipid MRS intensity.

Summary of Results: Disease severity scores ranged from 0–40. Plasma cholesterol and 7-DHC concentrations ranged from 8–131 and 0.2–18 mg/dL, respectively. Negative correlations were found between plasma cholesterol levels and the overall clinical assessment of disease severity (r=–0.632, p=0.025), gross structural abnormalities of the brain (r=–0.813, p=0.004), and the size of the brain’s lateral ventricular system (r=–0.748, p=0.006). In addition, a positive correlation was found between plasma 7-DHC levels and brain lipid MRS intensity (r=0.738, p=0.012).

Conclusions: The data show that clinical and biochemical indices of SLOS disease severity correlate with brain abnormalities. The finding of a positive correlation between 7-DHC and the MRS measure of brain lipid intensity corroborates an earlier anecdotal report of abnormally large brain MRS lipid peaks in patients with SLOS - a measure that was reported to decrease following cholesterol supplementation therapy. This possibility that brain lipid MRS signal intensity may have diagnostic and prognostic value in
SLOS warrants further study as it may prove useful as an outcome measure and biomarker in clinical trials.

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46,XY DISORDER OF SEX DEVELOPMENT: REPORT OF A FAMILY WITH AN APPARENTLY NOVEL X-LINKED FORM

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Case Report: Disorders of sexual development (DSD) are defined by discrepancy between external genitalia, gonadal and/or chromosomal sex. Various forms have been reported to be inherited in autosomal dominant, recessive, and more rarely, X-linked fashion. We report a family in which two genetically male, maternal cousins presented with ambiguous genitalia associated with impaired testicular function. Pedigree analysis is consistent with X-linked inheritance and known forms of X-linked DSD have been ruled out. The proband presented at birth with ambiguous genitalia characterized by microphallus, bilateral undescended testes and a pigmented midline raphe extending posteriorly over fused scrotal/labial structures. Small mullerian duct remnants were seen on cysotscopy. Endocrine workup in the neonatal period included a low testosterone level and undetectable anti-mullerian hormone, implying a Sertoli cell dysfunction or anorchia. After 3 doses of hCG, testosterone level did not increase. At orchiorphy, a small testis was noted on the right and only remnant tissue was found on the left. Based on the physical and laboratory findings, endocrinology suggested that this child’s condition was most consistent with “vanishing testes”. The proband’s mother’s sister’s fetus was noted to have discrepant karyotype (46,XY) and genitalia (female appearing) on prenatal ultrasound. Pregnancy was terminated at 22 weeks. Ambiguous genitalia was noted on external examination of the fetus. 105k oligonucleotide array CGH, performed on fetal tissue to rule out NR0B1 duplication, was normal. This apparently X-linked form of 46,XY DSD is not consistent with any of the three well described causes of X-linked DSD: alpha thalassemia/X-linked mental retardation, androgen insensitivity syndrome, and duplication of NR0B1 formerly known as DAX1 which is associated with congenital adrenal hypoplasia. This appears to be a novel, X-linked form of DSD with two 46,XY maternal cousins presenting with ambiguous genitalia associated with impaired testicular development. Genetic studies of the family are underway to identify a locus/loci that may be associated with the familial disorder. The underlying genetic etiology for the findings in this family may shed light on one of the causes for “vanishing testes”.

76
XP11.4 DUPLICATION IN A SIMILARLY AFFECTED MOTHER AND SON: A NEW RECOGNIZABLE SYNDROME WITH EXPRESSION IN THE FEMALE

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Purpose of Study: Identify phenotype of Xp11.4 duplication. Generally, Xp duplications of an extensive degree such as Xp11-Xp23 have not resulted in clinical abnormalities in females. Only 3 cases in females involving Xp11- Xp14 duplication have resulted in varied clinical abnormalities plus developmental delay and speech difficulties. This was explained on skewed X inactivation, both random and preferential. Could smaller Xp duplications be the cause of recognizable phenotype? A mother/son pair offered proof that this was so.

Methods Used: Phenotype analysis of mother and son. A 6-year-old girl was seen for developmental delay, dysmorphic facial features, speech difficulties, and behavior problems. He was the product of a normal pregnancy and he had normal prenatal and postnatal growth (ht., wt., h.c.). He had been healthy except for dislocation of the same elbow on 3 occasions. His mother had speech problems, particularly in expressive language, and she was manic depressive, but of normal intellect. Both the son and his mother had similar facial features which included triangular face, broad forehead, blue sclera, malar hypoplasia, prominent nasal tip, narrow palate, and smooth skin. Two differences were the mother had micrognathia and the son had pectus excavatum and bicuspid aortic valve and mildly dilated aortic anulus and root. An X-linked disorder was considered because the mother was somewhat milder, but no phenotype match was found. Summary of Results: Both mother and son had Xp11.4 duplication as an explanation for their physical features including speech difficulties and possible psychiatric problems. After 4 years of follow-up a CGH ARRAY was performed and it showed a duplication at Xp11.4 which was 0.07Mb to a maximum of 0.131 Mb in size. The mother showed the same duplication and is presently being studied for skewed X inactivation. The Xp11.4 duplication does duplicate the TSPAN7 gene which is known to cause non-syndromic mental retardation. Conclusions: Phenotype of Xp11.4 duplication results in a recognizable phenotype with variable effect on mentation plus expressive language difficulties, behavioral/psychiatric problems, and possible connective tissue abnormalities.

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OSTEOGENESIS IMPERFECTA AND GENERALIZED ARTERIAL CALCIFICATIONS OF INFANCY

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Case Report: The purpose of this abstract is to describe an infant afflicted with two unrelated genetic disorders, Osteogenesis Imperfecta and Generalized Arterial Calcification of Infancy. Osteogenesis Imperfecta,OI, is an autosomal dominant disorder of type 1 collagen causing bone fragility, short stature, blue sclera, scoliosis, progressive long bone deformity, hearing loss and dentoingenesis imperfecta. For OI type II, the most severe form, most infants die in the perinatal period from respiratory insufficiency. Generalized Arterial Calcification of Infancy, GACI, is a rare fatal autosomal recessive disorder thought to be caused by decreased inorganic phosphate production leading to increased hydroxyapatite deposition in blood vessel walls. This in turn leads to progressive arterial stenosis and decreased elasticity in vessel walls. Most infants afflicted with GACI die within the first year of life due to progressive stenosis of the coronary arteries resulting in myocardial infarction. Interestingly both these disorders have been treated with bisphosphonates with varying success as reported in the medical literature. The drug’s presumed mechanism of action in the two disorders differs. Bisphosphonates are synthetic analogues of pyrophosphate and pyrophosphate is both an inhibitor of bone resorption and an inhibitor of hydroxyapatite deposition. We present a male infant diagnosed with OI type II/III based on clinical evaluation, and confirmed with mutation analysis (splice site mutation COL2A1 IVS37+1G>A) and collagen analysis in fibroblasts. Echocardiography during the first days of life showed circumferential calcification of the pulmonary artery root. Repeat echocardiography at 11 weeks of age showed progression of calcifications in the pulmonary artery with the development of moderate pulmonary stenosis. Gated CT scan demonstrated calcifications in other large and small arteries most consistent with a diagnosis of GACI. Treatment with bisphosphonates was initiated and patient is now 4 months of age. Both of these conditions are very frequently fatal in the neonatal period. It is interesting to speculate whether the mechanism of disease in either of these disorders may alter the clinical course or potentially even palliate the other.

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EHLERS-DANLOS TYPE VIII, PERIODONTITIS-TYPE: FURTHER DELINEATION OF THE SYNDROME IN A 4-GENERATION PEDIGREE

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Case Report: The Ehlers-Danlos Syndromes (EDS) are a family of inherited disorders of connective tissue that share the common feature of joint hypermobility and skin abnormalities, significantly impacting the health and well-being of the affected individual. To date, at least six subtypes with distinct phenotypic features have been delineated; these include classic, hypermobility, vascular, kyphoscoliotic, dermatosparaxis, and arthrochalasia types. A few other rare subtypes have been described, including periodontitis, valvular, and progeroid types. Due to the rarity of these entities, at present, it is not clear whether they exist as autonomous forms of EDS. The periodontitis type of Ehlers-Danlos syndrome (EDS VIII - OMIM: 130080), was first described by McKusick in 1972 and recognized as a distinct EDS subtype by Stewart et al in 1977. EDS type VIII is distinguished from other subtypes by severe gingival recession and periodontitis leading to premature loss of permanent teeth and resorption of alveolar bone by the second to third decades of life, as well as lack of vascular and organ rupture. A limited number of patients and pedigrees with this condition have been described. We
report a 4-generation EDS VIII kindred that has been followed over 12 years, whose phenotype is generally characterized by joint hypermobility, normal connective tissue, cutaneous scar formation, critical scar atrophy, and severe periodontal disease. Descriptive statistics is presented for each clinical manifestation including dental, musculoskeletal, dermatologic, facial features and other evidence of tissue fragility. Similar to other subtypes of EDS, the age of onset and severity of symptoms was variable amongst affected individuals. Our initial studies on this pedigree do not show linkage to the previously reported EDS VIII locus at 12p13, consistent with previously reported genetic locus heterogeneity.

### 79 COMPARTMENT SYNDROME, A RARE COMPLICATION OF EHLERS-DANLOS SYNDROME TYPE IV

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**Purpose of Study:** Ehlers Danlos Syndrome (EDS) type IV, also known as vascular type, is caused by mutations in COL3A1 and is characterized by extreme tissue fragility. Affected individuals display thin, translucent skin, easy bruising, and develop severe aneurysms. Vascular rupture or dissection and organ rupture are the presenting signs in 70% of adults, and the median age of death is 48 years. Compartment syndrome as a complication of EDS type IV is rare. We present a case of a 37 year old female with EDS type IV who developed numerous episodes of compartment syndrome as a result of aneurysms or vascular rupture following minor trauma. She had 25 episodes involving various extremities, six of which had a documented elevation in compartment pressure. She underwent 42 surgeries to treat the compartment syndrome, including fasciotomies. A literature search revealed only three other cases of compartment syndrome associated with EDS type IV. We compare our case to those previously published and examine their varying presentations, area of involvement, treatment modality, clinical course, and other associated clinical features.

**Methods Used:** Retrospective chart review and literature search.

**Summary of Results:** Patient age ranged from 27–47 years. There were 2 males and 2 females. Areas of involvement included the arm, leg, hand foot, abdomen, and gluteus. Ruptured arteries were the posterior tibial, gluteal, and bilateral renal. Documented compartment pressure ranged from 15–80 mmHg. All patients were treated with fasciotomies except the individual with abdominal compartment syndrome who underwent abdominal decompression. One patient also had arterial embolization prior to fasciotomy. Patients had long term follow-up and were still living more than 5 years after their episode. Only our index case had recurrent episodes; the others were isolated incidents.

**Conclusions:** Compartment syndrome as a result of aneurysmal rupture is a rare but serious complication of EDS type IV. Prompt recognition and treatment are critical. Recurrence of compartment syndrome appears to be uncommon and was seen in only 1 of 4 cases in this series.

### 80 DNA CpG DEMETHYLATION AT IGF-1 INTRON 2 SAT5b ENHANCER SITE OCCURS AT ONSET OF ADOLESCENCE

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**Purpose of Study:** Insulin growth factor 1 (IGF-1) mediates neurodevelopment, growth, and insulin sensitivity. Hepatic production of IGF-1 determines serum levels. Starting in adolescence, growth hormone (GH) regulates IGF-1 expression through multiple STAT5b enhancer sites within the IGF-1 gene. The STAT5b enhancer within intron 2 (STEin2) appears to be particularly important. In the newborn mouse, STEin2 is flanked by DNA CpG methylation. In the adolescent mouse, STEin2 undergoes significant histone covalent modifications to allow GH signaling. However, little information exists about if and when STEin2 undergoes DNA demethylation. This information is important because 1) histone modification changes occur in conjunction with changes in DNA methylation; and 2) diseases such as intrauterine growth restriction affect hepatic IGF-1 expression. Characterizing the normal epigenetic regulation of GH-mediated IGF-1 will contribute to determining how deregulation can lead to disease. We therefore hypothesize that DNA CpG demethylation occurs within STEin2 occurs during adolescence.

**Methods Used:** Livers from C57B6 and B6.D2 mice were collected and flash frozen at days 7, 21, 28, 35 and 90. Four CpG sites on both strands of the above features, who also has the previously unreported abnormality of around the IGF-1 intron 2 STAT5b enhancer were analyzed by bisulfite sequencing.

**Summary of Results:** Overall, the IGF-1 SAT5b intro 2 enhancer was hypermethylated (80% meCpG) at day 7 and relatively hypomethylated (25% meCpG) by day 90. A dramatic decrease of DNA methylation occurred from day 21 (36% meCpG) through day 35 (15% meCpG). Percentage of methylation on sense and antisense strands in both mouse strains were equivalent.

**Conclusions:** We conclude that the majority of DNA CpG demethylation occurs in a relatively brief 2 week period between day 21 and 35 of life. This period provides a window of study on which to focus upon the chromatin modifying complexes that regulate IGF-1 expression in normal individuals, as well as determine which of these complexes (or their components) are disrupted by intrauterine growth restriction.

### 81 DUPLICATE GENETIC TESTING IN AN INTEGRATED HEALTHCARE SYSTEM

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**Purpose of Study:** Genetic testing for germline mutations is unique in that a test only needs to be done once in a patient’s life. The purpose of this study was to determine if two, commonly-ordered genetic tests were being performed more than once in our system.

**Methods Used:** The tests used for the analysis were Factor V Leiden (FVL) and the Prothrombin Variant 20210A (PTM). Six years of data were extracted from the electronic data warehouse (EDW). Duplicate tests were defined as the specific test code being present 2 or more times in the unique patient record separated by an interval of time. Data were also collected about the facility where testing was done, the location within the facility, the name and specialty of the ordering physician(s). Root cause and a variety of statistical analyses were performed to identify patterns as well as system factors leading to duplicate testing.

**Summary of Results:** Over the six-year period 4% of the FVL and 3.4% of the PTM tests were duplicated. This resulted in nearly $50,000 (using Utah Medicare rates) of waste for these tests over this period. Surprisingly, preliminary analysis showed that 20% of the duplicate tests occurred within one week of the initial test. This suggests that communication in care transitions (e.g. inpatient to outpatient or ICU to inpatient ward) may play a significant role in this problem. Additional analyses are currently underway to better characterize the predictive factors. Based on this information, root cause analysis will be applied to identify the system failure that results in duplicate testing.

**Conclusions:** Duplicate genetic testing is a problem in our system. Elimination of this duplication would save money and improve impact on patient care. Persistence of DT suggests there is a need for educating some providers about the appropriate use of genetic testing for germline mutations as well as exploring system changes that would prevent DT ordering.

### 82 DE BARSY SYNDROME; REPORT OF A PREVIOUSLY UN-DESCRIBED CLINICAL FEATURE THAT SUGGESTS NEW DIRECTION FOR ETIOLOGIC INVESTIGATION

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**Purpose of Study:** Explore the etiology of de Barys Syndrome.

**Methods Used:** Literature review and clinical evaluation.

**Summary of Results:** De Barys syndrome is a rare autosomal recessive progeroid syndrome of unknown etiology. Consistent features in the fewer than 30 reported patients include; pre and postnatal growth retardation, progeroid appearance from birth, large fontanelles, large ears, clinched hands, congenital hip dislocation, hyperextensible joints, severe mental impairments, DNA and cloudy corneas. Additional connective tissue abnormalities have been reported less frequently including scoliosis, easy bruising, thin translucent skin, and other joint dislocations. In a number of reports authors have identified tissue abnormalities in biopsies from individual cases including abnormalities of both elastic fibers and collagen. Despite these studies, the genetic etiology of this disorder remains unknown. Here we report a patient with phenotype of de Barys syndrome including all of the above features, who also has the previously unreported abnormality of...
tortuous cranial arteries in this patient with de Barsy syndrome.

Conclusions: Tortuous cranial arteries in this patient with de Barsy syndrome is an addition feature of connective tissue dysfunction. The commonality of this feature with Menkes disease could suggest new direction of investigation as to the etiology of de Barsy syndrome.

83 ELUCIDATING THE COMPLEXITY OF EPILEPSY: CLUES FROM 2Q23.1 DELETION

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Case Report: Epilepsy is one of the most common and genetically intricate neurological disorders in children. Previous studies have helped elucidate genes, which impart susceptibility to epilepsy. Since the introduction of the array CGH, additional deletion and duplication syndromes have been delineated. Current research suggests an estimated 10–30% background risk for copy number variations in patients with seizures. We report two patients who presented with seizures, developmental delay and dysmorphic features due to chromosomal deletions at 2q23.1. Case 1 is a 3 year old male with seizure disorder, deafness, microcephaly and dysmorphic features: microcornea, curly eyelashes, bushy eyebrows, hypoplastic nose, low-set ears with an over folded anterior helix, prominent halluclae creases, single transverse palmar creases, absent patella and severe neurological impairment. Cytogenetic analysis revealed a deleted region of 2q23 to 2q31.1. Case 2 is a 2 year old female with developmental delay, dysmorphic features, hypotonia who presented with new onset seizures. Dysmorphic findings included: Darwinius tuberence, hypotonic facial features with short upturned nose, and thickened tongue. An oligonucleotide microarray showed a 0.3 Mb deletion involving 6 oligonucleotides within 2q23.1. Common physical features included bifrontal narrowing and low anterior hairline, short neck with redundant neck skin, fifth digit clinodactyly, with generalized brachydactyly. In a literature review of 2q23.1 deletion, the critical region that has been implicated as a seizure gene is MBDS5, which seems to be responsible for regulating DNA methylation in central nervous system tissue. Our first patient’s larger area of deletion also involves the SCN gene cluster and DLX1/2 genes. Abnormal SCN1A channels affect GABA neurons and the associated seizures respond optimally to antiepileptic drugs that bind to the GABA receptor. The DLX genes play a major role in controlling craniofacial patterning and differentiation and survival of forebrain inhibitory neurons, suggesting that haploinsufficiency might indeed be responsible for our patient’s phenotype (i.e. seizures, microphthalmia). Chromosomal microarray serves not only as a diagnostic tool, but also as a window into the pathophysiology and treatment of genetic encephalopathies.

84 A PRENATAL DILEMMA; ELLIS-VAN CREVELD AND NEUROFIBROMATOSIS

Jelin AC, Perry H, MacLean J, Rauen K UCSF, San Francisco, CA.

Case Report: We report a case of a couple who presented for prenatal counseling because of a maternal history of café-au-lait macules, axillary and inguinal freckling and multiple cutaneous neurofibromas. On exam, findings were consistent with a clinical diagnosis of Neurofibromatosis Type 1 (NF1). Surprisingly, her partner was found to have a phenotype that consisted of dwarfism, dolicocephaly, multiple lower lip frenula, a narrow trunk, short limbs, and postaxial polydactyly. He also had a history of a partial AV canal with an ostium primum atrial septal defect and Eisenmenger's physiology. His presentation was concerning for Ellis-van Creveld (EVC). Neither had previously undergone molecular genetic testing. Originally the couple was mainly concerned about a possible diagnosis of NF1 for their unborn child. After examination and counseling the risk of EVC was also presented to the family who then underwent molecular genetic testing.

The male patient was found to be homozygous for a c.1238delA mutation of the EVC2 gene consistent with Ellis-van Creveld. The female patient was heterozygous for a truncating mutation (c.3567delA) in the NF1 gene. The female then elected to also have testing for EVC carrier status. An amniocentesis was performed and a targeted mutation analysis for the disease causing NF1 gene was requested.

Ellis-van Creveld (EVC) syndrome is an autosomal recessive skeletal dysplasia resulting from a mutation in either the EVC or EVC2 gene. The exact protein responsible for the phenotype has yet to be characterized. Patients have disproportionate dwarfism with short limbs, short ribs, thoracic dysplasia and postaxial polydactyly. Cardiac defects occur in 60% of patients and are usually in the form of an atrio-ventricular septation or a single atrium.

This case highlights the importance of joint couple counseling in prenatal cases. Although this couple originally presented for counseling in regards to a diagnosis of NF1, the paternal exam was concerning for another syndrome for which we were also able to offer molecular genetic testing. Although characteristics of EVC may be identified on prenatal ultrasound, the ability to perform a molecular diagnosis provides a definitive result to concerned patients.

Health Care Research 1
Concurrent Session
8:30 AM
Friday, January 28, 2011

85 BONE SCAN OVERUSE IN THE STAGING OF PROSTATE CANCER

Palvolgyi R1, Daskivich T1, Kwan L1,2, Chamie K1,3, Litwin M1,2,3 1UCLA David Geffen School of Medicine, Los Angeles, CA; 2UCLA School of Public Health, Los Angeles, CA and 3UCLA, Los Angeles, CA.

Purpose of Study: With contemporary widespread PSA screening, more patients exhibit low- and intermediate-risk prostate cancer, thus reducing the need and yield of bone scan imaging. We aimed to determine the utilization and subsequent positivity rates of bone scan imaging in a contemporary Veterans Affairs (VA) cohort of men with prostate cancer.

Methods Used: We retrospectively reviewed the charts of 1597 men diagnosed with prostate cancer between 1997 and 2004 at the Greater Los Angeles and Long Beach VA Medical Centers. We used univariate analysis to measure association between patient (age, race, comorbidity) and tumor (PSA, clinical stage, Gleason score) characteristics with bone scan use and positivity. We conducted the analysis for scans that were and were not clinically indicated, according to established AUA guidelines.

Summary of Results: Out of 1121 men not indicated for nuclear imaging, 452 (40%) received a bone scan with only 6 (1%) returning positive. Among the same group, increasing PSA, clinical stage, Gleason score, and subsequent D’Amico tumor risk, were all positively associated with bone scan overuse, but not with corresponding positivity rates. Univariate analysis showed age, PSA, clinical stage, Gleason score, and treatment type as significant predictors of bone scan overuse.

Conclusions: Bone scan overutilization in men with clinically localized prostate cancer results in unnecessary patient anxiety, time consumption, and significant economic waste for the VA. If the 40% overutilization rate for clinically localized prostate cancer reflects national overuse patterns, then implementing a quality improvement initiative to minimize overutilization would translate an annual savings of $9,409,680 for the VA, without compromising oncologic outcomes.
86 SCREENING FOR LYMPH SYNDROME USING COLORECTAL CANCER IMMUNOHISTOCHEMISTRY

Khaki A, Madlensky L University of California, San Diego School of Medicine, La Jolla, CA.

**Purpose of Study:** Current guidelines recommend that colorectal cancer (CRC) patients with abnormal immunohistochemical (IHC) staining for mismatch repair proteins be referred for genetic consultation to determine if germline genetic testing for Lynch Syndrome is appropriate. Our objective was to summarize patterns of IHC among CRC patients seen at a single institution over a 6 year period.

**Methods Used:** Chart reviews were conducted on all CRC adenocarcinoma cases at UCSD Medical Center from 2004–2009. Covariates included age and sex of patient, family history of CRC, type of insurance, year of diagnosis, tumor location and other histopathological features.

**Summary of Results:** A total of 651 patients were identified in the hospital tumor registry as having a CRC diagnosis in the study time period, of whom 331 had their tumor reviewed by pathology. Of these, 154 (46.5%) had IHC completed, with younger patients, those with a relative with CRC, and those with Medicare or private insurance more likely to have IHC completed. Of those with IHC, patients with right-sided tumors or positive CRC family history were more likely to have abnormal staining. Patients with abnormal staining were twice as likely to be referred for a genetics consultation than those with normal IHC (42.4% vs 20.3%, p = 0.009).

**Conclusions:** Patients who are more likely to have Lynch Syndrome can be efficiently identified through IHC and appropriately referred for a genetics consultation. In addition, some patients in the study with abnormal IHC did not have typical risk factors, and so systematic IHC for all patients may be appropriate.

87 CLINICAL AND ECONOMIC IMPACT OF FORMULARY RESTRICTION ON PHACOEMULSIFICATION

Burns JD, Tsai PL, University of Arizona College of Medicine, Tucson, AZ and University of Arizona College of Medicine, Tucson, AZ.

**Purpose of Study:** Our purpose was to examine the effect of formulary restriction on clinical outcomes and perioperative costs in phacoemulsification (phaco).

**Methods Used:** Retrospective analysis of 79 eyes that underwent phaco to determine whether subjects’ use of non-preferred fluoroquinolones (ofloxacin and moxifloxacin) or NSAIDs (bromfenac and diclofenac) due to formulary restriction over preferred agents (gatifloxacin and ketorolac) had an impact on clinical outcomes or personal costs. The change in visual acuity (VA), the absolute value of difference between post-operative and expected spherical equivalent (abs MRx diff) or IOP postoperatively compared to preoperative IOP (IOPp), rate of endophthalmitis or other serious adverse events, and the difference in the subjects’ cost were compared between the groups.

**Summary of Results:** Subjects who received ofloxacin (n=34, −0.70, p=0.008) and moxifloxacin (n=6, −0.54, p=0.034) had greater improvements in LogMAR VA over the gatifloxacin group (n=39, −0.32); however, they began with significantly worse vision than the gatifloxacin group [ofloxacin: (0.79, p=0.003), moxifloxacin: (0.66, p=0.005), gatifloxacin: (0.37)] prior to surgery when compared to HIC patients with right-sided tumors or positive CRC family history were more likely to have abnormal staining. Patients with abnormal staining were twice as likely to be referred for a genetics consultation than those with normal IHC (42.4% vs 20.3%, p = 0.009).

**Conclusions:** Patients who are more likely to have Lynch Syndrome can be efficiently identified through IHC and appropriately referred for a genetics consultation. In addition, some patients in the study with abnormal IHC did not have typical risk factors, and so systematic IHC for all patients may be appropriate.

88 INSURANCE STATUS AND EMERGENCY DEPARTMENT UTILIZATION IN PATIENTS WITH ACUTE STONE EPISODES

Han DS, Schroeder G, Richards G, Tenggjarajda C, Bowman R, Agarwal G, Baldwin D, Loma Linda University, Loma Linda, CA and Loma Linda University Medical Center, Loma Linda, CA.

**Purpose of Study:** According to the U.S. Census Bureau, 50 million people in the United States are uninsured. It has been suggested that those who are uninsured follow-up in the Emergency Department (ED) at a higher frequency due to inability to access specialized care. The primary objective of this study is to examine the relationship between insurance status and ED usage among patients during an acute stone episode and evaluate their follow-up care.

**Methods Used:** This is a single-center, IRB approved, retrospective study from January 2007 to July 2009. Patients presenting to the ED with possible acute stone episodes were identified by ICD-9 codes for nephrolithiasis, ureterolithiasis, renal colic, and abdominal pain. Patients were excluded if they did not have radiographic data confirming the presence of upper urinary calculi. Follow-up location, number of stones, and number of ED follow-up visits for the same stone episode were collected by chart review and telephone contact and stratified by insurance status. Data were analyzed using two-tailed Fisher’s exact and independent samples t-Test with a p<0.05 considered significant.

**Summary of Results:** Six hundred and ten patients were identified. Of these 321 patients were identified that met the criteria for an acute stone episode. In 136 of these patients follow-up location could not be identified. One hundred and eighty-five patients were included for analysis based on nephrolithiasis or ureterolithiasis seen on CT and a chief complaint of abdominal or flank pain. Of the 136 patients with unknown follow-up, 103 were insured (38% of total insured) compared to 33 who were uninsured (66% of total uninsured), p=0.001. In total, 143 of the insured patient population (85%) had at least one follow-up visit (ED, primary care, and/or urology) after initial presentation for an acute stone episode. In contrast, only 9 of the uninsured population (53%) had a follow up visit, p<0.003.

**Conclusions:** Those without insurance utilize the ED at a higher frequency for follow-up of acute stone episodes. Furthermore, those without insurance are less likely to follow-up with a urologist after an acute stone episode. Knowledge of follow-up patterns can guide health care systems to maximize patient care.

89 VARIABLES ASSOCIATED WITH HOSPITAL READMISSION OF PATIENTS WITH CONGESTIVE HEART FAILURE

Yusufzai M, Singh N, Sirohi R San Joaquin General Hospital, Stockton, CA.

**Purpose of Study:** Hospital readmission rates are high for patients with Congestive Heart Failure (CHF). Hospitals are required to reduce 30-day readmission rates. This study suggests that renal failure is an important factor in readmission of CHF patients.

**Methods Used:** Two hundred and two patients were admitted to inpatient service of a public hospital from June 2009 to June 2010 for CHF (males n=138, females n=64). The medical records were reviewed in a retrospective analysis to determine factors associated with readmission within 30 days. There were age and sex matched controls (n=153) and study group readmitted for CHF (n=49). Chi-square and t tests were used for statistical analysis.

**Summary of Results:** Out of 202 patients, 110 were readmitted with total 240 readmissions, of which 49 were readmitted for CHF with 82 readmissions. Out of the eighty-two, 41 readmissions were within 30 days. Readmission is found related to many variables. Age, sex, smoking, alcohol and drug abuse were non-significant risk factors. Renal failure was the most significant factor for readmission within 30 days. Out of the 49 patients who were readmitted for CHF, 69.4% (34/49) had renal failure compared to 44.4% (68/153) of controls (p<0.002) (RR=2.52, CI=1.10–5.77). Patients with CHF readmissions had a lower left ventricular ejection fraction (LVEF) and higher Serum creatinine levels as compared to controls (p<0.001). Patients non-compliant with medications and those having non-commercial state and county assistance insurance implying lower socio-economic status had higher rates of readmissions (p=0.003). In the total group of 202 patients,
There is a significant increase in readmission rate in patients with renal failure and the severity of renal failure may have prognostic significance. Follow-up appointment, diet, drug compliance and CHF education should be given to all patients at hospital discharge. Special consideration should be given to those with renal failure and limited access to health care.

**90**

**EFFECT OF FAXING EMERGENCY DEPARTMENT PRESCRIPTIONS ON PHARMACY WAIT TIME AND SUBSEQUENT HEALTH CARE UTILIZATION**

Fernando TJ, Baraff LJ David Geffen School of Medicine at UCLA, Los Angeles, CA.

**Purpose of Study:** The primary objective of this study is to assess whether faxing prescriptions directly to a pharmacy leads to decreased pharmacy wait times and greater satisfaction with the process of obtaining medication. Secondary objectives are whether faxed prescriptions are associated with decreased return Emergency Department (ED) visits or primary care visits.

**Methods Used:** Patients discharged from the UCLA Emergency Department with prescription medications were approached for study consent. Study participants were randomized into control or treatment groups. Participants in the control group were discharged with the standard prescription, while participants in the treatment group had their prescriptions faxed to a pharmacy. All study participants were contacted 7 days after ED discharge for a telephone interview.

**Summary of Results:** Data analysis was performed on 60 patients (23 males, 37 females) who completed the telephone interview. Study participants had a median age of 36 years (range=6–86). 31 patients were prescribed 1 medication, 24 were prescribed 2 medications, 4 were prescribed 3 medications, and 1 was prescribed 4 medications. Five patients did not pick up their medication from the pharmacy. Technical problems occurred with 9 faxed prescriptions. Data collection is ongoing.

**Conclusions:** After blocking for technical difficulties that arose with faxed prescriptions, we found a significant difference between the two groups in pharmacy wait time (Wilcoxon $p = 0.009$) and whether medications were ready for immediate pick up (Fischer’s exact $p < 0.001$). There was no significant difference in patient satisfaction with obtaining medication. Faxing prescriptions did not significantly change the number of return ED or follow-up primary care visits.

**Pharmacy Wait Time**

<table>
<thead>
<tr>
<th></th>
<th>Median Wait Time</th>
<th>IQR (25%-75%)</th>
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<tbody>
<tr>
<td>Faxed Rx</td>
<td>2 min</td>
<td>0-15 min</td>
</tr>
<tr>
<td>Not Faxed Rx</td>
<td>15 min</td>
<td>10-20 min</td>
</tr>
</tbody>
</table>

$p$-value = 0.009

**Percentage of Medications Ready For Pick Up**

<table>
<thead>
<tr>
<th></th>
<th>Meds Ready On Arrival</th>
<th>Waited For Meds</th>
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</thead>
<tbody>
<tr>
<td>Faxed Rx</td>
<td>74%</td>
<td>20%</td>
</tr>
<tr>
<td>Not Faxed Rx</td>
<td>15%</td>
<td>85%</td>
</tr>
</tbody>
</table>

Fischer’s Exact = 0.000

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**LOWERING LIPOPROTEINS AMONG EMPLOYEES: PROGRESS AT WORK**

Raymond LW1,2, Pankowski J3, Shiflett H2 1Univ of North Carolina, Chapel Hill, Charlotte, NC and 2Carolinas HealthCare System, Charlotte, NC.

**Purpose of Study:** Blood lipoproteins (BL) are an important reversible determinant of cardiovascular diseases which can impact worker health, job performance and health care costs. However, few reports have addressed the impact of worksite health promotion (WHP) on BL levels. We evaluated the effect of WHP on BL in employees of three companies which expressed interest in this outcome.

Methods Used: Workers at a lumber yard (N=250), an electric co-operative (N=150) and an engineering design firm (N=115) were invited to participate in free, non-fasting BL screening, as part of WHP. Serum analytes by autoanalyzer included total cholesterol (TCh) and high-density lipoprotein (HDL), and triglycerides (TG). Low-density lipoprotein (LDL) was derived using the Friedewald equation. Counseling on exercise, weight loss, dietary optimization and smoking cessation were included in the WHP program, and a physician sent each participant an individual letter with BL results, along with advice on what to do about the results.

**Summary of Results:** 168 men and 24 women (mean ages 45 and 46 years; 37% of invitees) provided baseline BL samples, and follow-up samples were available in 82 men and 16 women. TG and LDL were unavailable in only 5 of 196 blood samples with lipemic plasma due to hypertriglyceridemia. The ratio of TCh to HDL exceeded 5.5 in 33 participants at baseline, and in 24 after 19+8SD months of follow-up. Significant reductions also occurred in mean values of total, LDL and non-HDL cholesterol and in the ratio of TCh to HDL.

**Conclusions:** WHP activities were associated with significant near-term reductions in BL in these workers. Whether such reductions will be sustained and be accompanied by less cardiovascular disease remains to be determined. Non-participants remain a challenge.

**Baseline and Follow-up Lipoproteins in 98 Workers at 3 Companies**

<table>
<thead>
<tr>
<th></th>
<th>TCh</th>
<th>LDL</th>
<th>HDL</th>
<th>Ratio, cholesterol to HDL</th>
<th>Triglycerides</th>
<th>Non-HDL lipoproteins</th>
</tr>
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<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>188 +/- 38SD</td>
<td>126</td>
<td>40</td>
<td>5.0</td>
<td>T &lt; 1.5</td>
<td>119 +/- 88</td>
<td>147 +/- 36</td>
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<tr>
<td><strong>16-month follow-up</strong></td>
<td></td>
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<tr>
<td>176 +/- 36</td>
<td>114</td>
<td>40</td>
<td>4.7</td>
<td>T &lt; 1.5</td>
<td>116 +/- 84</td>
<td>130 +/- 37</td>
</tr>
<tr>
<td>p &lt; 0.001</td>
<td>0.003</td>
<td>0.78 NS</td>
<td>0.01</td>
<td>0.67 NS</td>
<td>0.001</td>
<td></td>
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</tbody>
</table>

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**AN EVALUATION OF THE ORAL HEALTH STATUS OF RURAL UGANDAN SCHOOL CHILDREN ENROLLED IN ‘BRIGHTER SMILES AFRICA’**

Meredith C1, Berg T2, Ashaba B2, Choi W1, Zhang M1, Musinguzi N2, Nambatya J, Nyairo S, Cannon W1, Kasangaki A1, Macnab A1 University of British Columbia, Vancouver, BC, Canada and 2Makerere University, Kampala, Uganda.

**Purpose of Study:** To document the oral health status of a cohort of rural Ugandan primary school children enrolled in an on-going school-based health-promotion program ‘Brighter Smiles’, with reference to the distribution of caries and incidence of significant oral pathology.

**Methods Used:** A Ugandan/Canadian student/faculty team conducted the program’s annual examinations using a validated dental survey (DMFS Scores), and health questionnaires; oral pathology and atypical variation were photographed, and comparison made of the number of anterior versus posterior teeth affected by caries and the extent of the decay. Education was given to address the dental problems found.

**Summary of Results:** 294 children aged 5–13 years were examined; 218 (74.2%) had oral pathology present. Anterior caries was present in 19 (6.5%) and posterior caries in 95 (32.3%). Other significant atypical findings and oral pathologies identified included gum recession in 4.4%, gingivitis in 12.9% and calculus in 36.4%. Malocclusion, Treacher-Collins Syndrome, tetracycline staining, peg lateral incisors, malnourished, herpetiform stomatitis, candida, ankyloglossia, transposition and germination were also present. Workshops were delivered to teach better oral hygiene techniques for posterior teeth, and suggest health practices to improve other pathologies where possible.

**Conclusions:** Posterior caries was more prevalent than anterior disease and the range of pathologies extensive. Delivery of the Brighter Smiles Program in rural schools is lowering the incidence of periodontal disease, reducing caries, and promoting knowledge and healthy practices in a country with limited health and dental care. The program is also providing valued collaborative educational and research opportunities for the teams involved.
REPUTITIVE STRESS SYMPTOMS IN RADIOLOGISTS IN THE DIGITAL AGE

Wood JP University of New Mexico, Albuquerque, NM.

Purpose of Study: With a switch to digital workstations, time spent in the same position has increased significantly. PACS has increased efficiency, but possibly at a physical cost to radiologists. This led to the development of this pilot study: Evaluate the prevalence of repetitive stress symptoms by surveying radiologists in Albuquerque.

Methods Used: We sent our questionnaires to 92 radiologists throughout Albuquerque (2 private practices, radiology residents and Attending Radiologists at Univ. of New Mexico). We used EpiInfo and SAS v9.2 for entry & analysis, and Fisher’s Exact Test due to small N-size.

Summary of Results: Overall, 58/92 responded (63%). 76% reported that they experience pain, stiffness, soreness, or cramping in extremity, back, or neck). When response was “yes” to experiencing pain, we asked them to specify the type and severity (Likert Scale 1–5, 5 being most severe). The Mean ranged from 2.2–2.4 for: Back, Neck, Arm/Shoulder, Wrist/Hand, or Leg. Less than 9% reported some sort of ergonomic training in the past year. When divided into private, academic attending, and resident, there was a significant difference among the groups spending greater than eight hours per day at a PACS/workstation (59%, 21%, 53% respectively). When comparing these 3, academic Attendings reported the highest pain frequency of at least once per month (86% compared to 76% in private and 67% in residents). There was no difference amongst the other groups in age and pain frequency.

Conclusions: As a pilot study, repetitive stress symptoms were common. The total N-size made statistically significant differences difficult, although some observations were evident. There were enough interesting qualitative findings (equal distribution of pain sites, no difference in pain correlating with age) to make this study worth repeating with a larger population. Future studies could include: the effect of documented ergonomic training on improvement in symptoms, error rate-pain symptom correlation, or error rate-break freq correlation.

MISSION, SENSEMAKING, AND TOOLS: EXPLORING THE CAPACITY OF COMMUNITY HEALTH CENTERS TO IMPLEMENT EVIDENCE-BASED INTERVENTIONS

Terasaki D 1UW School of Medicine, Seattle, WA and 2University of Washington, Seattle, WA.

Purpose of Study: Community health centers (CHCs) make up a large segment of the health care safety net in the United States. Due to their patient demographics and placement in underserved areas, CHCs are well positioned to decrease health disparities through evidence-based interventions. A multidisciplinary research group has developed a conceptual framework (Practice Change model) that is useful for exploring change in primary care settings. A section of the model labeled “Adaptive Reserve,” which includes 7 characteristics of healthy work relationships, held particular interest to our research team. We aimed: 1) To assess the applicability of the Practice Change model to CHCs, focusing on Adaptive Reserve, and 2) To gain insight into the implementation of evidence-based programs at CHCs.

Methods Used: Key informant interviews of administrative and clinical leaders were conducted at 4 CHCs and 1 national association. Interviews were recorded and transcribed verbatim. Three research personnel independently coded each transcript and then convened to discuss emerging themes. The final set of codes was subsequently entered in Atlas.ti for further analysis.

Summary of Results: Findings from 14 interviews elucidated 3 domains in the Practice Change model - inside/outside motivators, dynamic local ecology, and sensemaking - that merited special emphasis with regards to CHCs. In contrast to mainstream practices, for CHCs: 1) their mission serves as a major inside/outside motivator; 2) their dynamic local ecology is greatly influenced by the communities they serve; and 3) tools that facilitate sensemaking and key implementation steps enhance their capacity for change. Informants underscored “Trust” as the most significant work relationship and described their work place as a “family-like community.”

Conclusions: Our findings support the importance to design interventions in partnership with CHCs that: 1) align with CHC’s mission and investment in their community; and 2) utilize effective tools to aid in various stages of implementation, including sensemaking. This may achieve greater buy-in from CHC personnel and, ultimately, facilitate the implementation of evidence-based interventions that reduce health disparities where the need is greatest.

TRANSPARENT REPORTING OF GAMBLING EFFECTIVENESS STUDIES

Parhami I, Campos MD, Fink A, Fong T University of California - Los Angeles, Los Angeles, CA.

Purpose of Study: Gambling disorders are a significant health concern because they are associated with cardiovascular disease, major depression, substance abuse, financial troubles, and crime. This study systematically reviews the transparency of reports on interventions to ameliorate the adverse consequences of problem and pathological gambling. Transparent reporting is essential to the assessment of study quality.

Methods Used: Using specific inclusion and exclusion criteria, we identified 25 articles from Pub Med, PsyCINFO, and the Web of Science published between January 2000 and August 2010. Two researchers independently reviewed each article’s reporting using 58 items adapted from the American Public Health Association’s and CDC’s TREND (Transparent Reporting of Evaluations of Nonrandomized Designs) Statement. Twenty-two items represent particularly important criteria, and all should be reported. Differences in interpretation between reviewers were adjudicated by a third reviewer.

Summary of Results: Only 8 articles met at least 70% of the 58 general reporting criteria, (median=64%; range: 41–84%). None of the studies met all 22 of the important reporting criteria. Examples of particularly important criteria met by at least half the studies include descriptions of eligibility rules for participation, methods for handling missing data, and methods for establishing baseline study group equivalence. Fewer than half the studies described the barriers encountered in implementing the experimental interventions, methods for increasing compliance, and methods for addressing differences in baseline characteristics of participants who were lost to follow-up compared to those who were retained. No differences in transparency were found between articles published before and after the initial dissemination of the TREND criteria (2004).

Conclusions: Published studies reporting on the effectiveness of interventions for gambling related disorders tend to vary in their transparency with many important reporting criteria unmet. Transparent reporting is a prerequisite for determining if evidence is available to support implementing specific programs or treatments to address gambling disorders and the untoward medical and social consequences with which they are associated.

Hematology and Oncology I
Concurrent Session
8:30 AM
Friday, January 28, 2011

SURVIVAL OUTCOMES OF ADULTS WITH ACUTE MYELOID LEUKEMIA-A STUDY OF NATIVE AMERICAN, HISPANIC, AND NON-HISPANIC WHITES TREATED IN NEW MEXICO

Armojo B, Libby E, Quintana D, Rivera N, Wiggins C, Lee S, Abdul-Jaleel M University of New Mexico, Albuquerque, NM.

Purpose of Study: The goal of this study is to identify potential differences in outcome between ethnic groups from urban or rural areas in patients with newly diagnosed acute myelogenous leukemia (AML) treated at this center from 1986-2006.

Methods Used: A retrospective chart review was performed on patients identified through a New Mexico tumor registry search of all adult patients (≥ 18 years of age) with a new diagnosis of AML treated at this center from 1986 through 2006. 144 charts were available for review. Electronic, microfilm and paper-based medical records were reviewed. Data was collected for race/ethnicity, sex, zip code, age at diagnosis, pathologic diagnosis, date of diagnosis, cytogenetics, treatments received, total number of chemotherapy cycles, achievement of complete remission, and overall survival from time of diagnosis and from achievement of remission after induction.
chemotherapy. Cancer cases who resided in New Mexico counties with large population centers were designated as urban residents and the remaining cases were designated as rural residents. Statistical analysis was performed to examine the effect of the variables mentioned above on overall survival.

**Summary of Results:** The analysis included 144 patients (mean age 49.98, range 18-85). For those patients who received chemotherapy the standard treatment was 7-3 followed by high dose cytarabine (1-4 cycles) or similar regimen. There were no statistical differences between survival rates when comparing the three ethnic groups, Caucasians 78 (52%), Hispanics 43 (28%), and Native Americans 23 (15%) (P=0.2496). In agreement with previous reports, statistically significant differences were seen for treatment versus palliative care, cytogenetics and age. The study included 61 urban patients with a mean survival of 426 days and 83 rural patients with a mean survival of 278 days. Analysis revealed a statistically significant survival for urban patients who achieved complete remission after induction chemotherapy (P=0.0443).

**Conclusions:** Survival was improved for urban patients who achieved a complete remission after induction chemotherapy versus rural patients (P= 0.0443). Further study should be undertaken to identify the causes of this disparity.

**VALUE OF CONTROL GROUPS IN PHASE 2 CLINICAL TRAILS**

Moseley J\(^1\)-2, Pagel J\(^1\), Othus M\(^2\) \(^4\)University of Washington, Seattle, WA and \(^5\)Fred Hutchinson Cancer Research Center, Seattle, WA.

**Purpose of Study:** Each year, numerous abstracts describing new therapies for acute myeloid leukemia (AML) are presented at the American Society of Hematology (ASH) meeting, 63/91 abstracts submitted from 1993–2006 suggested therapeutically promising results; these 63 abstracts covered 37 drugs. However, 34/37 (92%) drugs initially viewed positively at ASH were found to have negative results with further study or have disappeared from the clinical trials arena. Walter et al note that absence of a control group contributes to this preponderance of false-positive, misleading results.

We encountered a specific example in a single-arm trial of a 4-drug combination (FLAM) investigated at Johns Hopkins and UW/FHCRC in 72 patients with “poor prognosis”, newly-diagnosed AML. The initial conclusion was that “the salutary results of FLAM in poor-risk pts will be extended to adults with non-poor risk AML and compared with traditional cytotoxic chemotherapy.” Our aim was to determine if the same held true when a control group was included in the analysis.

**Methods Used:** We identified a control group of 229 newly-diagnosed patients given standard therapy at either UW/FHCRC or in Southwestern Oncology Group trials. We next collected information on relevant prognostic factors in this group: age, performance status, cytogenetic findings, and whether disease onset was immediate or delayed. Finally, we combined the FLAM and control patients and used multivariate analysis to examine whether, after accounting for possible differences between FLAM and control in the distribution of prognostically-relevant covariates, the rate of complete remission (CR) was higher with FLAM.

**Summary of Results:** The multivariate analysis suggests that the probability that FLAM is >10% superior to standard therapy is < 1%. Additionally, FLAM does not appear more effective in any particular prognostic subgroup.

**Conclusions:** While not a substitute for a randomized trial comparing standard therapy and FLAM, our analysis indicates it is highly unlikely that FLAM is meaningfully superior to standard therapy. Our results motivated cancellation of a planned randomized trial. This will enable other drugs to be studied rather than devoting patients and other resources to further study of FLAM.

**SURVIVIN AS A POTENTIAL SENSOR OF OXIDATIVE STRESS IN HUMAN BREAST CANCER**

Pervin S\(^1\)-2, Tran A\(^1\), Tran L\(^1\), Urman R\(^1\), Chaudhari G\(^1\), Singh R\(^1\)-2 \(^3\)Charles Drew University of Medicine and Science, Los Angeles, CA and \(^4\)UCLA, Los Angeles, CA.

**Purpose of Study:** Breast cancer remains a poorly understood heterogeneous disease. Survivin, a unique member of the inhibitor of apoptosis (IAP) gene family, is over expressed in aggressive tumors and its down regulation sensitizes cancer cells to various therapeutic agents. The purpose of this study is to examine the role of survivin as a potential sensor of oxidative stress in human breast cancer.

**Methods Used:** Oxidative stress was induced in a panel of breast cancer cell lines by treating with DETA-NONOate (1-3nmM), a nitric oxide (NO) donor and expression of MAP Kinase Phosphatase-1 (MKP-1), an early stress-response gene, was examined. Survivin levels were either up regulated by over-expression of full-length human survivin cDNA or inhibited using small inhibitory RNA (siRNA) to examine the sensitivity of cells to oxidative stress. Relationship between survivin and MKP-1 was also examined in fresh and paraffin sections of human breast tumors by quantitative western blot, real-time PCR and immunohistochemistry.

**Summary of Results:** NO-induced oxidative stress down regulated cytosolic survivin and induced MKP-1 expression in estrogen receptor negative (ER-) breast cancer cell lines. This down regulation of cytosolic survivin protein was an early event and preceded the induction of MKP-1. High level of oxidative stress was required to induce MKP-1 in survivin over-expressing cells. In addition, reduction of basal survivin levels rendered these cells sensitive to low oxidative stress. However, ER+ cell line had low levels of only nuclear survivin but high MKP-1 levels. We further demonstrate that ER+ fresh human breast tumors also had low survivin, but high levels of MKP-1 expression. This inverse relationship between survivin and MKP-1 was also detected in fresh and paraffin-sections of ER- human breast tumors.

**Conclusions:** Reduction of survivin levels was essential for sensitizing the breast cancer cells to stress by upregulating MKP-1. High levels of survivin render ER- aggressive breast tumors insensitive to oxidative stress. We therefore, conclude that survivin levels determine the sensitivity of human breast cancer cells to NO-induced oxidative stress.

**RASSFIC OVER-EXPRESSION PROTECTS LUNG CANCER CELLS FROM BETULINIC ACID EFFECTS ON CELL PROLIFERATION**

Jo J, Franke E, Amaar Y, Reeves M Loma Linda University, Loma Linda, CA.

**Purpose of Study:** Ras association domain family 1 (RASSF1) gene is a Ras effector that plays an important role in carcinogenesis. The gene encodes two major proteins derived from alternate mRNA splicing. RASSF1A is a tumor suppressor. The function of RASSF1C remains to be fully elucidated.

Our lab has evidence suggesting RASSF1C may promote lung cancer cell growth. We showed that RASSF1C over-expression increases proliferation of non-small cell lung cancer (NSCLC) cells, KCi-H1299. We discovered that RASSF1C stimulates expression of interesting genes including the stem cell self-renewal gene PIWIL1 (also known as HIWI) in NSCLC cells.

Betulinic Acid (BA) has been shown to inhibit cell proliferation and migration, induce apoptosis in different human cancer types including lung cancer, and to down regulate PIWIL1 expression in gastric adenocarcinoma. In light of these findings and our findings on RASSF1C, we wish to determine if the down regulation of PIWIL1 gene expression and anti-cancer activities exerted by BA can be reduced by RASSF1C over-expression.

**Methods Used:** Cell proliferation: KCi-H1299 was transduced with MLV back-bone vector (NCI-BB) as a control or RASSF1C (NCI-IC) gene. Cells were cultured, counted, and plated onto 96 well plates. The cells were acclimated to serum-free media and exposed to 20–50μg/ml BA for 24 hr, and then analyzed by Alamar Blue assay. A plate reader analyzed fluorescence levels to indirectly measure cell proliferation or suppression.

RT-PCR: Cells were plated in 6-well plates and treated with 20–50μg/ml BA for 24 hr and then collected. RNA was isolated and used for RT-PCR analysis using RASSF1C and PIWIL1 gene specific primers.

Western Blot analysis: Cells were plated in 10 cm plates and treated with 20–50μg/ml BA for 24 hr. Cells were collected and used to prepare cell lysates for Western blot analysis using PIWIL1-antibody.

**Summary of Results:** Over-expression of RASSF1C seems to provide NCI-H1299 lung cancer cells with protection against the actions of BA. RT-PCR and Western blot analysis show that BA does not down-regulate PIWIL1 gene expression in NCI-H1299 cells.

**Conclusions:** RASSF1C over-expression reduces the anti-cancer activities exerted by BA on lung cancer cell proliferation. BA does not affect PIWIL1 gene expression in lung cancer cells unlike what has been reported in gastric adenocarcinoma cells.
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THE PENTOSTATIN PLUS CYCLOPHOSPHAMIDE (PC) NON-MYELOABLATIVE REGIMEN INDUCES DURABLE HOST T CELL FUNCTIONAL DEFICITS AND PREVENTS MARROW ALLOGRAFT REJECTION
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Purpose of Study: Although pentostatin [P] and fludarabine [F] have each been incorporated into non-myeloablative regimens, it is unknown whether these purine analogs differentially modulate host immunity prior to transplantation.
Methods Used: To address this, we utilized murine models to directly compare pentostatin and fludarabine for their ability to: (1) operate synergistically with cyclophosphamide [C] to induce host T cell depletion; (2) induce host T cell suppression, as defined by modulation of cytokine secretion in vitro and abrogation of host-versus-graft reactivity (HVGR) in vivo; (3) constrain host T cell recovery post-chemotherapy; and (4) prevent the rejection of T-cell depleted (TCD), fully MHC mismatched bone marrow allografts.
Summary of Results: Relative to single-agent regimens, combination PC and FC regimens, which were given over 3 days or 14 days, worked synergistically to deplete host CD4+ and CD8+ T cells; PC and FC regimens were developed that yielded similar levels of host T cell and myeloid cell depletion. In the setting of these generally comparable states of host T and myeloid cell depletion, the PC regimen was found to be more immune suppressive, as evidenced by reduced host T cell capacity to: (1) secrete IL-2 and IFN-γ in vitro; (2) mediate HVGR in vivo; and (3) recover numerically and functionally during a two-week observation period post-chemotherapy. Finally, using B6 hosts treated with the 14-day chemotherapy regimens, the PC regimen more consistently prevented the rejection of BALB/c TCD-allografts than the FC regimen (rate of allograft rejection, 14/15 [93%] of PC-treated recipients vs. 8/14 [57%] of FC-treated recipients; p<0.05).
Conclusions: Pentostatin and fludarabine were thus similar in their ability to operate synergistically with cyclophosphamide to induce marked host T cell depletion. However, the pentostatin-based regimen more profoundly suppressed host T cell cytokine potential, alloantigen-driven clonal expansion, numerical and functional recovery, and ability to reject fully MHC-disparate marrow allografts.

101 YIN YANG 1 REGULATES THE TRANSCRIPTIONAL REPRESSION OF SURVIVIN
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Purpose of Study: Survivin is a member of the Inhibitor of Apoptosis (IAP) family of proteins, and is highly expressed in all cancers but absent in normal tissue. Expression level correlates with chemos- and radioresistance, as well as poor prognosis in cancer patients. The mechanisms for upregulation of Survivin in cells undergoing stress associated with tumor development and the tumor microenvironment are not well understood. The putative stress response transcription factor Yin Yang 1 (YY1) was hypothesized to contribute to the upregulation of survivin in tumor cells.
Methods Used: In order to study regulation with luciferase reporter assays, U2OS cells were transfected with nested deletions of the survivin promoter, ranging from short (<230 bp) to long (>6280 bp). YY1 involvement in survivin promoter repression was confirmed using siRNA directed against YY1. A U2OS cell line containing a stable YY1 Tet-off system was used to determine whether a temporal increase in YY1 expression affects Survivin protein levels. To further evaluate the role of YY1 regulation of survivin expression, reporter constructs containing mutated putative YY1 binding sites in the proximal survivin promoter were used in reporter assays.
Summary of Results: When YY1 was overexpressed, luciferase expression was repressed 5–10 fold. Further studies showed that knockdown of YY1 releases the survivin promoter from the observed repression and leads to a 3–5 fold increase in promoter activity above basal levels. Furthermore, a low to moderate decrease in Survivin protein was observed in Tet removal experiments. Site-directed mutagenesis confirmed the involvement of proximal survivin promoter sites in YY1 overexpression-induced reporter repression. Mutation of these binding sites on the survivin promoter showed abrogation of the observed repression of survivin promoter activity.
Conclusions: These data suggest that YY1 is a novel repressor of survivin transcription. A better understanding of how YY1’s regulation of survivin expression in vivo affects cell cycle and cell death may provide new directions in therapeutics designed to prevent survivin overexpression in cancer.

102 SLUG mRNA IS HIGHLY EXPRESSED IN SQUAMOUS LUNG CARCINOMA AND ASSOCIATED WITH EXPRESSIONS OF EGFR AND ZEB1 IN PATIENTS WITH NON-SMALL CELL LUNG CANCER (NSCLC)
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Purpose of Study: Understanding biological markers for disease progression and metastasis in lung cancer is a key step for developing new treatment. The Epithelial-to-Mesenchymal Transition (EMT), a mechanism involved in the processes of invasion and metastases and sensitivity to EGFR Tyrosine Kinase Inhibitors, is characterized by the upregulation of the transcription repressors slug (a member of the snail family) and ZEB1 (Zinc finger E-box Binding homebox 1) and the resulting downregulation of E-cadherin.
Methods Used: In the present study, we performed quantitative RT-PCR analysis on resected tumors from 121 patients with NSCLC to measure the mRNA expressions of slug, ZEB1, and EGFR using beta-actin as the normalization signal.
Summary of Results: Slug mRNA was found to be significantly higher expressed in squamous lung carcinoma as compared with adenocarcinoma with a mean level of expression of 1.41 vs. 0.61 (p < 0.0009), respectively. In addition, slug was positively correlated with EGFR (Spearman r = 0.33, p = 0.0011) as well as with ZEB1 (Spearman r = 0.24, p = 0.028).
Conclusions: Our results demonstrate that slug mRNA is more elevated in squamous lung carcinoma, indicating that slug could be a potential target for the treatment of this type of lung cancer. The positive correlation between slug and EGFR based on patient tumors are consistent with the previous cell line data that showed decreased expressions of slug and ZEB1 when EGFR pathways were inhibited. Our results offer new insights into the pathways of EMT activation, and suggest a synergistic relation between EGFR activation, its downstream Myc product in the MEK/ERK pathway, and slug induction.

103 DETECTION OF INFLAMMATORY BIOMARKERS IN THE BLOOD OF PATIENTS WITH BREAST CANCER METASTASIS
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Purpose of Study: Breast cancer is the most common type of tumor and the second leading cause of cancer-related death for women in North America. The main cause of these deaths is not the primary tumor, but rather is from distant metastases. While standard treatment modalities have improved the overall outlook for women with breast cancer, there is still a need for better therapeutic and diagnostic approaches for patients that face metastasis. Clinical and experimental data have shown that pro-inflammatory cytokines and systemic inflammation can facilitate tumor metastasis, suggesting a correlation between inflammation and cancer metastasis. Therefore, the aim of this study is to detect new inflammatory biomarkers in the blood, which would potentially lead to earlier diagnoses and more successful outcomes for breast cancer patients with metastasis.
Methods Used: We evaluated the relationship between blood inflammatory biomarkers and breast cancer recurrence using blood samples from the Women Healthy Eating and Living (WHEL) study at UCSD. We obtained 20 case-control pairs. The cases are women who had distant metastasis. Controls are matched to cases based on date of diagnosis and stage of the primary breast cancer tumor and age at diagnosis of the primary cancer. Blood samples were acquired from both case and control women 1–2 years after their primary tumors were resected and before any signs of metastasis were present in the case women. RNA was extracted from buffy coats in order to perform quantitative RT-PCR to evaluate expression of selected inflammatory markers.
104 INFLAMMATORY MEDIATORS REGULATE IMP-3 EXPRESSION IN HEAD AND NECK SQUAMOUS CELL CARCINOMA (HNSCC)

Anastasiou C1, Luo J2, St. John M1 1David Geffen School of Medicine at UCLA, Los Angeles, CA and 2UCLA Health Center, Los Angeles, CA.

Purpose of Study: Insulin-like growth factor-II mRNA binding protein 3 (IMP-3; also known as homology domain-containing protein over-expressed in cancer and L523S), is an oncofetal protein believed to regulate translation of the potent growth factor and apoptosis inhibitor, insulin-like growth factor-II. IMP-3 is over-expressed in many HNSCCs and correlates with higher histologic grade, lymph node metastases (LNM), and advanced stages of disease.

HNSCCs are highly inflammatory and notably aggressive cancers. Prolonged exposure of human neoplasms to inflammatory mediators has been shown to promote epithelial-mesenchymal transition (EMT), tumor growth, invasion, angiogenesis, genetic instability, and metastasis. Among the myriad of inflammatory mediators elevated in the HNSCC microenvironment and associated with EMT and tumor growth is interleukin-1 (IL-1). We examined the effects of IL-1β on IMP-3 transcription and translation.

Methods Used: Western blot and real-time reverse-transcriptase polymerase chain reaction (RT-PCR) were utilized to determine how IL-1β affects IMP-3 expression in two well-characterized HNSCC lines Tu212 and Tu686, and in NFκB knock-down cells.

Summary of Results: IL-1β treated HNSCC cells showed significantly increased IMP-3 protein expression. This effect was diminished when NFκB knockdown cells were treated with IL-1β.

Conclusions: Our study demonstrates that inflammatory mediators in the tumor microenvironment increased IMP-3 expression in HNSCC. This is the first evidence that IL-1β may play a role in IMP-3 regulation, and that NFκB may be an intermediate in this regulatory pathway. Unraveling pathways in which IMP-3 operates may help identify novel targets for use in cancer prevention and therapy.

105 IS RACE A DETERMINANT OF SURVIVAL IN PATIENTS WITH CASTRATION RESISTANT PROSTATE CANCER TREATED WITH DOCETAXEL?

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Purpose of Study: The aim of this study was to identify ethnic differences between Hispanics (H), Whites (NH), and Native Americans (NA) with Castration Resistant Prostate Cancer (CRPC) in response to Docetaxel.

Methods Used: After Institutional Review Board approval, medical records of 59 CRPC patients treated with Docetaxel between 1999–2010 at the University of New Mexico Cancer Center and Hematology Oncology Associates were reviewed. Collected data included race, age, prior treatments, site of disease, and Gleason score. Docetaxel outcome variables were concurrent chemotherapy, baseline, nadir, and 12 week PSA; number and toxicity of Docetaxel courses; and addition of other treatments to Docetaxel. Progression of disease was defined as the duration of treatment with Docetaxel before the identification of a new lesion (by RECIST criteria) or PSA progression. Primary end point was overall survival (OS). Secondary endpoints was progression free survival (PFS).

Summary of Results: Median age of our cohort of patients was 71 years. There were 34 NHW, 16 H, 5 NA, 1 each of Asian, Black and Unknown. The median FWS was 24.6 weeks. The median overall survival was 44 weeks. The Gleason score at diagnosis did not have a statistically significant effect on OS or PFS of these patients. The OS was highest among NHW (75 weeks), followed by NA and worst for H (42.4 weeks). However, these results were not statistically significant (p-value=0.2778). PFS was greatest for NHW, followed by NA and then H (not statistically significant with a p value of 0.2778). Statistically positive variables for survival included PSA values at all time points, number of docetaxel courses and level of neutropenia following the first course of Docetaxel. The addition of thalidomide to Docetaxel had a favorable effect both on OS and PFS (p=0.0001 and p=0.0384, respectively).

Conclusions: In conclusions, ethnicities other than Black do not influence outcome of patients with CRPC, but known variables, such as PSA values and dose of chemotherapy did. Most interestingly, the addition of thalidomide significantly improved outcome in all New Mexican ethnicities.

106 DO WE KNOW WHAT CHILDREN RECEIVING LEUKEMIA/LYMPHOMA THERAPY HAVE VARICELLA ZOSTER IGG ANTIBODY PROTECTION?

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Purpose of Study: Varicella Zoster (VZ) infection can still be a life threatening illness in patients (pts) receiving anticancer treatment (Tx). Difficulty in determining the VZ antibody (Ab) status of pts led to the question: Do we know what pts have VZ Ab protection (Pro) at diagnosis (Dx) and during Tx?

Methods Used: A review of the records of all pts under 21 years who were treated for leukemia or lymphoma (L/L) at Doernbecher Children’s Hospital between 1/1/2003 and 12/31/2005 was done. Pt activity was followed for at least 4 years. Data reviewed included Dx, age, Tx, VZ Ab Pro at Dx, VZ Ab status (at least 4 months after onset of Tx) and clinical course.

Summary of Results: 118 were studied. At Dx, 73 pts were tested for VZ Ab; 45 were not. 49 pts had Ab Pro. 25 pts were tested at least 4 months after Tx was started: 7 pts Ab Pro at Dx were Ab Pro. 6 pts not tested at Dx were Ab Pro, 12 pts were Ab negative. 33 pts were never tested. Review of immunizations recorded was not helpful. 2 pts developed Varicella: one pt was never tested and the other Ab negative. 2 patients developed Zoster.

Conclusions: The VZ Ab status at Dx was known in 73 L/L pts (62%). The VZ Ab status, 4 or more months after Dx was known in 25 L/L pts (21%). The VZ Ab status of 33 (L/L) (28%) pts was never known.

During L/L Tx, there is a significant population of pts, whose VZ Ab status is not known.

Metabolism I
Concurrent Session
8:30 AM
Friday, January 28, 2011

107 PREVALENCE OF METABOLIC SYNDROME (METS) IN A MALE VETERAN CIGARETTE SMOKING POPULATION

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Purpose of Study: We have previously found that Mets is present in nearly one half of our local Veteran population at the Loma Linda Veterans Administration Medical Center. Smoking has been reported to be casually associated with Mets and may therefore contribute to this high prevalence in Veterans. We determined the proportion of smokers that met the criteria for Mets. In addition to waist circumference, BMI was used as a measure of central obesity in an attempt to examine the utility of BMI in comparison to the gold standard of waist circumference as a criterion for Mets.

Methods Used: We accessed computerized patient record systems for 500 consecutive male veterans enrolled in the “Break the Chains” smoking cessation class (August 2008–June 2009). We used the harmonized definition of Mets as published in Circulation (2009) for 239 patients (any 3 criteria of triglycerides >150 mg/dL, HDL cholesterol <40 mg/dL, systolic BP >130 and/or diastolic BP >85 mmHg, fasting plasma glucose >100 mg/dL and waist circumference >40 inches†). For all 500 patients we used BMI >30 kg/m2 as an alternative to waist circumference to evaluate the diagnostic utility of using BMI**.

Summary of Results: The prevalence of Mets in the subject populations was 57.2% using the BMI criterion and 63.2% using the waist circumference criterion. Of the 239 subjects with both measures, the sensitivity
and specificity to detect an increased central obesity using waist circumference as the gold standard was 80.8% and 84.9%, respectively.

Conclusions: Smokers are at high risk population for MetS and may gain weight after stopping smoking. While, waist circumference as a measure of central obesity identified a higher prevalence of MetS than BMI; sensitivity and specificity of BMI were high in this population of smokers and should be considered as an alternative measurement. Smokers need to have more aftercare for chronic disease risk management after achieving long term abstinence.

* Any of the above components of the metabolic syndrome could be satisfied alternatively by drug treatment or recorded clinical diagnosis.

**A BMI of 30 was chosen based on the WHO 1998 classification of MetS criteria.

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TESTOSTERONE DEPRIVATION ACUTELY DIMINISHES INSULIN SENSITIVITY IN YOUNG, HEALTHY MEN

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Purpose of Study: In men with prostate cancer, androgen deprivation therapy (ADT) alters glucose metabolism. Whether sex steroids modulate insulin sensitivity in healthy men remains unclear, as does whether such effects result from changes in testosterone or its active metabolite estradiol. We evaluated the effects of acute sex steroid withdrawal on insulin sensitivity in healthy men and determined the relative contributions of testosterone (T) and estradiol (E) to these effects.

Methods Used: We recruited 24 healthy men, 18–55 years old, with normal baseline T levels. All subjects received the GnRH antagonist acyline (300 mcg/kg/2 weeks × 2). In addition, subjects were administered either placebo transdermal gel and pills (Group 1), transdermal T gel 10g/day plus placebo pills (Group 2), or transdermal T gel plus the oral aromatase inhibitor anastrozole 1 mg/day (Group 3) daily for 28 days. Body weight, fasting serum hormones, adiponectin, glucose and insulin levels were measured bi-weekly.

Summary of Results: Group 1 subjects were medically castrate with treatment (T=0.8±0.8 nmol/L, E=32±11 pmol/L) whereas normal, baseline sex steroid levels remained unchanged in Group 2 subjects. Group 3 subjects maintained normal T levels while their serum E levels were equivalent to those in Group 1 (E=37±14 pmol/L). Group 1 subjects exhibited no changes in body weight or fasting plasma glucose, but their fasting insulin concentrations increased with treatment (P=0.02 vs. baseline) as did HOMA-IR (P=0.04 vs. baseline). Interestingly, adiponectin levels substantially increased in Group 1 (P=0.004 vs. baseline). None of these metabolic changes were observed among subjects in Groups 2 and 3.

Conclusions: Our data demonstrate that acute T withdrawal reduces insulin sensitivity independent of changes in body weight. Moreover, in the setting of androgen deprivation, increases in adiponectin do not exhibit the expected association with enhanced insulin sensitivity, thereby suggesting that adiponectin may be regulated directly by T. Changes in insulin sensitivity were not evident among subjects with a selective reduction in estradiol, indicating that diminished insulin sensitivity was attributable to androgen deprivation. Whether androgen replacement in hypogonadal men can improve insulin sensitivity merits further investigation.

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PROTECTIVE EFFECT OR TESTOSTERONE AGAINST HIGH FAT DIET INDUCED NON-ALCOHOLIC FATTY LIVER DISEASE IN T-DEFICIENCY RATS MAY NOT BE INSULIN-DEPENDENT

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Purpose of Study: NFALD is associated with obesity, diabetes, insulin resistance (IR) and Metabolic Syndrome (Met S). Testosterone (T) deficiency is a risk factor for developing diabetes and Met S, but the role of testosterone deficiency in hepatic steatosis has not been well studied. Our laboratory has shown that high fat diet (HFD)-induced NAFLD was attenuated by T replacement in castrated rat. In this study we examined whether T amelioration of NAFLD is related to changes in IR. We studied the 4 key biomarkers of insulin action including adipose triglyceride lipase (ATGL) and hormone sensitive lipase (HSL) and sterol regulatory element-binding protein-1 (SREBP-1) and fatty acid synthase (FAS).

Methods Used: Male rats were randomly placed into four groups: castrated rats on high-fat diet (HFD), castrated rats with T replacement on HFD, intact rats on HFD, and intact rats on regular chow diet (RCD). The HFD provided 71% energy from fat; RCD provided 16% of energy from fat. The rats were fed ad libitum for 15 weeks then animals were killed and liver tissue collected and kept at ~80°C. Western blot was used to determine the changes in expression of ATGL, HSL, SREBP-1, and FAS.

Summary of Results: As previously reported (Nikolaenko et al. ENDO 2010), serum T levels were not detectable in castrated rats, and T replacement led to higher serum T levels than in intact rats. No statistical difference was detected in serum glucose or insulin level between groups. The four insulin-regulated proteins, ATGL, HSL, SREBP-1, and FAS were not changed with castration after T treatment in rats fed a high fat diet.

Conclusions: As we reported previously, T treatment in castrated rats fed a HFD reduces hepatic steatosis and fibrosis compared with castrated animals. Insulin level and insulin-regulated proteins were not changed after T replacement. Our data suggest that androgen deficiency contributes to the severity of hepatic steatosis and that T plays a protective role in liver fat accumulation and NAFLD development. Insulin resistance may not play an important role in this rat model of HFD-induced NAFLD and the protective effect of T in reversing NAFLD.

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RAPAMYCIN ADMINISTRATION IMPROVES METABOLIC ABNORMALITIES AND DECREASES ADIPOSE TISSUE INFLAMMATION IN DIET-INDUCED OBESITY


Purpose of Study: Obesity is often associated with insulin resistance, which may promote and increase the risk of cardiovascular disease. However, the exact molecular mechanisms by which obesity leads to insulin resistance are poorly understood. A hallmark of visceral obesity is the accumulation of adipose tissue macrophages which can contribute to insulin resistance. Aberrant, chronic activation of the intracellular nutrient sensing protein, mammalian target of rapamycin (mTOR), a downstream effector of the insulin signaling pathway, has been implicated in the pathogenesis of insulin resistance. We hypothesized that oral administration of rapamycin, a potent and specific inhibitor of mTOR, could reduce insulin resistance and improve adipose tissue macrophage accumulation.

Methods Used: C57BL/6J mice were placed on a high fat, high carbohydrate “diabetogenic” diet with rapamycin or placebo for 16 weeks. Rapamycin was administered admixed in the diet a microencapsulated form and levels were detectable only in the drug-treated group.

Summary of Results: Mice that received rapamycin showed lesser weight gain (p<0.01) compared to placebo fed animals despite equivalent food intake. Body composition analysis showed decreased body fat mass in the drug treated group. Rapamycin-fed animals had lower plasma glucose (p<0.05) and modest improvement in insulin sensitivity on insulin tolerance testing. Plasma and hepatic triglyceride levels were reduced and morphologic analysis of the liver revealed decreased fat accumulation in hepatocytes in the rapamycin group compared to the control animals. Immunohistochemical analysis of periglandular (visceral) adipose tissue showed decreased staining for macrophage Mac2 in the mice treated with rapamycin. Gene expression analysis of adipose tissue revealed decreased expression macrophage F4/80 and TNFa mRNA, while adiponectin gene expression was increased in the rapamycin treated group.

Conclusions: Taken together, these data show that rapamycin improves the metabolic profile and decreases adipose tissue inflammation in this mouse model of diet-induced obesity. These results suggest that rapamycin and/or its analogs have the potential for development as anti-obesity therapies.
diabetes care. We examined the psychological factors underlying the unwillingness to begin insulin therapy among low-income Hispanic and African-American (AA) diabetic patients. 

Methods Used: We conducted a cross-sectional survey of 156 non-insulin-treated type 2 diabetic patients receiving care at a diabetes referral clinic in inner city Los Angeles. The Survey for People Who Do Not Take Insulin (SPI) was completed anonymously by consecutive patients during scheduled clinic visits from February to May 2010. Analyses were restricted to insulin-naive patients, and included the differential responses between Hispanic and AA patients, as well as correlations between demographic characteristics with the prevalence of unwillingness or the responses to each survey item.

Summary of Results: A total of 136 respondents (87%) were insulin-naive (57% female; 69% Hispanic, 24% AA). Mean age and diabetes duration were 51.1 ± 10.3 and 6.9 ± 6.9 years, respectively. Median monthly household income and highest educational level categories were $200–$1000 and grade 8–12, respectively. Overall, 41.9% reported a complete unwillingness to begin insulin. Compared to AA, Hispanic respondents were younger, lived fewer years in the U.S., had less education, were significantly more likely to be completely unwilling (46.6% vs. 26.5%, p=0.039), and reported a more negative attitude to 8 of the 9 survey items (p<0.01 for each). Overall, fewer years in the U.S. predicted greater unwillingness and a more negative attitude on 8 of the 9 survey items (p=0.03 for each); less education predicted greater feelings of unfairness (p=0.01); Hispanic ethnicity predicted greater feelings of failure (p=0.04); and males reported greater feelings of lifestyle restriction (p=0.04).

Conclusions: Complete unwillingness to begin insulin use and negative attitudes toward insulin are highly prevalent among low income, Hispanic type 2 diabetic patients. These data may help to better guide culturally appropriate patient counseling regarding insulin use.

113 EFFECT OF INSULIN ON SPILLOVER OF MEAL DERIVED FATTY ACIDS

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Purpose of Study: Spillover, or release of chylomicron triglyceride fatty acids by lipoprotein lipase (LPL) into the circulation as free fatty acids (FFA), may contribute to the elevated total FFA seen in insulin resistant (IR) states. A previous study in dogs suggested that spillover is regulated by intracellular lipolysis. The current study tested if exogenous insulin infusion during meal absorption in IR adults, which produces greater FFA suppression than the meal alone, results in reduced spillover.

Methods Used: Nondiabetic, IR adults (n=7) were studied on two occasions, a control day (C) and an insulin infusion day (I), using a non-randomized design. Following a 5 day controlled diet and an overnight fast, subjects were given a liquid meal made from a commercial nutrition supplement with additional vegetable oil to total 35% of calories as fat, 15% as protein, & 50% as carbohydrate. Subjects sipped 1–2 ounces of test drink every 15 minutes for 6.5 hours to achieve steady state. A commercial lipid emulsion labeled with [3H] triolein was infused to serve as a chylomicron tracer, and [1C] oleate was infused to measure the rate of appearance of FFA and [3H] oleate - the latter to allow calculation of spillover. The study was repeated 2 weeks later in identical fashion except that the subjects received insulin at 20 mL/m2/min.

Summary of Results: Plasma FFA concentrations were higher on the C day compared to I (124±13 v 75±8 μmol/L, p < 0.01). Glucose concentrations were not different between C compared to I (113±2 vs 113±1, p=NS). Glucose infusion rate on I averaged 20±4 g/h. Plasma triglyceride concentration was similar on C compared to I, both at baseline and during meal absorption (181±23 v 168±29, p=0.50 & 257±22 v 230±35 mg/dL, p=0.12). Oleate rate of appearance did not differ on C v I (31±27 v 29±53 μmol/min, p=0.3). Spillover was the same on C and I (28±3% vs 29±3%, p=0.77). Oleate clearance was less with C than I (9±1 v 12±1 mL/min, p=0.02).

Conclusions: Our data indicate that suppression of intracellular lipolysis with insulin does not reduce LPL-mediated spillover in humans during meal absorption. Regulation of spillover may be related to noninsulin FFA suppression or other factors.

114 SITAGLIPTIN VERSUS THIAZOLIDINEDIONES AS A THIRD-LINE ORAL ANTI-HYPERGLYCEMIC AGENT IN TYPE 2 DIABETES MELLITUS


Purpose of Study: To compare sitagliptin and thiazolidinediones (TZDs) as a third-line oral anti-hyperglycemic agent among poorly controlled, ethnic minority type 2 diabetic patients.

Methods Used: We treated 108 insulin-naive type 2 diabetic subjects who were sub-optimally controlled on maximum tolerated doses of metformin plus sulfonylureas with addition of sitagliptin 100 mg daily, and compared their responses against a group of 104 similar patients treated with rosiglitazone (8 mg) or pioglitazone (45 mg) daily as their third-line oral agent, using an identical treatment algorithm. Subjects were assessed bi-monthly, and those who achieved HbA1c < 7.5% by 4 months were continued through 12 months of follow-up. Pioglitazone 45 mg daily was used as rescue therapy for subjects who failed to achieve and/or maintain HbA1c < 7.5% at or beyond 4 months.

Summary of Results: At baseline, sitagliptin- and TZD-treated subjects had identical HbA1c (9.4 ± 1.8% and 9.4 ± 1.9%, respectively) and similar known diabetes duration (6.7 ± 5.0 and 7.6 ± 5.8 years, respectively). HbA1c was reduced in both groups at 4 months (p<0.001), but the reduction was greater with TZDs than sitagliptin (−2.0 ± 1.7% vs −1.3 ± 1.8%, p=0.06); was the proportion of subjects achieving HbA1c < 7.5% (61.5% vs 46.1%, p=0.026). Of all subjects achieving HbA1c < 7.5% at 4 months, the same proportions of subjects in each group successfully sustained their HbA1c < 7.5% by 12 months (59.1% vs. 57.8%). Of the sitagliptin subjects who failed to achieve or maintain HbA1c < 7.5% and were given pioglitazone 45 mg daily as rescue therapy, 27.3% successfully achieved HbA1c < 7.5% after an average of 4 months on pioglitazone. Sitagliptin was well tolerated, with no significant weight gain, hypoglycemia, or other adverse events.
Conclusions: Among ethnic minority type 2 diabetic patients very poorly controlled on maximum tolerated doses of metformin and sulfonylureas, third-line add-on therapy with a TZD controlled hyperglycemia more effectively than sitagliptin after 4 months. Our findings may have implications for clinical practice recommendations.

115 INSULIN RESISTANCE IN ADOLESCENTS WITH TYPE 1 DIABETES: ROLE OF HYPERGLYCEMIA

Pryor L1, Hull A2, Green MC1, Forster J3, Reusch JE1,2, Nadeau KJ1 UC Denver, Aurora, CO; 2UC Denver, Denver, CO; 3DV/AMC, Denver, CO and 4UC Denver, Aurora, CO.

Purpose of Study: Insulin resistance (IR) and associated metabolic syndrome (MetS) are likely responsible for the increased risk of cardiovascular disease (CVD) in type 2 diabetes, but the etiology of accelerated CVD in type 1 diabetes (T1D) is unclear. We recently reported the paradoxical presence of IR in lean T1D youth despite absence of the MetS. This IR may contribute to CVD in T1D, but its mechanism is unknown. A potential cause of IR in T1D may be hyperglycemia. A study reported IR in T1D adults that was unrelated to HbA1c, but this study excluded marked hyperglycemia (HbA1c > 9.5 excluded, mean HbA1c 7.6%) and has not been replicated in youth. Thus, the question of glucose’s role in the IR of T1D remains. We aimed to determine if acute or chronic glycemia contributes to IR in T1D adolescents, a group notorious for hyperglycemia.

Methods Used: We enrolled 66 lean, sedentary (<3 hours exercise/week), T1D youth (46% male, age 15.5±2.2 years, HbA1c 8.6±1.5%, HbA1c range 6.2-11.9%, BMI Z-score 0.38±0.96, %fat 24.9±7.5%) and 14 matched, lean, sedentary non-diabetic controls. Following a 3-day control diet and overnight insulin infusion, IR was determined by a 80 μM/min hyperinsulinemic euglycemic clamp.

Summary of Results: Fasting glucose prior to the clamp was 122.0±23.7 mg/dL in T1D youth and 83.9±5.1 mg/dL in control youth. T1D youth were significantly more IR than controls (mean glucose infusion rate 8.3±3.3 vs. 15.3±3.7 mg/kg/min, p<0.01; 11.6±4.1 vs. 19.8±4.6 mg/lean/kg/min, p<0.01) despite similar final glucose and insulin concentrations. By simple linear regression, age, Tanner stage, BMI Z-score, and % fat mass, but not gender, were significantly associated with IR in mg/kg/min among T1D subjects (p=0.005, p=0.01, p=0.001, p=0.0001 respectively). While controlling for age, BMI Z-score, and percent fat mass, there was no significant association between fasting glucose prior to the clamp or HbA1c, and IR (expressed either per kg or per lean kg).

Conclusions: Similar to adults with lower HbA1c, T1D adolescents have IR unrelated to acute or chronic glycemia or the MetS, suggesting the existence of alternative unique mechanisms of IR in T1D. Future studies will aim to detect these factors to target CVD risk-reducing therapies.

116 A UNIQUE CASE OF A 34 YEAR OLD WOMAN WITH TYPE-2 DIABETES PRESENTING WITH DIABETIC KETOACIDOSIS SECONDARY TO INSULIN ANTIBODIES

Garimella M, Schade DS University of New Mexico, Albuquerque, NM.

Case Report: High titer insulin antibodies are an unreported cause of diabetic ketoacidosis. We describe the novel case of a 34 year old woman with type 2 diabetes for 10 years (A1C=10.9), controlled on diet and oral medications alone, who presented with severe diabetic ketoacidosis. Patient noted that two weeks prior to admission she had a viral prodrome of fever and rash along with unusually elevated blood glucoses in the 400s. This prompted her to take 70/30 human insulin for 5 days. She had previously used 70/30 human insulin only during her two pregnancies, five and seven years prior to admission. Initial insulin requirements on admission were 40 units/kg/day (150 units/hour) intravenously for correction of the hyperglycemia and ketoacidosis. Further evaluation revealed an extremely high titer of insulin antibodies. When subcutaneous insulin was finally initiated on day 6, she relapsed into diabetic ketoacidosis, indicating the continued presence of high titer insulin antibodies. Her daily hospital chemistries and antibody levels are shown in the table below.

Conclusion: The temporal relationship between the decline in elevated insulin antibodies and the improvement of diabetic ketoacidosis strongly suggest a causal relationship which has hitherto never been reported. Previous intermittent use of parenteral insulin and viral illness are likely precipitating factors for the development of insulin antibodies. Physicians need to include insulin antibodies in the differential of precipitating causes of diabetic ketoacidosis and be cautious about weaning intravenous insulin rapidly in these patients.

<table>
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<tr>
<th></th>
<th>Day1</th>
<th>Day2</th>
<th>Day3</th>
<th>Day4</th>
<th>Day5</th>
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<tr>
<td>Glucose (mg/dL)</td>
<td>512</td>
<td>406</td>
<td>305</td>
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<td>247</td>
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<tr>
<td>HCO3 mmol/L</td>
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<tr>
<td>Insulin U/hr</td>
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<td>960</td>
<td>140</td>
<td>120</td>
<td>320</td>
</tr>
<tr>
<td>Insulin antibody mmol/L</td>
<td>5565</td>
<td>2498</td>
<td>880</td>
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</tbody>
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Normal levels of insulin antibodies are <0.02 nmol/L.

117 SYNERGISTIC EFFECT OF FRUCTOSE AND INSULIN ON GLUCOSE METABOLISM IN HEPG2 CELLS

Patterson ME1,2, Wahjudi P2, Mao C1,2, Yee JK1,2, Lee PW1,2 Harb-UCLA Medical Center, Torrance, CA and 1LA BioMed, Torrance, CA.

Purpose of Study: The phenomenon that fructose is a better substrate for glycogen synthesis than glucose is known as the glucose paradox. It has been postulated that the increase in glycogen synthesis is the result of increases in glucose uptake and gluconeogenesis in hepatocytes in the absence of insulin. Whether the addition of insulin can further stimulate glycogen synthesis is unknown. To test this possibility, we investigated the effect of fructose on glycogen synthesis in HepG2 cells.

Methods Used: HepG2 cells were grown to confluence and incubated for 4 hours in medium containing 100 mg/dL glucose, 10 mM 50%U-13C3 lactate, and 1 mM sodium pyruvate. 4 sets of conditions were tested: no fructose or insulin; with fructose, no insulin; no fructose, with insulin; and with fructose and insulin. The medium concentrations of fructose and insulin were 3 mM and 100 nM, respectively. Glucose uptake and cell glycogen quantitation were carried out by GC/MS analysis with U-13C glucose as the internal standard.

Summary of Results: Glucose uptake, which was slightly increased by fructose and insulin compared with controls, was highest in the fructose plus insulin group. Glycogen (μg) had a similar trend with markedly increased levels in the fructose (40.67 ± 4.21) and insulin (18.43 ± 3.5) groups compared with controls (10.7 ± 2.2) with the largest increase in the fructose plus insulin group (66.11 ± 6.34). Glucose release (mg/dL) decreased with the addition of insulin (9.65 ± 1.09) and increased with fructose (13.9 ± 0.65) compared with glucose only (10.81 ± 1.03). The group with fructose and insulin showed a further decrease (8.64 ± 0.61). Increased fatty acid synthesis was seen with fructose (9.06 ± 0.12%) and insulin (9.89 ± 0.35%) compared to the control group (7.76 ± 0.11%) with the greatest increase in the fructose plus insulin group (11.05 ± 0.22%).

Conclusions: Our results show that the combined effect of insulin and fructose increase glucose cycling and fatty acid synthesis, abnormalities commonly found in metabolic syndrome and insulin resistant diabetes. Whether these metabolic changes are the underlying mechanisms for the association between high fructose consumption and the development of insulin resistance in metabolic syndrome remains to be investigated.

Neonatal – Pulmonary I

Concurrent Session

8:30 AM

Friday, January 28, 2011

118 A NOVEL METHOD FOR THE DETECTION OF NITRITE-DERIVED NITRIC OXIDE IN WHOLE BLOOD

Goltiao D1, Barceló LE1, Blood AB2, Power GG3 Loma Linda University, Loma Linda, CA and 2Loma Linda University, Loma Linda, CA.

Purpose of Study: Nitric oxide (NO) is a potent vasodilator important to blood flow regulation. Recent studies suggest that nitrite (NO2−), a compound found in plasma at ~150 mM concentrations, can be converted into NO by the following reaction with deoxyhemoglobin: deoxyHb + NO2− + H+ → metHb + NO + OH−.

Yet, as this reaction is more than a million times slower than the rates at which NO itself is scavenged by reactions with hemoglobin, it is still unclear whether this reaction can create vasodilating amounts of NO. Using a highly sensitive method to detect small quantities of NO would help verify this reaction in vivo, providing a new method for the detection of nitrite-derived NO in whole blood.

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sensitive method of measuring NO gas in liquid, this study aimed to measure NO production from nitrite in whole blood. We hypothesized that nitrite injections into blood at near-physiological concentrations would produce vasodilating amounts of NO.

**Methods Used:** A closed 300-mL circuit with gas exchanger, heater, pump, pressure and flow rate sensors, was filled with either phosphate buffered saline (PBS) or with sheep blood circulating at 300 mL/min and deoxygenated by continuously perfusing the gas-phase side of the exchanger with N2 and CO2. Gas exiting the exchanger was sampled for NO using a chemiluminescence NO analyzer (NOA) sensitive to ~5 parts per billion. The sensitivity for detecting NO in the liquid phase was determined by injecting free NO into circulating PBS. The production of NO from nitrite and hemoglobin was measured by injecting sodium nitrite (50 to 500 μM) into circulating blood. Methemoglobin was measured via spectrophotometry.

**Summary of Results:** Injections of NO into PBS resulted in linearly proportional (R2 = 0.995) detection of NO by the NOA from <10 nM to >10 μM concentrations. Although injection of nitrite into whole blood resulted in the production of methemoglobin as predicted by the above equation, NO production in blood was not detected until nitrite concentrations reached concentrations ~1000 times higher than physiologic concentrations.

**Conclusions:** Using novel methods of detecting low nanomolar concentrations of NO in liquid, we were unable to detect NO production from nitrite unless nitrite concentrations were ~1000-fold higher than the physiological range. These data suggest that the vasodilating properties of nitrite are derived by mechanisms other than the reaction of nitrite with deoxyhemoglobin.

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**119 NITRITE INFUSION AT PHYSIOLOGIC CONCENTRATIONS REDUCES CAROTID VASCULAR RESISTANCE IN FETAL SHEEP**

Truong G1, Schroeder H2, Bragg S1, Power GG2, Loma Linda University Medical Center, Loma Linda, CA and 1Loma Linda University Medical Center, Loma Linda, CA.

**Purpose of Study:** Nitrite, an anion present in mammalian plasma at mid-nanomolar concentrations, can be converted to the vasodilator nitric oxide by reacting with deoxyhemoglobin. The reaction occurs faster with fetal hemoglobin than adult, and may regulate fetal vascular tone under hypoxic conditions. This experiment tests the hypothesis that nitrite influences fetal carotid artery vascular resistance under physiological conditions.

**Methods Used:** We surgically instrumented fetal sheep for continuous measurement of arterial blood pressure and carotid artery flow, from which carotid resistance was calculated. After 4 to 5 days of recovery, ewes and fetuses both received intravenous L-NNA to block nitric oxide synthase activity. Nitrite was then infused into the fetal carotid artery (via a lingual artery catheter) in doses increasing from 10 nM to 33,333 nM in 15 minute steps. Samples for blood gases and nitrite concentrations were obtained periodically throughout the experiment. One-way ANOVA was used to determine significant changes over time.

**Summary of Results:** A significant increase in carotid resistance was observed after L-NNA infusion (p<0.01). Carotid artery resistance decreased following initiation of nitrite infusion, reaching statistical significance during the infusion of 333 nM nitrite (p<0.05).

**Conclusions:** We conclude that nitrite infusion to the carotid artery at near-physiological concentrations significantly reduces carotid vascular resistance to flow.

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**120 PRENATAL ADMINISTRATION OF ROSIGLITAZONE TO RAT PUPS DOES NOT ALTER THE ADULT METABOLIC PHENOTYPE**


**Purpose of Study:** To study the metabolic profiles of adult rats exposed prenatally to RGZ.

**Methods Used:** Pregnant Sprague-Dawley rat dams were administered either diluent, RGZ (0.3mg/kg), RGZ (3mg/kg), or Dexamethasone (0.25mg/kg), intraperitoneally (IP) once daily x 2 doses, 24h apart, starting at embryonic day 18. At 11~12 wks of age glucose and insulin tolerance tests were performed after a 12h fast (glucose administered at 1g/kg IP, and insulin administered at 1U/kg subcutaneously). De novo fatty acid synthesis was analyzed by deuterium incorporation (DI) [99.9% deuterated water (D2O)]. D2O was injected IP once at a dose equivalent to 4% of body weight, followed by free access to drinking water containing 6% D2O for 7 days] and mass spectrometry analysis at 14 wks. At 15 wks, the animals were sacrificed and livers, lungs, liver, skeletal muscle and fat were collected for Western hybridization (WH) for PPARy (peroxisome proliferator-activated receptor gamma) and adiponectin. Similarly, triglycerides, cholesterol, insulin, glucagon and troponin-I assays revealed no significant differences among all 4 groups, (all p values > 0.05).

**Conclusions:** Prenatal administration of RGZ does not alter the adult metabolic phenotype. We speculate that RGZ therapy is a safe alternative to dexamethasone for accelerating lung maturation and in preventing broncho-pulmonary dysplasia in the premature infant.

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**121 LUNG LIPOFIBROBLASTS (LIFs) ACTIVELY RECRUIT, STORE AND RELEASE RETINOIC ACID IN RESPONSE TO PGE2**

Truong N, Sakurai R, Rehan V, Torday J Harbor-UCLA, Torrance, CA.

**Purpose of Study:** Retinoic Acid (RA) is necessary for epithelial phenotypic expression and alveolar barrier function, yet treatment with exogenous RA has not been found to consistently promote and protect the alveolar epithelial barrier, either experimentally or clinically. We have previously shown that the alveolar type II (ATII) cell increases PGE2 secretion when stretched, stimulating Lipid Droplet (LD) secretion from neighboring lipofibroblasts (LIFs) at PGE2 concentrations comparable to those produced by ATII cells in culture. Since RA is also stored in LIF LDs, we hypothesized that PGE2 would concomitantly stimulate RA and lipid secretion by LIFs, coordinating the release of ATII surfactant lipid substrate with RA, the latter being an agonist for both surfactant phospholipid synthesis and surfactant protein expression. Such a pleiotropic mechanism of RA action would coordinate surfactant phospholipid and protein production with alveolar distension, preventing atelectasis.

**Methods Used:** Uptake, storage and release of RA by cultured fibroblasts was determined based on the kinetics of 3H-RA in cultured Wister human embryonic lung fibroblasts, primary fetal rat lung fibroblasts and adult lung fibroblasts. Cells were treated with Prostaglandin E2 (5 × 10−7 M) as a positive control for the uptake and release of RA from LIFs. The sensitivity and specificity of the PGE2 effects on LIF RA kinetics were determined using time-course and dose-response curves in combination with the use of specific molecular inhibitors (phloretin) and stimulators (butaprost) of the PGE2 receptor.

**Summary of Results:** LIFs actively recruit RA over a ~1–2 hour period, and store it in lipid droplets. PGE2 (5 × 10−7 M) caused release of RA, whereas baseline secretion was minimal.

**Conclusions:** LIF uptake and release of RA is coordinately regulated by PGE2 signaling from ATII cells. This effect has novel implications for understanding the molecular targets for the treatment of Respiratory Distress Syndrome. Bronchopulmonary Dysplasia, Interstitial Pulmonary Fibrosis and Emphysema. (Partially supported by grants from the NIH (HL-55268, 5R01HL094526-02A1).
Fibroblast growth factor 10 (FGF-10), an important growth factor that regulates lung branching and alveolarization, is decreased in BPD. On the other hand, our lab has recently shown in a rat model that BPD can be prevented by PPARγ agonists such as prostaglandin J2 (PGJ2). How PGJ2 affects FGF-10 signaling is not known. The purpose of the study is to determine the effects of FGF-10 on alveolar fibroblast differentiation and determine if PGJ2 blocks hyperoxia-induced changes in FGF-10 signaling.

Methods Used: To determine the effects of FGF-10 on alveolar fibroblast differentiation, both e19 (primary) fetal rat lung fibroblasts and human embryonic (WI38 cells) were treated with FGF-10 (1-100 ng/ml) for 24h. Following which, markers of fibroblast differentiation (PPARγ, ADRP, SREBP1c, p2, leptin and triolein uptake) were analyzed. In separate experiments, neonatal Sprague Dawley rat pups were categorized in the following groups: normoxia (control, 21%O2), normoxia + PGJ2 (0.3mg/kg), hyperoxia (exposure to 95%O2 for 7d), hyperoxia + PGJ2 (0.3mg/kg). PGJ2 was administered i.p. once daily. At the end of experimental period, the rat pups were sacrificed and protein was extracted from whole lung samples. Using Western blotting, we probed for FGF-10, and its receptor FGF receptor 2, PPARγ, ADRP, leptin, fibronectin, Bax, Bcl-2 and cleaved caspase-3.

Summary of Results: FGF10-treated fibroblasts showed dose-dependent increases in markers of differentiation. Under in vivo conditions, PGJ2 blocked hyperoxia-induced decreases in FGF-10, PPARγ, leptin and increases in FGF-receptor 2 and fibronectin levels. Furthermore, hyperoxia-induced changes in apoptotic markers (increases in Bax and cleaved caspase 3 and decrease in Bcl-2) were also blocked in the PGJ2-treated group.

Conclusions: Our data demonstrate the down-regulation of FGF-10 signaling and increase in apoptosis on exposure to hyperoxia, both of which were blocked by PGJ2. We speculate a direct interaction between FGF-10 and PPARγ signaling pathways in the maintenance of alveolar growth and differentiation (Grant support: NIH-HL55268, HL075405, HD058948, HD051857) and TRDRP (15T0250, 17R0170).

**123 URINARY NITRIC OXIDE METABOLITES (NOx) AND CYCLIC GUANOSINE MONOPHOSPHATE (cGMP) IN VENTILATED EXTREMELY LOW BIRTHWEIGHT (ELBW) NEWBORNS RECEIVING INHALED NITRIC OXIDE**


**Purpose of Study:** ELBW newborns requiring mechanical ventilation after 7 d of age have > 70% incidence of bronchopulmonary dysplasia (BPD). In the NO CLD Trial, treatment with inhaled nitric oxide (iNO) increased survival without BPD (Ballard R, New Engl J Med 2006, 2007) and increased levels of NOx in plasma and tracheal aspirate in a dose-dependent manner (Posecheg J Perinatol 2009). The effect of infant iNO treatment on cGMP, which mediates many responses to NO, is not known. The objective of this study was to assess concentrations of NOx and cGMP in urine of a new cohort of ELBW infants.

**Methods Used:** 379 urine samples were collected at intervals from 38 infants ≤ 50 wk (mean 25.4 wk) in a pilot trial of late surfactant treatment. All infants received 24 d of iNO per the NO CLD protocol. NOx was assayed with a Sievers’s NO Analyzer and cGMP was measured by Elisa (Cayman). All data were normalized to urine creatinine (cre, Cayman) and expressed as median values.

**Summary of Results:** Baseline values, defined as concentrations after stopping iNO, were NOx 359 pmol/μg cre and cGMP 8.3 nmol/μg cre. NOx increased in dose-dependent fashion with iNO exposure, plateauing between 10 and 20 ppm: 2 ppm 2.6-fold, 5 ppm 2.4-fold, 10 ppm 3.3-fold, and 20 ppm 2.9-fold (all ≤0.001). Corresponding values for cGMP were 1.1-fold (NS), 1.8-fold, 2.5-fold and 3.1-fold (each ≤0.002). The fold increase at 10 to 20 ppm was inversely correlated with baseline values for NOx and cGMP (r= -0.50 ~ -0.48, p=0.004). Using mean infant values, urinary cGMP was correlated with NOx at both baseline (r=0.43) and at iNO doses of 10~20 ppm (r=0.35). Treatment with late surfactant did not affect either NOx or cGMP.

**Conclusions:** We conclude that iNO treatment of ELBW infants increases levels of both NOx and cGMP in the urine, reflecting delivery of NO to the lung, stimulated cGMP production, and diffusion of both biomarkers into plasma. Urinary NOx and cGMP are non-invasive measures of endogenous and exogenous NO activity.

**124 LIPOFIBROBLAST LIPID DROPLET TRAFFICKING CO-REGULATES ALVEOLAR TYPE II CELL (ATII) SURFACTANT PHOSPHOLIPID:PROTEIN EXPRESSION**

Williams J, Sakurai R, Rehan V. Torday J Harbor-UCLA, Torrance, CA.

**Purpose of Study:** The mechanism corregulating surfactant phospholipid and protein by ATII is largely unknown. Distension of lipofibrolasts (LiFs) releases lipid droplets. We hypothesized that lipid droplets coordinately stimulate surfactant phospholipid and protein.

**Methods Used:** Adipocyte differentiation-related protein (ADRP)-GFP lipid droplets (LDs) normalized to their triglyceride content were incubated with AS49 cells. Subcellular localization of LDs was monitored by confocal microscopy. The effects of LDs on SP-B expression was determined by PCR and Western analysis. LD stimulation of saturated phosphatidylcholine (satPC) synthesis was determined by 3H-choline incorporation into satPC. LDs were injected i.p. to mechanically ventilated rats to determine if ADRP localized in the lung and stimulated surfactant synthesis.

**Summary of Results:** LDs localized in AS49 nuclei within 10 min, appearing as prominent cytoplasmic inclusions over the next two hours. Uptake of the GFP-ADRP LDs was dose-dependent, increasing SP-B mRNA expression by 80% at 100 μg/ml, and blocked by co-incubation with actinomycin D. LDs also increased SP-B protein expression in a dose-dependent fashion, increasing SP-B by 70% at 100 μg/ml, blocked by co-incubation with cycloheximide. Incubation of AS49s with LDs for 24 h stimulated satPC synthesis by 57-fold; co-incubation with ADRP antibody caused a dosimetric decrease in LD-induced satPC synthesis. Uptake of LDs administered to mechanically ventilated adult rats (30 min) resulted in a dose-dependent increase in lung ADRP content ranging from 5% at 45 μg/kg to 20% at 4600 μg/kg. SP-B protein increased between 5% at 45 μg/kg and 20% at 4600 μg/kg.

**Conclusions:** LDs rapidly translocate to ATII nuclei, stimulate SP-B expression and surfactant phospholipid synthesis in an ADRP-dependent manner. In vivo administration of LDs results in a dose-dependent increase ADRP in lung, and a dosimetric increase in SP-B, consistent with the in vitro findings. Therefore, stretch-regulated ATII-fibroblast interaction causes secretion of LDs that coordinate stimulus surface active phospholipid and surfactant protein production. (Partially supported by grants from the NIH (HL-55268, HL075405, HD058948, and HD-051857) and the TRDRP (14RT-0073, 15T0250, 17R0170).
doses of surfactant (callfactant) plus INO to improve surfactant composition in ventilated preterm infants.

Methods Used: Randomized, blinded trial of ELBW (≤ 1000g and 30 0/7 wk GA) ventilated at 73–14 d. Infants received INO plus 5 dose procedures of surfactant (SURF) or sham (CTRL). SP-B content (% of total protein) was quantified by immunoblot assay in large aggregate surfactant isolated from tracheal tracheal aspirate samples. 85 infants were enrolled; 48 were treated every 3–4 d (SURF n=28 and CTRL n=20) and the remainder were treated weekly after the first 2 doses.

Summary of Results: Infants treated every 3–4 d that received SURF (vs. CTRL) were less mature (25.0 vs. 25.7 wks, P=0.05) and more likely male (68 vs. 40%, P=0.06). SP-B content at study entry was lower in SURF (1.06%) vs. CTRL (2.34%, P=0.05). Compared to baseline levels, SP-B content increased 0.69% (P=0.0003) and 0.05% (P=0.11) on days 1 and 2 after SURF; content decreased slightly in CTRL. The change in SP-B content on d 1 for treated infants was correlated with the mean levels of SP-B content (r=0.60, P=0.004), possibly indicating enhanced SP-B degradation in deficient infants. There was no difference (SURF vs. CTRL) in PDA ligation (41 vs. 28%, P=0.37), NEC (11 vs. 15%) or IVH 3/4 (4 vs. 5%) after enrollment. 201 doses of surfactant were administered with adverse event frequency ≤ 2% (reanimation, or significant bradycardia or respiratory decompensation).

Conclusions: Late surfactant increased SP-B content. Administration of late surfactant is well-tolerated and appears safe with regard to co-morbidities of prematurity.

126 CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASE 2 AS A POTENTIAL MEDIATOR OF BETAMETHASONE-INDUCED FETAL LUNG MATURATION

Nelson JJ, Li C, Li A, Chan B, Ramanathan R, Minoo P. Keck School of Medicine, University of Southern California, Los Angeles, CA.

Purpose of Study: Antenatal Steroids are in common clinical use for promoting maturation and hence improving perinatal outcome in preterm neonates. The precise mechanism by which steroids enhance lung development & improve respiratory functions remains unknown. Calcium/calmodulin-dependent protein kinase (CaMK2) may be an important mediator of corticosteroid-induced fetal lung maturation. The current study examined the relationship between the maturational impact of betamethasone on fetal lung and activation of CaMK2 in a murine model.

Methods Used: The pattern of CaMK2 activation (phosphorylation) was examined during lung development by western blot analysis. Antenatal betamethasone was administered to pregnant mice on day 14 (E14) of gestation. The course of the regimen was chosen to resemble what is used in humans. Two doses of betamethasone separated by 24 hours were administered. Saline was used as control. Fetuses at E16 were delivered by hysterectomy and the lungs isolated for characterization. The lungs were examined for gross morphology and expression of a number of pulmonary cell differentiation makers including Clara cell-specific K10 protein, beta tubulin, alpha smooth muscle actin, surfactant proteins B and C, and Tia1. Activation of CaMK2 was conducted by detection of Phospho-CaMK2 by western blot analysis.

Summary of Results: CaMK2 activation correlated with the onset of sacular stage during lung development. Betamethasone treatment was associated with enhanced fetal lung maturation, cell differentiation and concomitant activation of CaMK2.

Conclusions: To our knowledge, these are the first data demonstrating a close association between CaMK2 activation and lung maturational stages. The results also show CaMK2 as a target of betamethasone, a well known stimulator of lung maturation in clinical use. We speculate that CaMK2 may be an important mediator of betamethasone-induced lung maturation.

127 THIRDHAND SMOKE ADVERSELY AFFECTS FETAL LUNG GROWTH AND DIFFERENTIATION

Chien K, Sakuri R, Torday J, Rehan V. Los Angeles Biomedical Institute, Torrance, CA.

Purpose of Study: Though nicotine is the main cigarette smoke constituent linked to altered lung structure and function in utero smoke exposure infants, other constituents have not been thoroughly evaluated. This is particularly true for thirdhand smoke (THS), defined as tobacco smoke that sorbs onto indoor surfaces and dust, where semi- and non-volatile chemicals and particulates can undergo modifications to produce new toxics that remain on the surfaces, or later desorb and re-appear in the micro-environment. We tested the hypothesis that THS exposure alters the development and differentiation of the lung by affecting the alveolar PPARγ signaling known to be essential for normal lung development.

Methods Used: Embryonic day 19 fetal rat lung explants were exposed to diluent, nicotine (1 X 10–8M or 1 X 10–5M), or 4–(methyltrinitrosamino)-1-(3-pyridyl)-1-butanone (NNK) (1 X 10–8M or 1 X 10–5M), the main tobacco-specific N-nitrosamine constituent of THS, for 24h. Cell proliferation (BrDU incorporation), cytotoxicity (LDH assay), and expression of key markers of alveolar differentiation (surfactant protein B and C and PPARγ expression, choline incorporation into saturated phosphatidylcholine and triolein uptake) were determined.

Summary of Results: Cell proliferation decreased and cytotoxicity increased significantly with NNK compared to nicotine (p<0.05), suggesting more adverse effects of NNK. With NNK, choline incorporation into saturated phosphatidylcholine decreased significantly (p<0.05), while it increased significantly with nicotine. Triolein uptake increased significantly with both nicotine and NNK (p<0.05), though more robustly with NNK (p<0.05 vs. nicotine). Western analysis showed significantly decreased PPARγ and increased fibronectin levels (p<0.05) with both NNK and nicotine exposure.

Conclusions: NNK affects lung growth and differentiation more robustly than nicotine, possibly contributing significantly to in utero smoke exposure-induced pulmonary damage. These data can be used in designing specific preventive and/or therapeutic strategies against the deleterious effects of in utero THS exposure on lung development [Supported partially by grants from the TRDRP (15FT-0250, 17RT-0170)].

128 MECHANICAL VENTILATION OF PRETERM LAMBS LEADS TO MORE APOPTOSIS AND LESS PROLIFERATION OF NEURONS AND GLIA

Alvord J, Wint A, McCoy MJ, Dong L, Dahl M, Callaway C, McKnight R, Yoder BA, DiGernimo R, Lane RH, Albertine K. University of Utah, Salt Lake City, UT.

Purpose of Study: Prolonged mechanical ventilation (MV) of the preterm human neonate is associated with lung injury. Frequently, the brain also is injured. Our studies, using chronically ventilated preterm lambs, indicate that MV changes histone marks in the brain. These changes could affect regulation of cell processes, such as apoptosis and proliferation of neurons and glia. The present studies purpose was to test this possibility. We hypothesized that prolonged MV increases apoptosis and decreases proliferation among neurons and glia in the brain of preterm lambs.

Methods Used: Preterm (PT) lambs, treated with antenatal steroids and postnatal surfactant, were managed by MV or high-frequency nasal ventilation (HFNV; akin to bubble nasal CPAP (n=4) for each) for either 3d or 21d. We use HFNV as the positive gold-standard for alveolar formation in the lung. At the end of 3d or 21d of ventilation, cortical brain tissue from the temporal lobe was fixed and analyzed by double immunofluorescence to colocalize cleaved caspase 3 or PCNA within neurons (neuronal marker-positive), immature oligodendrocytes (O1-positive), or reactive astrocytes (GFAP-positive).

Summary of Results: Neurons in gray matter had significantly (p<0.05) more colocalization of cleaved caspase 3 in the MV group compared to the HFNV group at 3d (22±3* vs 7±3/mm3) and at 21d (22±3* vs 11±4). Conversely, neurons had significantly less colocalization of PCNA in the MV group compared to the HFNV group at 3d (17±3* vs 23±7) and at 21d (27±7* vs 16±1). Among glial cell types, the only colocalization difference was for immature oligodendrocytes with cleaved caspase 3 at 21d. Significantly more for immature oligodendrocytes were apoptotic in the MV group compared to the HFNV group (29±3* vs 16±1).

Conclusions: We conclude that MV for 3d or 21d is associated with a shift in balance between cell death and proliferation in the brain. The shift is toward more apoptosis and less proliferation of neurons and immature oligodendrocytes. These results are consistent with less brain-derived neurotrophic factor in the same MV preterm lambs compared to We speculate that imbalance results from epigenetic alterations in chromatin structure of genes that regulate apoptosis and proliferation. (HL62875, HL56401, HD11075).
Neonatology – General I

Concurrent Session

8:00 AM
Friday, January 28, 2011

129 TRACT BASED SPATIAL STATISTICS OF NEONATAL HYPOXIC-ISCHEMIC ENCEPHALOPATHY: SELECTIVE INVOLVEMENT OF CORTICAL SPINAL TRACT

Weiner A1,2, Czeschin R1, Wnioskowski J1, Tokar E2, Nagasunder A2, Bluml S3, Ramanathan R1,2, Paquette L1,3, Panigrahy A1,4, USC, Los Angeles, CA; 2CHLA, Los Angeles, CA; 3Children’s Hospital of Pittsburgh of UPMC, Pittsburgh, PA; 4CHLA, Los Angeles, CA and 5UC, Los Angeles, CA.

Purpose of Study: HIE in the newborn is heterogeneous with respect to both neuroimaging patterns and etiologies. In this study, diffusion tensor imaging (DTI) combined with a TBSS-based analysis were used to test the hypothesis that selective white matter (WM) tracts in the newborn brain are vulnerable to HIE despite the pattern of injury seen on conventional MRI or etiology.

Methods Used: 12 neonates with HIE were scanned between 2005–2009 using a standardized neonatal imaging protocol and a neonatal head coil on a 1.5T GE magnet. The inclusion criteria included having a pattern of central and/or peripheral HIE on conventional and diffusion sequences. A group of term clinical controls with normal conventional MRs was used for comparison. The DTI protocol included 25 directions at a b-value=700. Data analysis was performed using Oxford University's FMRIB FSL software. Voxel-wise statistics were performed using Threshold-Free Cluster Enhancement and corrected for multiple comparisons. Diffusivity metrics were calculated using a ROI approach and DTI studio. Clinical variables were recorded.

Summary of Results: The etiologies for HIE in this group were heterogeneous and included perinatal depression, traumatic birth injury, metabolic disease, non-accidental trauma, and hypotension. In the HIE group, the one minute Apgar ranged from 1–3, the five minute Apgar from 0–5 and the pH of the first ABG ranged from 6.81–8.66. TBSS revealed in the HIE cases selective reduction of anisotropy of the posterior limb of the internal capsule (PLIC) when compared to term controls (p<0.05). No selective patterns related to the diffusivity metrics were found.

Conclusions: TBSS reveals selective microstructural WM injury in the PLIC in neonates with different neuroimaging patterns and etiologies of HIE, suggesting a common vulnerability of the cortical spinal tract fibers. This could be related to the high metabolism of these structures at birth as documented by other imaging modalities including PET.

130 ELECTRICAL FIELD STIMULATION OF THE SUPERIOR CERVICAL GANGLION ATTENUATES CEREBRAL BLOOD FLOW IN TERM AND PRETERM LAMBS

Czynski AJ1, Blood AB1, Buchholz FL2 Loma Linda Univ., Loma Linda, CA and 2Loma Linda Univ., Loma Linda, CA.

Purpose of Study: The role of the superior cervical ganglion (SCG) in controlling cerebral autoregulation is controversial. Most studies involve adult human or animal models. There are no reports describing SCG function in autoregulation of preterm infants. We hypothesis that SCG regulates cerebral blood flow (CBF) in premature lambs.

Methods Used: Premature lambs 120–124 d (n=6) and 2-week-old term lambs (n=5) were anesthetized, mechanically ventilated, and instrumented. Arterial PCO2 and Pao2 were maintained in a constant range. CBF was monitored using laser Doppler flow (LDF) technique. After placement of LDF probes, the left or right SCG was randomly selected, and platinum electrodes were placed. Following a stable baseline of CBF, the SCG was electrically stimulated for 30 seconds 3–6 times with a return to baseline between stimulations. Distribution of values for each CBF time point were analyzed using Friedman 2 Way ANOVA. Differences at specific time points compared to baseline were determined by Wilcoxon Signed Rank test.

Summary of Results: With SCG stimulation CBF decreased (p<0.01) on the ipsilateral side, but was unchanged on the contralateral side, and no change in BP. After stimulation stopped CBF increased but remained lower than baseline (p<0.018).

Conclusions: We conclude SCG contributes to regulation of CBF in our model. Our study suggests stimulating the SCG exerted localized vasocostriction of CBF. Better understanding of the vasoconstrictor effect of the SCG in regulation of CBF may lead to effective treatment of premature babies at risk for IVH.

131 COMPARISON OF UMBILICAL VENOUS AND INTRAOSSEOUS ACCESS DURING SIMULATED NEONATAL RESUSCITATION

Rajani A, Chitkara R, Halamek L Stanford University School of Medicine, Palo Alto, CA.

Purpose of Study: Current Neonatal Resuscitation Program guidelines recommend placement of an umbilical venous catheter (UVC) for any neonate with persistent bradycardia in spite of appropriate cardiopulmonary resuscitation or hypovolemic shock. UVC placement is complex, imposes significant space constraints on the resuscitation team, and is only required in 0.12% of all births. Successful intraosseous needle (ION) placement and use in neonates is well described, and animal models suggest that epinephrine administered intraosseously has similar effects to other intravenous routes. This study compares UVC and ION placement in the delivery room setting to understand time to placement, ease of use and errors in placement for each device.

Methods Used: Forty physicians were recruited (8 attending neonatologists, 5 neonatal hospitalists, 6 fellows in neonatology, 5 neonatal nurse practitioners, 16 residents in pediatrics). Participants were shown an instructional video of UVC and ION placement and were allowed to practice placement with both modalities. Using a realistic delivery room and neonatal mannequin in a pediatric simulation center with a confederate nurse and respiratory therapist, participants were asked to attend the two deliveries requiring intubation or hypovolemic shock. UVC placement is complex, imposes significant space constraints on the resuscitation team, and is only required in 0.12% of all births. Successful intraosseous needle (ION) placement and use in neonates is well described, and animal models suggest that epinephrine administered intraosseously has similar effects to other intravenous routes. This study compares UVC and ION placement in the delivery room setting to understand time to placement, ease of use and errors in placement for each device.

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Summary of Results: ION placement was significantly faster than UVC placement by 46 seconds (p<0.0001). Overall, there was no difference in perceived difficulty between IO and UVC placement. Residents in pediatrics (n=16) found ION placement to be significantly easier than UVC (p=0.003). There was no significant difference in the number of errors between UVC and IO placement.

Conclusions: ION placement can be performed more quickly than UVC without any increase in overall perceived difficulty or rates of error. ION insertion should be considered as a first line option for epinephrine administration in the delivery room, especially for practitioners who do not routinely place UVCs in the intensive care setting.
132 COMPARISON OF CARDIAC OUTPUT MEASUREMENTS BETWEEN ELECTRICAL CARDIOMETRY (EC) AND ECHOCARDIOGRAPHY

Kayabayb RG1, Bhombal S2, Ebrahimim M1, Seri I1,2 1LAC+USC Medical Center, Los Angeles, CA and 2Childrens Hospital Los Angeles, Los Angeles, CA.

Purpose of Study: To investigate the correlation of cardiac output measurements obtained by the non-invasive continuous cardiac output monitor (electrical cardiometry, Aescolun®) and echocardiography in term and preterm infants.

Methods Used: This is a prospective observational study. Left ventricular output (LVO) was measured by echocardiography and EC simultaneously. As EC estimates LVO by assessing blood flow in the ascending aorta, measurements were performed on term and preterm infants with a patent ductus arteriosus irrespective of the potential hemodynamic significance of duc-tal shunting. Measurements were obtained from day 1–72 of postnatal life.

Summary of Results: There were 46, paired measurements performed on neonates with a gestational age of 23–41 and birth weight of 375–4330gms. LVO by ECHO and EC was 653±188, and 658±263 mL/min, respectively (P=0.5).

Conclusions: Despite the relative imprecision of L VO measurement by echocardiography, there is a good correlation between the cardiac output measurements obtained by EC and echocardiography in neonates. There is a potential for continuous monitoring of cardiac output with the use of EC in these infants but additional studies simultaneously investigating changes in CO in response to treatment are needed to confirm these findings.

133 TRACT BASED SPATIAL STATISTICS (TBBS) OF ECMO-RELATED NEONATAL BRAIN INJURY: INITIAL EXPERIENCE

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Purpose of Study: Neonates treated with ECMO (extra-corporeal membrane oxygenation) are at risk for long-term neurodevelopmental sequela.

The precise mechanism of brain injury is not yet understood. In this study, we used diffusion tensor imaging (DTI) combined with a TBBS-based analysis to test the hypothesis that ECMO results in selective microstructural injury. The precise mechanism of brain injury is not yet understood. In this study, we used diffusion tensor imaging (DTI) combined with a TBBS-based analysis to test the hypothesis that ECMO results in selective microstructural injury.

Methods Used: 28 neonates who had been on ECMO were scanned between 2005–09 using a standardized neonatal imaging protocol and neonatal head coil on a 1.5T GE magnet. There were 18 veno-venous ECMO patients and 10 veno-arterial. The reason for ECMO included primary PPHN (n=6), cardiac arrest (n=2), sepsis (n=5), congenital diaphragmatic hernia (n=8), meconium aspiration syndrome (n=6) and dilated cardiomyopathy (n=1).

Due to technical limitations associated with scan acquisition and/or post-processing of the data, a total of 11 ECMO cases were used for the analysis. The comparison groups without ECMO included: (a) term and preterm clinical controls with normal conventional MRIs (n=60), (b) congenital heart disease (CHD) (n=45). The DTI protocol included 25 directions at a b-value=700. Data analysis was performed using Oxford University’s FMRIB FSL software. Voxel-wise statistics were performed using Threshold-Free Cluster Enhancement and corrected for multiple comparisons. Clinical variables were recorded.

Summary of Results: TBSS revealed no difference in fractional anisotropy of selected WM tracts in the ECMO cases compared to both preterm and term controls. When comparing ECMO cases to the CHD cases, there was decreased anisotropy in the splenium of the corpus callosum (CC) of CHD patients.

Conclusions: Our preliminary results show no definite anisotropic differences between ECMO neonates and clinical controls. This suggests that ECMO does not increase the risk for brain microstructural injury. In contrast, CHD patients did demonstrate decreased anisotropy in the splenium of the CC compared to ECMO patients, which is likely a neurodevelopmental aberration in CHD neonates.

134 THE EFFECT OF ANTITHROMBIN III ON BLOOD PRODUCT REQUIREMENTS DURING NEONATAL EXTRACORPOREAL MEMBRANE OXYGENATION SUPPORT

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Purpose of Study: Neonates receiving extra corporeal membrane oxygenation (ECMO) support frequently require transfusion of blood product. Due to their relative immaturity and their underlying medical and surgical conditions, neonates treated with ECMO are often deficient in Anti-thrombin III (ATIII). This in turn further enhances their propensity for blood product exposures during ECMO support. The purpose of this study is to determine whether ATIII administration alters the need for blood product administration during ECMO support.

Methods Used: Neonates requiring ECMO at the NICCU of Childrens Hospital Los Angeles were divided in a pre-ATIII (years 2006–2008) and post-ATIII (years 2009–2010) administration periods. One vial of ATIII is administered to patients for ATIII level typically less than 60%. Patient demographics, length of ECMO support and blood product [fresh frozen plasma (FFP), packed red blood cells (PRBC), cryoprecipitate and platelets] requirements were compared between the two time periods.

Summary of Results: In this ongoing study, so far five patients in the pre-ATIII periods (Mean birth weight = 3290 ± 225 g, gestational age = 38 ± 7 weeks, length of ECMO support = 205 ± 79 hours) and five patients in the post-ATIII periods (Mean birth weight = 3295 ± 307 g, gestational age = 38 ± 7 weeks, length of EMCO support = 234 ± 124 hours) were identified. During ECMO, patients in the post-ATIII period were exposed to significantly less FFP as compared to the pre-ATIII period (mean FFP volume = 48 ± 11 vs. 126 ± 44 mL, p = 0.04). No differences in the volume exposure of PRBC, cryoprecipitate or platelets were noted between the two groups.

Conclusions: Our preliminary data suggest that supplementation of ATIII during neonatal ECMO support may result in a reduction of FFP exposure.
as the therapist stood at bedside. Masking to treatment was maintained with a privacy screen. Weekly heart rate variability (HRV) data, from which high-frequency power (parasympathetic) and low-frequency power (sympathetic) were extracted, was acquired prior to the morning treatment session. The low-to-high frequency ratio (LF:HF) assessed ANS function with a lower ratio indicative of improved parasympathetic activity.

**Summary of Results:** 36 infants (DMT n=16/7M & Control n=20/10M) were studied. Infant demographic and anthropometric characteristics were similar between groups. DMT infants demonstrated significantly greater parasympathetic activity over time (F = 5.47, p = 0.03) (Figure 1).

**Conclusions:** Parasympathetic response is essential for recovery following stress-induced sympathetic activity. We demonstrate twice-daily DMT promotes greater parasympathetic activity in preterm infants. DMT may be a useful therapy for attenuation of stress in hospitalized, preterm infants.

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**137 EFFECT OF FLUORESCENT LIGHT EXPOSURE ON METALLOPORPHYRIN-TREATED NEWBORN MICE**

Schulz-Geske S, Kalish FS, Zhao H, Katayama Y, Champion KA, Vreman HJ, Vong WJ, Stevenson DK, Stanford University School of Medicine, Stanford, CA.

**Purpose of Study:** Neonatal hyperbilirubinemia is due to an increased bilirubin (BR) production and a decreased capacity to eliminate BR. Blue light (400–520 nm) is commonly used to reduce BR levels. However, a more strategic approach might be to prevent BR production. Metalloporphyrins (Mps) effectively decrease BR formation by competitive inhibition of heme oxidase (HO), the rate-limiting enzyme in heme degradation. Chromium mesoporphyrin (CrMP) and zinc deuteroporphyrin bis glycol (ZnBG) are promising Mps for use since they are absorbed orally, highly potent, and affect minimally other hemeoproteins. Because some Mps are photosensitizers, the phototoxicity of CrMP and ZnBG were studied in a 3-d-old mouse model.

**Methods Used:** 3-d-old FVB mice were given vehicle (CON), CrMP, or ZnBG (3.75–30.0 μmol/kg body weight (BW) IP). For light treatment, pups were placed in an open box lined with wet paper towels, set under 2 cool white and 1 blue fluorescent (TL52) tubes at an irradiance of 35.0±1.0 μW/cm²/nm for 3h (CON; CrMP: ZnBG-L), and wetted every 30min with water. Age-matched treated pups were returned to mothers and kept under ambient light (CON, CrMP, ZnBG). Kaplan-Meier plots were used to determine survival (n=10/group). Liver tissue HO activity, antioxidant capacity, and plasma markers of liver and cardiac muscle injury (AST, CK-MB) were measured in each group.

**Summary of Results:** ZnBG-L and CrMP-L treatment resulted in a dose-dependent mortality with an LD₅₀ of 20.0 and 21.5 μmol/kg BW, respectively. However, in contrast to ZnBG, there was no significance difference in mortality between CrMP-L and CrMP groups. ZnBG-L pups had significant weight loss (1.7±1.0%; decreased liver antioxidant capacity (4.7-fold, p<0.05), and increased AST (1.4-fold, p<0.05) and CK-MB (10.0-fold, p<0.05) levels compared to CON. Moreover, the inhibitory potency of ZnBG for HO decreased significantly by ~15% after light exposure. In summary, CrMP is not phototoxic, but shows chemical toxicity with an LD₅₀ of 22.5 μmol/kg BW; whereas, ZnBG is potentially phototoxic.

**Conclusions:** We conclude that at low doses (<3.75 μmol/kg BW), ZnBG has a high inhibitory potency, but low phototoxicity similar to tin mesoporphyrin (SnMP), and thus may still have potential use in the treatment of neonatal hyperbilirubinemia.

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**138 ROLE OFHEME OXYGENASE IN A MURINE MODEL OF EARLY NECROTIZING ENTEROCOLITIS**

Schulz-Geske S, Kalish FS, Jang KY, Zhao H, Huey M, Vreman HJ, Sylvester KS, Wong RJ, Stevenson DK, Stanford University School of Medicine, Stanford, CA and Stanford University School of Medicine, Stanford, CA.

**Purpose of Study:** Necrotizing enterocolitis (NEC) is typified by mucosal and intestinal necrosis. Three “stress” factors are usually present: a preceding ischemic event, bacterial colonization, and enteral feeding, causing increased intestinal permeability and injury followed by exaggerated inflammatory responses. Heme oxygenase (HO)-1 degrades heme to bilirubin (BR), free iron, and carbon monoxide (CO). Since BR and CO have antioxidant, anti-apoptotic, and anti-inflammatory properties, we propose that HO-1 may protect against the development of NEC.

**Methods Used:** 10d-old HO-1 heterozygote (Het, HO-1<sup>j</sup>) and WT mice were used. For NEC induction, all pups were removed from the mom, orally gavage with 200μL formula (2g Similac 60/40 in 10mL Esbilac)/5g BW q 4h x 2d and exposed to hypoxia (5%O<sub>2</sub> x 2min before feeding, 2%/d, n=5).

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At d3, pups were returned to moms. Control pups (WT; Het-CON) remained with moms and breastfed. At d4, all pups were sacrificed and a 1.0cm of distal ileum (IL) and proximal colon (C) were harvested for morphology.

To compare NEC severity, tissues were sectioned for H&E staining. Histological changes were blindly scored on a scale of 0 (normal) to 4 (severe inflammation/necrosis). Apoptosis in IL and C of CON and NEC-induced mice were assessed by TUNEL staining.

Summary of Results: In this pilot study, histological scoring showed morphological changes of IL and C for Het-CON vs WT-CON. NEC development was more advanced and severe in Het-NEC vs WT-NEC pups (Table). Interestingly, tissue damage was higher in the C than the IL for both NEC-induced genotypes. TUNEL staining showed an increase in apoptosis in Het-CON IL and C vs those of WT-CON. Moreover, apoptotic cells were strongly reduced in Het-NEC C vs WT-CON, Het-CON, and WT-NEC, which may be a sign of reduced proliferation and the presence of necrotic tissue.

Conclusions: A partial deficiency in HO-1 leads to an earlier, more progressive and severe development of experimental NEC. Also, it appears that the early development of NEC may originate in the proximal colon in this murine model.

140 RISK FACTORS FOR EPILEPSY IN TERM INFANTS WITH HYPOXIC ISCHEMIC ENCEPHALOPATHY
Hong KJ1, Bonifacio SL2, Barkovich AJ3, Sullivan JE2,3, Rogers EE1,2, Ferriero DM4,5, Glass HC2,3, *University of Washington, Seattle, WA; 2University of California San Francisco, San Francisco, CA; 3University of California San Francisco, San Francisco, CA; 4University of California San Francisco, San Francisco, CA.

Purpose of Study: Hypoxic ischemic encephalopathy (HIE) is a significant cause of neonatal death and adverse neurodevelopmental outcome, such as cerebral palsy, developmental delay, and epilepsy. The goal of this study was to determine neonatal predictors of epilepsy in term newborns with HIE using clinical data and MRI.

Methods Used: This was a cohort study of 195 term infants with birth asphyxia, born 1993 through 2009. Neonatal clinical data, such as neonatal seizures, encephalopathy scores, and MRI pattern of injury, were obtained through chart review. Parents of the infants were contacted by telephone and administered a seizure questionnaire. Neurodevelopmental data including cognitive scores, neuromotor scores (NMS), and neurological outcomes were extracted from standardized longitudinal follow up data.

Summary of Results: Of 121 children (10%) developed epilepsy. Univariate analyses showed significant differences between epilepsy and no epilepsy groups for neonatal seizures, EEG seizures, status epilepticus, encephalopathy scores of 5 or 6, and brain injury on MRI (P < 0.01). Of 49 infants with neonatal seizures, 12 (24%) developed epilepsy. The infants with EEG seizures (RR = 9; 95% CI = 3–27) and status epilepticus (RR = 11; 95% CI = 5–25) were more likely to develop epilepsy than those without. There was no significant association for clinical seizures (P = 0.5) or encephalopathy scores of 3 or 4 (P = 0.1) and epilepsy. The children with epilepsy had injury on MRI: 5 had basal ganglia patterns and 7 had watershed patterns. Children with epilepsy had an average cognitive score of 54 ± 11, whereas children with no epilepsy had a score of 97 ± 17 (P < 0.005). Median (range) NMS were 5 (0–5) for children with epilepsy and 0 (0–5) for children with no epilepsy (P < 0.005).

Conclusions: Term infants with HIE and neonatal seizures, specifically electrographic seizures and status epilepticus, evidence of brain injury on MRI, and more severe encephalopathy scores are at a higher risk of developing epilepsy. Our data provide information for clinicians to use when counseling parents on the possible outcome of epilepsy.
This study demonstrated induced axonal degeneration in re-

erial terminal sites exposed to Aβ. Therefore, the axonal terminal sites are located in the optic radiation in the

tical brain areas while the neuronal bodies of the RGC sit in the eye. Aβ peptides were injected into the right hemisphere optic radiation region of six 12 week old female C57BL/6 mice. DTI was conducted on each mouse at 1 and 3 months after Aβ injection.

Summary of Results: Among the measured white matter tracts, only optic tracts and optic nerves showed Aβ effects. The ipsilateral optic tract showed a significant 15% decrease in axial diffusivity, suggesting axonal damage caused by Aβ injection. Damage in the optic tract propagated further to the optic nerves, which showed significant 10% decreases of mean diffusivity. Histological examination will be performed to confirm the imaging findings.

Conclusions: This study demonstrated induced axonal degeneration in response to axonal terminal site exposure to Aβ peptides.

Percent Change in Oxygen Saturation

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<th>Number of Subjects</th>
<th>%ΔSaO2</th>
<th>% ΔACSO2</th>
<th>% ΔRSAO2</th>
<th>% ARSO2</th>
<th>% ΔACSO2 + % ΔRSAO2</th>
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Data is presented as mean(std).

Neuroscience I
Concurrent Session
9:00 AM
Friday, January 28, 2011

142 AMYLOID BETA INJECTION IN AXONAL TERMINAL SITES RESULTS IN MOUSE OPTIC TRACT DEGENERATION
Carrick D1, Sun S2,1 1Loma Linda University, Loma Linda, CA; 2Loma Linda University, Loma Linda, CA and 3Loma Linda University, Loma Linda, CA.

Purpose of Study: Amyloid plaques are a pathological hallmark of Alzheimer’s disease (AD) and the principal component of these plaques is amyloid beta (Aβ). Although Aβ has been implicated in AD pathogenesis, the mechanism of Aβ-induced neurodegeneration is not clear. In this study, we applied novel magnetic resonance diffusion tensor imaging (DTI) to identify white matter damage as a result of focal exposure of axonal terminals to Aβ.

Methods Used: In the mouse visual system, the optic nerve and the optic tract are extended axons originating from the retinal ganglion cells (RGC). Therefore, the axonal terminal sites are located in the optic radiation in the subcortical brain areas while the neuronal bodies of the RGC sit in the eye. Aβ peptides were injected into the right hemisphere optic radiation region of six 12 week old female C57BL/6 mice. DTI was conducted on each mouse at 1 and 3 months after Aβ injection.

Summary of Results: Among the measured white matter tracts, only optic tracts and optic nerves showed Aβ effects. The ipsilateral optic tract showed a significant 15% decrease in axial diffusivity, suggesting axonal damage caused by Aβ injection. Damage in the optic tract propagated further to the optic nerves, which showed significant 10% decreases of mean diffusivity. Histological examination will be performed to confirm the imaging findings.

Conclusions: This study demonstrated induced axonal degeneration in response to axonal terminal site exposure to Aβ peptides.

143 TEMPORAL ALTERATIONS IN LESION VOLUME IN A RAT MODEL OF REPETITIVE MILD TRAUMATIC BRAIN INJURY
Muellner M1,2, Coats J3, Mohd-Yusof A3, Negliaro K5, Obenaus A5,6, Huang L6,1 1Loma Linda University, Loma Linda, CA; 2Loma Linda University, Loma Linda University, CA; 3University of California, Riverside, Riverside, CA; 4California State University, San Bernardino, San Bernardino, CA; 5Loma Linda University, Loma Linda, CA and 6Loma Linda University, Loma Linda, CA.

Purpose of Study: Repetitive mild traumatic brain injury (rmTBI) is an important medical concern for active military personnel with 10–20% reporting neurological or psychological symptoms. In the present study, we characterized the neuropathological profiles of repetitive mTBI using a rat model where the first mild impact was followed by a second mTBI at intervals of 1, 3, or 7 days by non-invasive magnetic resonance imaging (MRI) correlated with histology.

Methods Used: Thirty-four Sprague Dawley adult male rats (2 mo old) were randomized into sham, single mTBI, and 3 groups with two episodes of mTBI: 1) 1 day apart; 2) 3 days apart; 3) 7 days apart. A craniotomy was performed followed by a mild controlled cortical impact (CCI) delivered by an electromagnetic driven piston (0.5 mm depth, 4 mm diameter tip at 6.0 m/s, 200 ms duration). A second identical impact was delivered at 1, 3 or 7 days after the first CCI event at the same location. Sham animals underwent the same surgical procedure without CCI. T2 weighted imaging (T2WI) was acquired on a 4.7T MRI (Bruker Biospin) at 24 hrs after each impact and a final MRI was acquired at day 14 after the initial injury. Results were correlated with ex vivo histology.

Summary of Results: In the mTBI groups of 1 and 3 days but not 7 days apart, a second CCI resulted in increased T2WI lesion volumes that persisted until 14 days (p<0.05 vs sham).

Conclusions: The brain appears to exhibit heightened vulnerability to a second mild traumatic insult up to 3 days after an initial mTBI event. Rat models of rmTBI may serve as a clinically relevant platform for evaluation of outcome parameters for testing experimental therapeutics. MRI is a sensitive neuroimaging biomarker for monitoring the pathological evolution after mTBI.

144 ELEVATED LEVELS OF CASPASE 14 IN PATIENTS WITH ALZHEIMER’S DISEASE
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Purpose of Study: Caspase 14 is a recently discovered aspartate protease that was found to be elevated in a patient who progressed from control to mild cognitive impairment and who subsequently progressed to and died of Alzheimer’s disease (AD). The purpose of this current study is to elucidate the significance of caspase 14 in the etiology of Alzheimer’s disease.

Methods Used: This study utilized flash-frozen brain samples from patients grouped (n=4 in each group) into control, AD-only, and AD with cerebral amyloid angiopathy (CAA) via post-mortem pathological diagnosis. Standard immunohistochemistry was used to compare the levels of caspase 14 in the brain parenchyma of each group and in brain parenchyma versus isolated brain vasculature. Standard immunohistochemistry was also performed on brain sections from each group in order to localize changes in caspase 14. Human polyclonal caspase 14 antibody was used for both techniques.

Summary of Results: LC-MS/MS demonstrated a 25-fold increase of caspase 14 from serum in a patient progressing from control to MCI. Immunohistochemistry of brain homogenate demonstrated a statistically significant increase (p=0.004) in caspase 14 in AD-only parenchyma compared to control and AD+CAA parenchyma. Immunohistochemistry of brain microvessels showed a strong non-significant trend towards increased caspase 14 expression in both AD-only and AD+CAA compared to control. Immunohistochemistry of AD-only brain sections were negative for intraneuronal staining of caspase 14 while control and AD+CAA brain sections stained positive. The vasculature of AD-only and AD+CAA brain sections was also prominently stained.

Conclusions: Our results suggest that AD+CAA cases follow a different progression of disease than AD-only cases. Future studies will aim to clarify the role of caspase 14 in Alzheimer’s disease.
145 SURGERY DURATION VERSUS BRAIN IN JURY IN THE NEONATAL RAT MODEL OF HYPOXIA-ISCHEMIA
Burris M, Chen H, Fajilan A, Zhang JH Loma Linda University School of Medicine, Loma Linda, CA.
Purpose of Study: In humans, hypoxia-ischemia causes brain injury which may lead to neurological deficits. For study of neonatal hypoxia-ischemia, preclinical models have been developed to allow testing of neuroprotective agents. The purpose of this study is to evaluate the relationship between the surgical duration and the severity of brain injury in the neonatal rat.
Methods Used: Unilateral carotid ligation was performed in postnatal day 7 and 10 rat pups, followed by 2.5 hours of enclosed-chamber hypoxia (8% oxygen). Time-to-ligation of each study group was set at 5, 7, 13 and 21 minutes.
Summary of Results: There was a statistically significant difference between the groups of surgical durations of 5 and 7 minutes compared with those of 13 and 21 minutes.
Conclusions: The operative time is a major determinant in the resulting cerebral infarction following unilateral carotid ligation and incubation in an hypoxic chamber in P7 and P10 rat pups.

146 INVESTIGATING THE CURRENT BIOLOGICAL MODEL OF ADDICTION
Lawhorn JK University of Washington School of Medicine, Seattle, WA.

147 INHIBITION OF ASTROCYTE GLT-1 IN THE VENTROMEDIAL HYPOTHALAMUS ENHANCES THE EPINEPHRINE RESPONSE IN HYPOGLYCEMIA COUNTERREGULATORY FAILURE
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Purpose of Study: Hypoglycemia is a major limiting factor in the achievement of tight glycemic control with intensive insulin therapy. With repeated exposure to hypoglycemia, CNS-mediated activation of hormonal counterregulatory responses (CRRs) that normally correct low glucose become impaired (Hypoglycemia-Associated Autonomic failure, HAAF). Glutamate signaling within the VMH is essential to the activation of hypoglycemia-induced CRRs. Thus, changes in how glutamate is regulated should be a mechanism to consider in the development of HAAF. We hypothesize that hypoglycemia-induced up-regulation in astrocyte GLT-1, a glutamate re-uptake transporter responsible for clearing extracellular glutamate and terminating signaling, may be a contributing factor in the development of CRR failure. Elevated GLT-1 expression would be expected to reduce the availability of glutamate, thereby impairing hypothalamic glutamate mechanisms mediating the activation of CRRs.
Methods Used: To test this hypothesis, we injected a rodent model with ditydrokainic acid (DHK; 1μM in 0.5 μl/side), a selective inhibitor of astrocyte GLT-1, bilaterally into the VMH and measured glucose and hormonal CRRs to single (SH) and recurrent (RH) hypoglycemia.
Summary of Results: In response to SH, epinephrine (EPI) levels were significantly elevated in response to DHK treatment (2,031±239) as compared to ACSF controls (1,134±152) (t=6.00; p<0.05). The recurrent hypoglycemia (RH) model, as expected, dramatically reduced EPI levels as compared to SH controls (630±72 vs. 1,134±152, l60; p<0.024). Injection of DHK into the VMH prior to the final bout of RH effectively restored the impaired EPI response to levels observed in SH controls (1,280±194 vs. 1,134±152, l60; p=0.027). Selective inhibition of GLT-1, in the VMH glucose-sensing site enhanced a key CRR to hypoglycemia, adrenal medullary epinephrine release, and prevented the typical downregulation in this CRR that occurs with repeated hypoglycemia.
Conclusions: These findings suggest that hypoglycemia-induced up-regulation in GSK3 expression and/or function may impair glutamatergic signaling required for the full activation of hormonal CRs and reveal a potentially important and novel astrocyte-mediated mechanism in the development of HAAF.

148 MEASURING DEPRESSION SEVERITY IN PATIENTS WITH EPILEPSY
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Purpose of Study: The purpose of the study was to identify the different factors which contribute to depression in patients with epilepsy and to compare relevant findings with patients who only have epilepsy.

Methods Used: Questionnaire packets were given to patients at local neurology clinics. These packets contained questions relating to the patient’s Quality of Life (QOL). Patients were asked to rate their QOL and social support scores. A statistical software package tabulated the data and determined different QOL scores from the patient surveys. The scaled responses of the patients provided quantitative data which was plotted and analyzed.

Summary of Results: Patients with both epilepsy and depression scored higher in perceived criticism, stigmatization, and lower self-efficacy. Compared to patients with epilepsy only, these patients were criticized more by family and friends, felt a greater sense of stigma in the community, and experienced much lower self-esteem. In another aspect of QOL, patients with epilepsy and depression also had lower social support scores, less appraisal, sense of belonging, stigma and tangible levels. They received less treatment for their condition, lacked familial support and social interaction, felt stigmatized in society, and had little access to transportation and financial aid. However, families of patients from both groups showed approximately the same level of emotional support.

Conclusions: Our data showed that patients with both epilepsy and depression experience a much lower QOL compared to patients with only epilepsy. This shows that depression serves as a comorbidity for patients with epilepsy. Our methods for acquiring patient data helped illustrate this point by using variables and scores to determine the QOL in these two different patient groups.

149 STUDY OF THE NEUROPHYSIOLOGY OF COGNITIVE DYSFUNCTION IN PARKINSON’S DISEASE
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Purpose of Study: Cognitive dysfunction affects 20-40% of persons with Parkinson’s disease (PD) at the time of diagnosis and is a significant risk factor for psychosis, dementia, nursing home placement and death. Among individuals with PD surviving 20 years or longer, cognitive dysfunction is the leading cause of nursing home placement and 75% eventually develop dementia. These statistics have not significantly changed since the 1920’s despite major advances in the treatment of motor symptoms. This study builds on recent advances in cognitive neuroscience and an innovative neuropsychiologic research technique to address this glaring gap in our knowledge and treatment of PD.

Methods Used: We will use magnetoencephalography (MEG) to investigate cognitive dysfunction in subjects with PD performing a demanding cognitive task (modfied cued Stroop paradigm). For each trial, subjects will receive an instructional cue, either “Color” or “Word”, indicating whether they are to name the color or read the word of the upcoming stimuli. The stimuli are color words written in colored font. The stimuli may be congruent (e.g. “red” written in red letters) or incongruent (e.g. “red” written in blue letters). Subjects will perform this task continuously for 30 minutes while in their on-medication state. We plan to collect data on 10-20 PD subjects and 10-20 age-matched controls.

Summary of Results: Preliminary evidence shows that the Stroop paradigm is an effective method of determining cognitive dysfunction mediated by executive control, indicating that this study is capable of demonstrating cognitive differences between PD and non-PD populations.

Conclusions: We hypothesize that top-down networks, particularly those related to medial frontal structures affected by PD, are critical in mediating cognitive dysfunction. We predict that cognitive dysfunction will correlate with medial frontal brain activity.

150 REPRODUCIBILITY OF GAMA-BAND RESPONSE RECORDED BY MAGNETOENCEPHALOGRAPHY AND ELECTROENCEPHALOGRAPHY
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Purpose of Study: Patients with schizophrenia often exhibit unusual sensory experiences, ranging from visual or auditory distortions to dynamic hallucinations. Oscillatory neuronal electrical activity in the range of 30-50 Hz (the gamma-band oscillation) has been proposed to involve feature binding or inter-regional communication within the brain and is critically dependent on inhibitory neurotransmission within the cerebral cortex. Gamma-band oscillatory power has been reported to be impaired in persons with schizophrenia, suggesting a heritable component. Magnetoencephalography (MEG) and electroencephalography (EEG) technologies have repeatedly shown this effect, making the measure an exciting potential tool that could be useful to other researchers. However, no one has yet performed test-retest measurements in the same group of subjects. This study is a necessity before such data can be used in large-scale projects such as genetic linkage analyses or as biomarkers in clinical trials.

Methods Used: Twenty healthy and normal hearing adults screened for personal and family history of mental illness and neurological disorders will be recruited. The subjects will be recorded in an auditory steady state response (ASSR) paradigm while having a 64-channel EEG recording followed by a 248-channel MEG recording. One week later, they will return and repeat the same procedures. We will be comparing two stimuli in the MEG and EEG runs - amplitude modulated white noise and click trains, binaurally presented at 75 dB SPL. Both produce strong 40 Hz responses. We will be looking at phase-locked response amplitude and phase-locking factors, in both sensor and source space. The reliability of the source localization will also be a secondary interest variable. Analysis of ASSR-derived measures of gamma-band power and inter-trial phase-locking for rest-retest reliability will ensue.

Summary of Results: With five of twenty subjects completed, no results have yet been analyzed.

Conclusions: We expect to see a high level of test-retest reliability with the MEG and expect it to be significantly greater than that seen in the EEG recording.

151 THE EFFECT OF PRENATAL ALCOHOL EXPOSURE ON SUBSEQUENT ETHANOL CONSUMPTION AFTER THE ADOLESCENT EXPERIENCE OF A TRAUMATIC EVENT IN MALE C57BL/6J MICE: A PILOT STUDY
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Purpose of Study: Fetal Alcohol Spectrum Disorder (FASD) affects 0.2-1% of the population (Sampson et al. 1997; US Department of Health and Human Services 2005). People with this disorder may demonstrate differential behavioral responses to stressful situations (Kodituwakku 2007, Hellmann 2010). As part of a series of studies on the interaction of stress and alcohol abuse, we aimed to test the hypothesis that prenatal exposure to ethanol predisposes individuals to the development of alcohol misuse following trauma exposure.

Methods Used: Female C57BL/6j mice were allowed to binge drink a 10% ethanol solution for the duration of pregnancy. Six male offspring from prenatal alcohol exposure (PAE) litters and six male offspring from saccharin control litters were provided access to 20% ethanol solutions according to the Drinking In the Dark model described by Rhodes et al (2005) beginning at age 45–50 days. Binge drinking before and after two cycles of withdrawal were recorded. The third withdrawal cycle was coupled with exposure of the animals to a traumatic event via predator odor exposure after which binge drinking was again assessed.

Summary of Results: Both PAE and control animals demonstrated a strong alcohol deprivation effect following the first withdrawal but not following the second withdrawal. Alcohol consumption increased in both groups following the traumatic exposure with the PAE animals trending toward a greater increase in drinking after trauma than the controls; however, this difference did not reach statistical significance (p = 0.1635). Trauma-exposed mice maintained drinking levels, which is not seen after withdrawal cycles prior to trauma.
There was a significant difference between the maintenance of drinking levels following the first, second and third withdrawal (with trauma) (p<0.0001) but, after the trauma and control groups did not significantly differ from each other.

**Conclusions:** Exposure to a traumatic event during adolescence increases binge drinking in both control and PAE C57BL/6J male mice. PAE mice show a heightened sensitivity to trauma, as evidenced by a greater increase in drinking which may reach statistical significance with a higher powered study.

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**MGMT BIOCHEMICAL ACTIVITY IS ASSOCIATED WITH MYELOXIDATION FOLLOWING THERAPY WITH TEMOZOLOMIDE**

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**Purpose of Study:** Inclusion of the methylating agent temozolomide (TMZ) during radiation therapy, then continuing TMZ as a single agent after radiation can significantly prolong patient life. However, treatment with TMZ may result in clinically significant leukopenia, neutropenia, anemia or thrombocytopenia in a minority of patients. Suppression of bone marrow function threatens the efficacy of TMZ therapy by necessitating dose reduction or discontinuance. Such clinical limitations emphasize the need to develop methods to identify patients susceptible to TMZ-induced myelotoxicity. The purpose of this study is to examine the association between clinically relevant myeloxidation and expression of O6-methylguanine-DNA methyltransferase (MGMT), the DNA repair activity that removes cytotoxic O6-methylguanine (O6-mG) DNA adducts induced by TMZ, in human peripheral blood leukocytes (PBLs).

**Methods Used:** To test our hypothesis, we assayed MGMT biochemical activity and determined MGMT promoter CpG methylation status, a surrogate measure of gene expression, in three patient populations (n=28): 10 glioma patients treated with TMZ showing no myelotoxicity; 8 glioma patients treated with TMZ showing myelotoxicity (defined as clinically relevant Grade 3 or higher myelotoxicity that resulted TMZ drug reduction, delay in therapy or discontinuance) and 10 disease-free, untreated controls. MGMT promoter CpG methylation status determined by methylation-specific PCR and MGMT activity was measured by standard biochemical assay that assays transfer of O6-[(3H)methyl]G from DNA to protein.

**Summary of Results:** Mean MGMT activity was 2-fold lower in patients with myelotoxicity compared to treated patients with normal counts (8.3 ± 3.9 vs 16.3 ± 7.8 fmol/106 cells; P = 0.014) and to disease-free, untreated controls (8.3 ± 3.9 vs 15.7 ± 6.8 fmol/106 cells; P = 0.011). All samples displayed unmethylated promoters.

**Conclusions:** These data suggest that [1] myeloxidation in TMZ-treated patients reflects a reduced capacity to remove TMZ-induced O6-mG; [2] promoter CpG methylation status is not indicative of MGMT expression in PBLs and [3] low MGMT activity be a marker for increased risk for TMZ-induced myeloxidation.

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**DIFFUSE OPTICAL SPECTROSCOPY MONITORING OF CYTOCHROME C OXIDASE REDOX STATE DURING PHYSIOLOGICAL CHALLENGES IN ANIMAL MODELS**

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**Purpose of Study:** Cyanide (CN) poisoning induces lethal histotoxic anoxia and stops aerobic cell metabolism by binding to active sites on and disabling the function of cytochrome c oxidase (CoO). When bound, the electron transport chain is arrested with the near infrared optically active copper core in reduced form. We developed a broadband diffuse optical spectroscopy (DOS) prototype system to measure bulk tissue chromophore concentrations and demonstrated that DOS can quantitatively assess tissue in vivo onset of CN toxicity and hemorrhage. In this study, we investigated feasibility of DOS detection of CoO redox states by subjecting animals to respiratory challenges during CN poisoning in a sublethal rabbit cyanide model and swine hemorrhage model where the relationship between hemoglobin and CoO should be divergent, thus enabling assessment of potential optical CoO signal cross-talk effects.

**Methods Used:** New Zealand White rabbits were used in this study. 10mg of sodium cyanide in 60cc normal saline was infused through the femoral vein at a rate of 1cc/min. Respiratory challenges, changes in inspired O2 levels from 100% to 21% and back to 100%, were applied before, during and after CN infusion, and changes in oxy- and deoxyhemoglobin concentrations and CoO redox states were monitored continuously with the DOS probe placed on the right inner thigh muscle. Four hemorrhage studies were conducted on swine (−25kg). Blood volume (30cc/kg) was removed over 20 minutes through the right femoral artery. DOS measurements were taken with the probe placed on the left inner thigh muscle.

**Summary of Results:** During the hemorrhage, CoO decreased concurrently with the decrease in oxyhemoglobin indicating that CoO redox state became reduced due to hypoxia. During CN infusion, CoO redox state also became reduced while oxyhemoglobin concentration increased due to histotoxic anoxia and resulting blockage in the electron transport chain.

**Conclusions:** Spectral similarities between hemoglobin CoO redox states make non-invasive spectroscopic distinction difficult. By a combination of physiological perturbations, DOS demonstrates detection of CoO redox state changes that are decoupled from hemoglobin concentration changes.

### 154

**ONTOGENY AND HIGH ALTITUDE INFLUENCE CA2+ SPARKS IN SHEEP PULMONARY ARTERIAL MYOCYTES**

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**Purpose of Study:** Ryanodine receptors (RyR) and associated Ca2+ spark events are important to pulmonary arterial (PA) tone with potential differences in their role in fetuses as compared to adults. RyRs are implicated in hypoxic pulmonary vasoconstriction (HPV) and ET-1 dependent contractility. Both HPV and ET-1 signaling are implicated in the development of pulmonary hypertension. Evidence also indicates HPV and PA tone are dysregulated by chronic hypoxia (CH) in fetuses and adults, and yet their combined influence on Ca2+ sparks and the relationship to PA tone is not known. We therefore tested the hypotheses that Ca2+ sparks are restricted before birth and that CH augments their function.

**Methods Used:** These hypotheses were tested on PA from near term fetal sheep or adult ewes at low altitude or following 100+ days at 3801 m (CH). Ca2+ sparks were measured in myocytes of Iflu-4 loaded PA strips.

**Summary of Results:** Ca2+ spark activity was far greater in adults relative to fetuses while CH inhibited spark activity in fetuses. Ca2+ sparks in pulmonary myocytes from adults had greater amplitudes as compared to fetuses and the sparks were wider and longer. Chronic hypoxia in comparison reduced spark amplitude but increased spark width and duration and slowed the time to peak amplitude. Selective RyR1 inhibition with 10 μM dantrolene reduced spark activity in normoxic fetuses and adults but not CH adults, while 10 μM ryanodine restricted spark activity in all three of these groups. Ca2+ spark activity was unaffected by dantrolene or ryanodine in myocytes from CH fetuses. Overall, RyR function appears developmentally regulated and altered by CH.

**Conclusions:** The influence of chronic hypoxia and maturation on the role of RyR activity during HPV remains unresolved, but this data suggests that RyR function may be blunted in fetus and absent in hypoxic fetus. The differential effect of CH on RyR activity in fetus and adult provides evidence that RyRs could be therapeutically relevant in the treatment of pulmonary hypertension.
cardiovascular events. Many factors affect flow-mediated vascular reactivity including plasma nitric oxide (NO) levels. Ischemic conditioning of the extremity (ICE) has cardio-protective effects and one of the mechanisms may be via NO. NO is also important in the physiologic responses to high altitude (HA) exposure but the effect of HA on FMD is unknown. The purpose of this study was to evaluate the effects of ICE and HA on FMD.

Methods Used: FMD was measured in 13 healthy runners at baseline, after daily ICE or sham-ICE at sea level (SL) and 60 minutes after completing a 12.8km competitive run to the summit of White Mountain, CA at 4342m. Each subject completed the course twice (with ICE and with sham-ICE) separated by 6 weeks in a randomized cross-over design. ICE was administered using blood pressure (BP) cuffs around the thigh inflated to 200mmHg for 5 minutes, deflated for 5 minutes and repeated for 4 cycles. The cuffs were inflated to 40mmHg for sham-ICE. This was done daily for 5 days at SL before ascent. Duplex ultrasonography with a linear array transducer was used to measure brachial artery diameter before and after 5 min of brachial artery occlusion. The effects of HA and ICE on FMD were evaluated using repeated-measures ANOVA.

Summary of Results: Complete data were obtained in 12 subjects. At SL, FMD was 15.5% ± 7.0% (mean ± SD) without ICE and 9.8% ± 6.1% with ICE. At HA, FMD was 11.5% ± 9.5% without ICE and 6.0% ± 5.1% with ICE. Compared to SL without ICE, FMD was attenuated by 36.5% with ICE at SL, 60.7% with ICE at HA and 25.5% without ICE at HA. Both ICE and HA blunted the FMD response (p<0.05 for ICE and P=0.01 for HA by ANOVA, ICE*HA interaction p=NS). Compared to SL without ICE, pre-inflation diameters were larger by 10.1% with ICE at HA and by 3.6% without ICE at HA.

Conclusions: FMD is significantly attenuated by both ischemic conditioning of extremity and high altitude. This finding is predominately due to larger pre-dilation artery diameters likely induced by vasodilators released by ICE and altitude exposure.

Conclusions: In the present study, FMD was attenuated by both ischemic conditioning of extremity and high altitude. This finding is predominantly due to larger pre-dilation artery diameters likely induced by vasodilators released by ICE and altitude exposure.

156 COMPARISON OF SVV TO SCVO2 IN PATIENTS UNDERGOING MAJOR SURGICAL PROCEDURES

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Purpose of Study: Major surgical procedures are accompanied by a risk of intraoperative blood loss and fluid shifting. Prior studies have demonstrated that intraoperative goal directed fluid therapy based on keeping stroke volume variation (SVV) below a critical threshold might result in increased cardiac output, stroke volume, and blood pressure. It has also been shown that increasing low central venous oxygen saturation (ScVO2) with fluid therapy in sepsis patients is associated with improved patient outcome. This study was designed to investigate the relationship between SVV and ScVO2 in the intraoperative, non-emergent, surgical setting.

Methods Used: This is a Departmentally sponsored, IRB approved prospective non-randomized sequential study in adult patients undergoing major surgical procedures with a pre-operative estimated blood loss of ≥15% total blood volume. SVV was measured using an arterial pressure cardiac output device (Vigileo, Edwards Lifesciences, Irvine CA). ScVO2 was continuously monitored using an oximetric central venous catheter (PreSep, Edwards Lifesciences, Irvine CA). Data from these devices were collected through an electronic interface to the electronic anesthesia record. Intraoperative fluid management was guided to maintain SVV <12%. Transfusion of blood products was at the discretion of the physicians caring for the patient. Data was analyzed for correlation between SVV and ScVO2 using JMP V8.0.2, with p<0.05 as statistically significant.

Summary of Results: Data was collected from 34 subjects. A total of 16187 time matched SVV to ScVO2 measurements were used for comparison. There was no correlation between SVV and ScVO2 paired samples (Pearson’s r = −0.146; p<0.02).

Conclusions: It has been reported that SVV is useful for guiding intraoperative fluid therapy, while ScVO2 has been used to guide fluid therapy in sepsis patients. This study shows no correlation between SVV and ScVO2 during the intraoperative phase of patient care. Although SVV and ScVO2 can be used independently as guides for fluid therapy in different settings, intraoperative ScVO2 appears to not be interchangeable with SVV for guiding intraoperative fluid therapy.

References:
Critt Care Clin. 2010;26:322

157 VITAMIN D RECEPTOR DELETION DISRUPTS NORMAL LUNG DEVELOPMENT AND LEADS TO AN ASTHMA PHENOTYPE

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Purpose of Study: There are strong epidemiologic data suggesting a link between vitamin D deficiency during pregnancy and childhood asthma. These data are complemented by experimental animal data showing that vitamin D is one of the local alveolar paracrine factors that spatiotemporally modulates perinatal pulmonary maturation. We have recently shown that vitamin D augments perinatal lung maturation such that its deficiency would perturb normal lung structural and functional development in a way that is consistent with the asthma phenotype. However, the mechanistic link between vitamin D deficiency during pregnancy and childhood asthma is not fully established. In this study, we aimed to determine the effect of vitamin D receptor deletion on key markers of lung maturation.

Methods Used: Control (wild type) and vitamin D receptor null mutant mice (C57BL6 background) were examined at 2 and 7 weeks postnatally. At sacrifice, lungs were perfused with phosphate buffered saline and collected for morphometry, RNA and protein analyses. Western blot and immunohistochemistry for key markers of lung differentiation were performed. Furthermore, using the Affymetrix Mouse Genome 430 2.0 array, the transcriptional profile of the lungs from the two groups at 7 weeks of age was determined.

Summary of Results: Both by Western analysis and by immunohistochemistry, at 2 and 7 weeks of age there was down-regulation of the intermediates for PTHrP/PPARγ signaling, a key signaling pathway for lung maturation, and up-regulation of Wnt signaling intermediates, a key pathway in asthma pathogenesis. Self-Organizing Map cluster analysis corroborated these data by showing up-regulation of the Wnt signaling pathway, down-regulation of the PTHrP/PPARγ signaling pathway in vitamin D receptor null mice compared to WT mice.

Conclusions: These data suggest that altered vitamin D signaling leads to down-regulation of homeostatic PPARγ signaling, and up-regulation of Wnt signaling, predisposing to a myogenic pulmonary phenotype that is consistent with the propensity to asthma in vitamin D deficiency.

158 SIMVASTATIN INHIBITS EXPRESSION OF EOTAXINS IN MOUSE AND HUMAN AIRWAY EPITHELIUM: IMPLICATIONS FOR THE TREATMENT OF ALLERGIC ASTHMA

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Purpose of Study: We have previously shown that systemic administration of simvastatin in a mouse model of allergic asthma inhibits eosinophilic inflammation and improves lung function. We wanted to evaluate the potential contribution of the airway epithelium to this observation. We hypothesized that simvastatin attenuates the expression of Th2 cytokines/chemokines important in human eosinophilic asthma.

Methods Used: Human bronchial epithelial cells (HBEC1) were grown under air-liquid interface (ALI) conditions until confluent. Mouse tracheal epithelial cells were harvested from naive BALB/c mice and grown to confluence in ALI for 4 weeks. HBEC1 cells were pre-treated with simvastatin (Sim, 20 μM) for 3 days, then stimulated with IL-13 (20 ng/mL) for 6 hours on the last day. Mouse cells were initially pre-treated with Sim 10 μM for 24 hours, then stimulated with IL-13 (20 ng/mL) and co-incubated with Sim for 48 hours. Expression of chemokines eotaxin-1, eotaxin-3, MCP-1, MCP-2, MCP-3, and CCR3 was analyzed by RT-PCR. Gene expression was assessed relative to housekeeping genes Hsp90ab1 (for mouse cells) and β-Actin (for HBEC1 cells).

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Summary of Results: In HBE1 cells, Sim treatment reduced IL-13-induced eotaxin-3 expression by 58% (p=0.00089), and eotaxin-2 expression by 60.6% (p=0.011) in mouse cells. Sim treatment reduced IL-13-induced eotaxin-1 expression by 92.6% (p<0.005), MCP-1 expression by 84% (p<0.005), MCP-2 expression by 53.8% (p=NS), and MCP-3 expression by 87.2% (p<0.05). Simvastatin (10 μM) also attenuated the mRNA expression of CCR3 by 62.03%, the receptor for chemokines important in eosinophil recruitment (i.e. RANTES, MCPs, and eotaxins).

Conclusions: These data suggest that simvastatin attenuates Th2 cytokines/chemokines important in eosinophilic inflammation pertinent to allergic asthma. At least some of the simvastatin effect may be occurring at the level of the airway epithelium. Thus, statins may be a novel therapy for allergic asthma that warrants additional study in humans.

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159 DEVELOPMENT OF A COBINAMIDE-BASED CYANIDE SENSOR TO RAPIDLY DETECT CYANIDE TOXICITY

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Purpose of Study: Cyanide is an extremely fast acting, potent and lethal agent and exposure may result from a number of different events including smoke inhalation, acts of terrorism and industrial accidents. Death can occur within minutes of exposure. Therefore, rapid diagnosis of cyanide toxicity is of great importance. Current methods to detect and quantify cyanide in blood, such as mass spectrometry, high-performance liquid chromatography, and spectrophotometry are slow (hours to perform), as they require off-gassing and trapping of cyanide in HCN gaseous phase. Here we present a simple, whole blood assay employing cobinamin and spectrophotometry without the use of toxic reagents or off-gassing of cyanide to rapidly detect cyanide in blood samples.

Methods Used: We used cobinamide, a precursor to cobalamin biosynthesis with an overall cyanide binding affinity of 1010 M-1, to bind with cyanide in whole blood to extract cyanide from erythrocytes. After cyanide was added to whole blood to simulate cyanide exposure, cobinamide was added to the specimen and mixed for 3 to 5 minutes. Plasma was then separated and absorbance was measured with a spectrophotometer and assessed for characteristic spectral changes of resultant dicyanocobinamide.

Summary of Results: When cobinamide binds to cyanide and forms dicyanocobinamide, a peak at 580 nm wavelength in absorbance predictably shifts based on relative cobinamic/dicyanocobinamide concentrations in the specimen. Results were reproducible in a rapid whole-blood assay requiring minimal equipment and non-toxic reagents.

Conclusions: This study demonstrates feasibility of concept for development of a rapid cobinamide-based cyanide assay for detecting cyanide levels and toxicity in whole blood. With completion of standard assay curves, accurate, rapid cyanide assays using cobinamide and simple spectrophotometry should be possible using this approach. In the future development of “diptest” based rapid field diagnostic testing kits may be possible using these principles.

160 TEMPORARY ARM OCCLUSION MAY REVEAL LEVEL OF CYANIDE TOXICITY

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Purpose of Study: Previously, we found that amplitudes of non-invasively detected oxy (OHb) / deoxyhemoglobin (RHB) concentration changes during respiratory challenge from 100% to 21% oxygen decrease as acute cyanide (CN) toxicity increases in an animal model. Because respiratory challenge cannot be used in CN exposed patients since oxygen supplementation is critical, we tested an alternative physiologic stress for estimating CN level using temporal occlusion on the upper arm.

Methods Used: New Zealand white rabbits were administered saline (n=5), 10 mg of NaCN (n=6) or 20mg of NaCN (n=6) in 60ml saline via the femoral vein at 1ml/min. After CN infusion, additional 90min of recovery was monitored. Upper arm occlusions using a finger cuff (130mmHg) were applied for 4 mins, then released before, during, and post cyanide infusion. Changes in O2Hb and RbHb concentration throughout the experiment were measured from lower arm muscles using near infrared spectroscopy (NIRS). The decrease/increase rate of O2Hb and RbHb concentration during occlusion was determined by calculating the half-time constant, τ12, using a Hill equation [O2Hb or RbHb = A(t/τ12 + 7)] where A is amplitude of O2Hb and RbHb changes and t is time.

Summary of Results: Tourniquet application caused a drop of O2Hb and increase of RbHb, which were reversed as the tourniquet pressure was released. Amplitudes of both O2Hb and RbHb changes lessened during cyanide infusion and returned to the baseline level at the end of recovery while control animals showed little changes. 20mg CN infused animals showed a much greater drop in the amplitudes of both O2Hb and RbHb changes than 10mg CN infused animals (50% vs 25% drop compared to the baseline). 20mg CN infused animals also showed that O2Hb decrease rate during the upper arm occlusion was 3.5 times slower compared to the baseline values during cyanide infusion, compared to 1.5 times from 10mg CN infused animals.

Conclusions: These results support our hypothesis and show that NIRS combined with temporal occlusion may be a potential non-invasive tool for estimating extent of cyanide toxicity in vivo.

161 LETHAL LEVEL CYANIDE POISONING REVERSAL BY INTRAMUSCULAR INJECTION OF COBINAMIDE

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Purpose of Study: Cyanide poisoning is a major worldwide threat. Military and civilian mass exposures to cyanide may occur through acts of terrorism and war. Current effective treatment methods, which require intravenous delivery, are ineffective in mass casualty situations. We are investigating intramuscular administration of Cobinamide, a novel water-soluble molecule that can bind 2 molecules of cyanide per molecule with higher affinity than currently approved hydroxocobalamin, for the treatment of mass casualty cyanide poisoning. In this study, we have evaluated the efficacy of IM cobinamide formulations in a rabbit model of cyanide poisoning.

Methods Used: Lethal level cyanide toxicity was generated in anesthetized and ventilated New Zealand white rabbits by infusion of 20mg cyanide solution intravenously over 60min. At the time of treatment, animals were injected intramuscularly with specific formulations of oxidized and reduced cobinamide, and compared to the control saline injected group. Quantitative analysis was done using continuous wave near infrared spectroscopy (CWNIRS) and diffuse optical spectroscopy (DOS) for CNS region and tissue oxygen and deoxyhemoglobin concentrations. Arterial blood was collected to measure the level of cyanide, blood gasses and cobinamide.

Summary of Results: IM injection of specific reduced cobinamide formulations has demonstrated the ability to reverse the effects of lethal CN poisoning, including fast recovery of oxygen and deoxyhemoglobin concentrations in cyanide exposed animal tissue. Blood plasma samples also indicated significant amounts of cobinamide absorption into the blood.

Conclusions: The result indicates that cobinamide is a rapid acting agent and appears to be effective in reversing the physiologic effects of cyanide poisoning when given intravenously to lethally CN poisoned subjects. Our additional study suggested that sulfur containing reducing agents may facilitate transportation of cobinamide into the blood more effectively. Future investigations on other additives that will allow rapid cobinamide transport are needed.

162 POSTNATAL MATURATION DECREASES THE ROLE OF RHO-KINASE IN ELECTROMECHANICAL COUPLING OF SHEEP PULMONARY ARTERIES

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Purpose of Study: L-type Ca2+ channels (Cav) as well as Rho-kinase are important in electrically induced contraction of pulmonary arteries, thus being therapeutically relevant in pulmonary hypertension. Evidence indicates that the role of Cav in pulmonary artery contraction is potentially reduced by chronic
Less than 300 cases of lung herniation have been reported and Rho-kinase inhibition was elicited in chronic hypoxia (3801 m) conditions for 100+ days. Pulmonary arterial rings were depolarized with cumulative doses from 5 to 125 mM of K+, or stimulated repeatedly with 125 mM of K+.

**Summary of Results:** One-hundred and twenty-five mM of K+ elicited the greatest force in pulmonary arteries from adults and this was not influenced by chronic hypoxia. The estimated EC50 for K+-induced contraction was similar in pulmonary arteries from all four animal groups. Ca2+ inhibition with 10 μM verapamil, diltiazem or nifedipine reduced, but did not ablate, K+-dependent contraction. Rho-kinase inhibition with 10 μM Y27632 reduced K+-contraction to a greater extent in pulmonary arteries from fetuses as compared to adults regardless of their gestational altitude. The combination of 10 μM nifedipine and 10 μM Y27632 dramatically reduced K+-contraction in both fetuses and adults.

**Conclusions:** Overall, electromechanical coupling appears augmented by maturation and unaffected by chronic hypoxia. Moreover, Rho-kinase related pathways appear to contribute to this coupling process in a maturationally dependent manner. This adds to a growing body of evidence regarding the importance of Rho-kinase in the treatment of pulmonary hypertension in newborns and adults.

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163 COUGH AND CHEST BULGE: A RARE CASE OF SPONTANEOUS LUNG HERNIATION

Giri PC, Anholm JD Loma Linda VA Healthcare System, Loma Linda, CA.

**Case Report:** Spontaneous lung herniation is a rare but often under-diagnosed entity.

**History:** 69 year old male with COPD, Gold Stage III presented with cough and shortness of breath for three weeks. He had an acute onset of right sided chest pain and swelling one week prior to admission after he felt “something give away” in his back.

**Physical Exam:** Chest wall: 10 x 10 cm bulge below right scapula, prominent with cough. Echymoses seen in lower half of chest wall.

**Imaging:** CT scans showed herniated lung through the right 8th intercostal space.

**Discussion:** Less than 300 cases of lung herniation have been reported of which around a 100 are spontaneous thoracic (intercostal) hernias. Most result from an acute increase in intrathoracic pressure due to coughing, sneezing, blowing on a musical instrument or heavy lifting classically in

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164 EFFECT OF KIDNEY REST ELEVATION ON POSTOPERATIVE CREATININE KINASE LEVELS IN HAND-ASSISTED LAPAROSCOPIC DONOR NEPHRECTOMY


**Purpose of Study:** Rhabdomyolysis (RM) during minimally invasive surgery has been reported in the literature and is a known cause of acute renal failure. Known risk factors in the perioperative period include positioning, operative time, and patient size. Our objective was to evaluate whether kidney rest elevation has an effect on postoperative creatinine kinase (CK) levels and risk for RM.

**Methods Used:** Serum CK was prospectively measured in all kidney donors at a single academic institution between February of 2003 and April of 2010. Thirty-one patients who underwent donor nephrectomy without kidney rest elevation were compared to forty patients who underwent donor nephrectomy with kidney rest elevation. Statistics were performed using either an independent sample t-test or a Mann-Whitney test, followed by multiple linear regression to determine independent variables with p <0.05 considered significant.

**Summary of Results:** Patient demographics and preoperative characteristics were not significantly different between the two groups. Laparoscopic donor nephrectomy with the kidney rest elevated was not a significant predictor of increased postoperative CK values compared to patients without kidney rest elevation (median CK 450 vs 458 IU/L; p = .871). Kidney rest elevation did not result in increased postoperative creatinine compared to patients without kidney rest elevation during laparoscopic donor nephrectomy (median creatinine 1.18 vs 1.17 mg/dL; p = .809). After adjusting for length of surgery and gender, the kidney rest was not a significant predictor of increased postoperative CK values (p = .834).

**Conclusions:** Kidney rest elevation does not affect the risk for increased postoperative CK and RM. Other factors, including surgeon preference and comfort with the surgical position, should determine whether or not the kidney rest is elevated during laparoscopic donor nephrectomy.

165 ADIPOSE DERIVED-STEM CELL ADHESION, PROLIFERATION, AND MIGRATION ON ALLODERM® MATRIX

Slack GC1,2, Hargaval SJ1,2, Zulk P1,2, Rahgozar F2, Yaghoubian A2, Kruger E2, Ehsani N2, Tabit C2, Bradley J1-2 1UCLA David Geffen School of Medicine, CA. CA and 2UCLA, Los Angeles, CA.

**Purpose of Study:** The use of acellular dermal matrices, like AlloDerm® Regenerative Tissue Matrix, is currently used in breast reconstruction after ablative mastectomy surgery. Adipose derived stem cells (ASCs) are multipotential cells that may differentiate into fat, bone, muscle or nerve cells. The aim of our study was to determine if AlloDerm® may act as a suitable scaffold for ASCs to assist faster integration of local tissue and graft and promote revascularization, of importance prior to radiation treatment for breast cancer.

**Methods Used:** ASCs, obtained from liposuction patients, were seeded onto the dermal face of the AlloDerm® scaffold by two methods: 1) pipet placement, 2) centrifugation (1000 rpm for 5 minutes). Constructs were
Apatite coated PLG scaffolds were formed by incubating successfully attach and proliferate over a 6-week interval. The present data indicate that a combination of apatite and BMP-2 do not simply enhance the osteogenic response of hMSCs, but act through different and possibly opposing pathways. Thus multiple signaling strategies may be necessary to achieve optimal bone regeneration.

**Summary of Results:** The iliohypogastric (IHG) nerve was found to be 2.9mm wide, 27.1mm from the twelfth rib (T12), and 46.2° from the lateral border of the psoas muscle. The ilioinguinal (ILI) nerve was 2.1mm wide, 43.3mm from T12, and 44.6° from the psoas muscle. The genitofemoral (GEN) nerve, located 93.7mm from T12, was found overlying the psoas with a width of 2.3mm. The lateral femoral cutaneous (LFC) nerve was 2.2mm wide, 188mm from T12, and 38.1° from the psoas. The 7.7mm wide femoral (FEM) nerve was located 150.2mm from T12 and 22.9° from the psoas.

**Conclusions:** In this study, we observe considerable anatomic variability associated with each lumbar plexus branch. The results of the study can improve outcomes in surgical intervention of post-hermorrhaphy inguinodynia.

**Table:**

<table>
<thead>
<tr>
<th>Nerve</th>
<th>Distance from T12 (mm)</th>
<th>Angle From Psoas</th>
<th>Width (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IHG</td>
<td>27.1 (4±4.0)</td>
<td>36.2°</td>
<td>2.9</td>
</tr>
<tr>
<td>ILI</td>
<td>43.3 (14±7.8)</td>
<td>46.6°</td>
<td>2.1</td>
</tr>
<tr>
<td>GEN</td>
<td>93.7 (55±10)</td>
<td>Overlie Psoas Major</td>
<td>2.3</td>
</tr>
<tr>
<td>LFC</td>
<td>118 (76±162)</td>
<td>38.1°</td>
<td>2.2</td>
</tr>
<tr>
<td>FEM</td>
<td>150.2 (117±97)</td>
<td>22.9°</td>
<td>7.7</td>
</tr>
</tbody>
</table>

**Figure 1. Schematic Representation of Retroperitoneal Lumbar Plexus Nerves**
delays. Providers also conducted ASA 11-pt airway, heart, and lung exams for the PAC and PATC groups. Laryngoscopic view was graded, with Grade 3 or 4 considered difficult view (DV). Difficult airway management (DA) was defined as >1 attempt by an experienced provider and/or the need for >1 type of laryngoscope.

Summary of Results: 174 participated (38 phone, 64 PAC, 72 PATC). Prediction of DV by PAC and PATC correlated well with the Staff Anesthesiologist’s evaluation on day of surgery. 19 patients (10 PAC, 19 PATC) had DV. PAC and PATC were equally likely to predict this. DA was present in 6: the Anesthesiologist’s evaluation was incrementally more predictive of this (67% vs 50%). No unexpected findings were noted on heart/lung exams on day of surgery, and patients/providers reported high satisfaction with PATC.

Conclusions: PATC is technically feasible and equivalent to current in-person evaluation, including the ASA 11-point airway exam, with additional potential to minimize patient inconvenience and costs of missed/cancelled appointments.

169 FAILURE STRENGTH OF TENDON REPAIR WITH VARYING STITCH COUNT IN THE PULVERTAFT WEAVE AND SIDE-TO-SIDE TECHNIQUES

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Purpose of Study: The Pulvertaft weave (PTW) is a common repair technique used during tendon transfers. This study was designed to compare the maximum load-to-failure of the PTW and side-to-side (STS) repair methods and to analyze the mechanical interaction between the number of weaves and stitches.

Methods Used: Six groups of repairs were performed using a porcine tendon model. Three PTW groups, PTW3, PTW4, and PTW5, had one, two, and three weaves, respectively (N=5). The others, utilizing STS technique (STS3, N=5; STS4, N=5; STS5, N=7), had no weaves, but equal amounts of stitches as in each PTW group. For PTW repairs, incisions were made at the tendon free end and the transfer tendon weaved through the hosting tendon. A stitch was placed at the free ends and at each weave point. One weave yielded three stitches with an additional stitch per additional weave. Overlapping the tendons and placing three to five equidistant stitches completed the STS repairs. Cross-stitches with four stitch points were used for all repairs. The specimens were tested to failure in tension using an Instron machine.

Summary of Results: All failures occurred within the repair region. The mode of failure for all groups was suture breakage. There were no significant differences among the three STS groups in maximum load-to-failure (STS3, 239±16.4 N; STS4, 262±19.1 N; STS5, 253±91.5 N). PTW4 (402±45.8 N) was not significantly different than PTW3 (309±21.4 N, p=0.08) and PTW5 (501±50.8 N, p=0.06). However, PTW5 was greater than PTW3 (p=0.05). PTW4 and PTW5 were greater than all STS groups (p<0.05). A linear regression of the number of weaves versus load for all groups yielded a slope of 80.84 (R\(^2\)=0.78, p<0.001). A linear regression of the suture count versus load-to-failure for STS groups yielded a slope of 1.6 (R\(^2\)=0.01, p=0.72) and for the PTW groups yielded a slope of 23.9 (R\(^2\)=0.82, p<0.001).

Conclusions: The PTW construct had better mechanical properties than the STS. This study suggests that when using a cross-stitch for tendon repair, the addition of weaves provides a dramatic increase in maximum load-to-failure capacity of the construct and that an increase in the number of stitches provides mechanical benefit only when the tendons are weaved.

170 OCULAR IMAGING TECHNIQUES: AN USEFUL TOOL IN THE DIAGNOSIS AND MANAGEMENT OF PATIENTS WITH ANTERIOR SEGMENT PATHOLOGY

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Purpose of Study: Ocular pathology affecting the anterior segment can present diagnostic problems because it often involves structures that are not readily accessible through routine examination methods. The goal of the study was to assess and compare the utility of ultrasound biomicroscopy (UBM) and anterior segment optical coherence tomography (AS-OCT) in the evaluation and management of patients with ocular pathology involving the anterior segment of the eye.

Methods Used: Retrospective review of 40 eyes with various conditions involving the anterior segment, examined with UBM (Sonomed, Inc, Lake Success, NY) and AS-OCT (Carl Zeiss Meditec Inc., CA) over a time period of 18 months.

Summary of Results: Abnormalities were found in 32 eyes of patients with the following etiologic diagnoses: anterior scleritis, intermediate uveitis, traumatic hyphema, foreign body, iris or ciliary body mass and intraocular lens. Indications for the UBM were: visualization of anterior chamber structures, visualization of posterior iris or ciliary body, assessment of intraocular lens position, pre-operative assessment in eyes with hypotony or trauma and for follow-up of documented abnormalities. Indications for anterior segment OCT were: assessment of the angle, measurement of corneal thickness, visualization of anterior iris and preoperative assessment.

Conclusions: The OCT and UBM imaging techniques offer a complete assessment of the anterior segment of the eye. Both are sensitive imaging modalities that provide excellent diagnostic clues in patients with anterior segment involvement. UBM is invaluable in accurately visualizing the posterior iris and ciliary body region. In the majority of cases the high-resolution imaging techniques confirmed the clinical diagnosis and were useful in patient follow up and surgery planning.

171 URETEROSCOPY WITHOUT INTRA-OPERATIVE FLUOROSCOPIC IMAGING AS A SAFE ALTERNATIVE


Purpose of Study: During the course of a ureteral stone episode, a patient is at risk for multiple doses of radiation. Although the doses are not routinely high enough to cause dose-dependent effects such as skin burns, the dose-independent effects such as cancer, could potentially be severe, warranting the reduction of radiation exposure whenever possible. This retrospective study assesses the safety and effectiveness of ureteroscopy for the treatment of kidney and ureteral stones without the use of fluoroscopic guidance in light of the potential risks to patients and medical personnel that are inherent to radiation exposure.

Methods Used: A retrospective review of ten patients who electively received ureteroscopy without intra-operative imaging between January 2009 and June 2010 was performed and the outcomes of the procedures were analyzed to determine their effectiveness. These ten patients and their outcomes were then compared with 25 patients who received standard low-dose fluoroscopy-guided ureteroscopy during that same time period.

Summary of Results: The two groups were found to have no significant differences in gender, age, BMI, stone laterality, stone size, stone location, postoperative complication rate, stone free rate, or repeat procedures (all P > 0.45). The greatest difference found was that operating time for the fluorless procedure was 9.7 minutes shorter than for the low-dose procedure (P = 0.26).

Conclusions: In select patients, a fluorless ureteroscopy technique can safely and effectively be employed by a skilled physician for the treatment of kidney and ureteral stones without exposing the patient to unnecessary and potentially harmful radiation.

172 RECONSTRUCTIVE OR COSMETIC PLASTIC SURGERY: FACTORS INFLUENCING THE TYPE OF PRACTICE ESTABLISHED BY CANADIAN PLASTIC SURGEONS

McInnes C\(^1\), Courtemanche D\(^2\), Verchere C\(^2\), Bush H\(^2\), Amoja P\(^1\) \(^1\)University of British Columbia, Vancouver, BC, Canada and \(^2\)British Columbia Children's Hospital, Vancouver, BC, Canada.

Purpose of Study: Recently, some in organizational plastic surgery have voiced concerns that the specialty is facing an identity crisis. Challenged by factors such as increasing competition in the cosmetic marketplace and decreasing reimbursement for reconstructive procedures, many American plastic surgeons have adopted increasingly cosmetic-focused practices. This study will investigate the currently unknown practice profiles of Canadian plastic surgeons to determine the reconstructive-cosmetic mix, as well as factors which influence the type of practice to see if a similar pattern is occurring in Canada.
Methods Used: An anonymous online survey was distributed to all 352 Canadian plastic surgeons with email accounts registered with the Canadian Society of Plastic Surgeons (CSPS) and/or the Canadian Society for Aesthetic Plastic Surgery (CSAPS) which contained questions regarding practice profiles.

Summary of Results: A 34% response rate was found (120 responses), of which 76% have reconstructive practices and 24% have cosmetic practices. Variables more common among reconstructive surgeons were female gender, younger age, pre-residency research experience, advanced degrees (Master’s/PhD) and higher educational debt, among others.

Conclusions: The field of reconstructive plastic surgery appears to be thriving in Canada. Compared to the US, a greater proportion of Canadian plastic surgeons have reconstructive practices, likely due to different funding models and competition. Compared to cosmetic surgeons, a greater proportion of reconstructive surgeons hold university positions, have more academic practices, work longer hours, and take more emergency call. When deciding which type of practice to establish, reconstructive surgeons were relatively more influenced by academic opportunities, and less influenced by financial and non-financial metrics.

Purpose of Study: Lumbar spinal stenosis is a disabling medical condition in which narrowing of the spinal canal compresses the spinal cord and nerves. Entrapment of the cauda equina roots often presents with difficulty walking, pain in the back and lower extremities, and weakness in the legs, a condition called neurogenic intermittent claudication (NIC). The disease continues to be a leading cause of morbidity in the elderly population and is the leading cause of lumbar spine surgery for adults over the age of 65. Historically, the standard operative procedure to treat NIC was laminectomy, an invasive surgery that removed the lamina of the spine. As laminectomy is more invasive with longer postoperative recovery, X-STOP, a minimally invasive interspinous process implant, was introduced to treat neurogenic intermittent claudication secondary to lumbar stenosis. This study assesses the outcome of patients treated with X-STOP up to the 4 year post-operative period.

Methods Used: IRB approval was obtained for this retrospective cohort study, in which the Zurich Claudication Questionnaire (ZCQ) via telephone was administered to an initial total of 23 patients who were implanted with X-STOP to assess their outcome measures in three domains: 1) Symptom Severity, 2) Physical Function, 3) Patient Satisfaction. The secondary outcome measure of the Visual Analog Scale (VAS) was used to assess post-operative trends in pain in X-STOP patients. Both the ZCQ and VAS scores were obtained in 18 patients at the 3 year post-op period and 5 patients at the 4-year post-op period. Five patients were lost in the follow-up process due to unavailability or deceased status.

Summary of Results: Based on the ZCQ and VAS scores, there was a 68% (15/22) success rate following the procedure. The VAS levels at three years were comparable to the one-year post-operative values as the pain levels stabilized over time. For the patients reaching the 4-year post operation period, ZCQ and VAS scores remained significantly low. There were no postoperative fractures of the spinous processes, implant dislodgement, or wound complications.

Conclusions: X-STOP is a safe and effective treatment for NIC that provides marked relief of symptoms with sustained beneficial outcomes at 4 years of follow-up.

Purpose of Study: X-rays are frequently used in medical practice and are associated with a cumulative radiation dose. Previous studies have calculated the average cumulative radiation doses received by physicians, but no previous studies have characterized the radiation exposure received by patients treated with X-rays.

Methods Used: A retrospective review was performed between January 2004 and April 2010 of patients with solitary renal tumors ≤ 4.0 cm treated with either percutaneous ablative technique. The procedure time, radiation exposure, and cost were compared using the Mann-Whitney U Test and Pearson Chi-Square with significance considered at p < 0.05.

Summary of Results: Fifty-three patients with small renal masses were treated with a percutaneous ablative therapy (23 PRA and 30 PCA). The tumor size, ASA score, and patient age were similar between groups. The billed charges were similar between PCA ($18,079) and PRA ($17,148); p=0.7. PCA had longer procedural time (88.2 vs. 58.6 min; p<0.001), greater number of probes (3.2 vs 1.3; p<0.001), more CT scans (16.2 vs 10.5; p=0.003), and greater total radiation exposure (2388 mGy-cm vs 1461 mGy-cm; p=0.02) compared to PRA.

Conclusions: No prior study has attempted to quantify the radiation dose received during PRA and PCA. Our study demonstrates that all patients received relatively large radiation dose during percutaneous ablation with PCA patients receiving the largest dosage. Physicians must consider this factor when selecting treatment modalities for young patients with renal masses and continue to work to reduce the radiation exposure received by these patients.
176 RELATIONSHIP OF ESOPHAGEAL MUCOSAL OXYGEN SATURATION TO GASTROINTESTINAL FUNCTION IN PATIENTS UNDERGOING CARDIOPULMONARY BYPASS: A PRELIMINARY REPORT
Blair BM, King NC, Applegate RL, Sanghvi C, Dorotta IR, Gatling JW, Loma Linda University Medical Center, Loma Linda, CA.
**Purpose of Study:** Visible light spectroscopy (VLS) allows measuring of esophageal mucosal oxygen saturation (StO2) during surgery, and reflects perfusion of the lower esophagus. This may provide information regarding GI tract perfusion during surgery since blood supply is shared from the splanchic circulation. Changes in GI perfusion may be associated with delayed recovery of normal GI function and longer hospital stay. We evaluated the use of esophageal StO2 in adults requiring cardiopulmonary bypass (CPB).
**Methods Used:** This is an ongoing IRB approved observational study in adult patients undergoing elective cardiac surgery requiring CPB. Continuous monitoring of esophageal StO2 (T-Stat, provided as an unrestricted material grant from Spectros, Portola Valley, CA) was used. Data was captured electronically. Analysis included Pearson correlation between average esophageal StO2 pre-, during, and post-CPB, the duration of CPB, events associated with CPB, return of normal GI function/bowel movement, and length of stay. Two-tailed significance was set at 0.05.
**Summary of Results:** Nine subjects have been analyzed. The magnitude of the difference between average esophageal StO2 pre-CPB to post-CPB correlated to delayed return of GI function (r = 0.808; p = 0.008) and to length of stay (r = 0.708; p = 0.033). In addition, esophageal StO2 levels decreased below pre-CPB baseline levels in the transition periods on and off CPB, but neither of these findings were statistically related to return of bowel function.
**Conclusions:** This preliminary analysis revealed esophageal StO2 monitoring has some limitations. Non-physiologic fluctuations in oxygen saturation levels can occur when a TEE probe is used, so measuring esophageal StO2 during TEE needs to be explored. Our results indicate that return of normal GI function is delayed in subjects in whom the average esophageal StO2 post-CPB is significantly higher than the average esophageal StO2 pre-CPB. This translates into longer hospital stays, as patient discharge is delayed until bowel function returns. We hypothesize that in this subset, higher average esophageal StO2 post-CPB compared to pre-CPB reflects hyperemia, which could lead to reperfusion injury of the GI mucosa.

Western Student Medical Research Forum
Student Session I - Global Health
8:30 AM
Friday, January 28, 2011

177 EVALUATION OF THE FEASIBILITY AND EFFECTIVENESS OF A COMPLEMENTARY FEEDING INTERVENTION FOR REDUCTION OF CHILDHOOD MALNUTRITION IN KENYA
Ashton R², McCalmont K², Tomedi A³, Rohan-Mijares F¹, University of New Mexico School of Medicine, Albuquerque, NM and ²University of New Mexico School of Medicine, Albuquerque, NM.
**Purpose of Study:** To study the operational feasibility and effectiveness of the distribution of locally-available foods to prevent malnutrition and improve child growth in Kenyan children.
**Methods Used:** A quasi-experimental design was chosen with an intervention group consisting of children in all of the villages in one region of arid, rural, Eastern Kenya, and a non-intervention comparison group of children in all of the villages in an adjacent region. Children included in the study were age 6 to 20 months with weight-for-height z score > -2 at baseline. The intervention was the distribution of a monthly food ration for the index child and separate rations for the family, and group education on appropriate complementary feeding and hygiene. At baseline and at the end of the 7-month intervention, community health workers visited every household to measure child length/height and weight. Each month a questionnaire was administered to the intervention household to assess receipt of supplemental food and degree to which the index child received the food.
**Summary of Results:** Children in the intervention (n = 141) and control (n = 185) groups had similar baseline anthropomorphic measures. The caretakers in the intervention group confirmed that the intended amounts of food supplements were received monthly, and child nutrient intake was significantly greater in the intervention group. During the 7-month intervention, the growth of the children, measured by mean z score, was significantly better in the intervention group than the control group, weight for age difference = 0.79 (p < .0001), weight for height difference = 1.17 (p < .0001). Compared to the control group, the intervention group had a lower prevalence of wasting (0% vs. 8.8%, p = .0002) and underweight (5.4% vs. 22.4%, p < .0001). Infectious morbidity (diarrhea and respiratory infections) was similar in both groups.
**Conclusions:** The findings suggest that the distribution of locally available foods is operationally feasible and improves child growth and decreases malnutrition in Kenyan children.

178 NUTRITION EDUCATION IN THE BHUTANESE NEPALI REFUGEE COMMUNITY OF TWIN FALLS, ID
Brito T, University of Washington, Seattle, WA.
**Purpose of Study:** The College of Southern Idaho Refugee Program (CSIRP) has resettled 2,500 refugees since 1980, empowering them to live successfully and independently in Twin Falls. In the last 2 years, many new arrivals have been Bhutanese Nepali. Post-migration diets of refugees tend to be heavily dependent on refined sugars, polished grains and nutrient-poor snacks. A presentation was designed to educate about making choices that support a balanced diet and healthy lifestyle.
**Methods Used:** Interviews of CSIRP staff members identified nutrition as a challenge for refugees. Review of the primary literature confirmed the effectiveness of a visually supported intervention based on the USDA Food Pyramid guidelines for an audience with varying degrees of English proficiency. Attendance at a Bhutanese Nepali festival provided cultural context for traditional diet. A poster based on the Food Pyramid with photographs illustrating each food group was created as part of a presentation for an ESL class; an interpreter was asked to attend.
**Summary of Results:** Twenty-five refugees attended the presentation during an ESL class. Though an interpreter was available, most attendees could follow the presentation in English with the aid of the poster. The target audience was engaged and asked clarifying questions about adapting traditional recipes. It sparked a discussion about the consequences of poor nutrition choices made by fellow refugees. Many asked for and were referred to the USDA website in order to create a personalized diet plan.
**Conclusions:** The presentation was well received; the audience was involved and interested as evidenced by the relevant questions and discussion. Translation by the interpreter was not necessary for audience comprehension; this was a success as the intent was for basic language and symbols on the visual to convey the message adequately to those without a mastery of English. The poster and presentation will be included as a part of future ESL classes. The refugee community in Twin Falls is very close knit and it is common for acquired knowledge to be passed on from old to new refugees as they are welcomed to their new life. Hopefully the information from this presentation can be part of that exchange. Though educational interventions aimed at a non-English-speaking population can seem intimidating, they are possible and often some of the most necessary.

179 ADDRESSING THE HEALTHCARE WORKER SHORTAGE IN RURAL UGANDA BY TRAINING VILLAGE HEALTH WORKERS
Chuka B, University of Washington, Seattle, WA.
**Purpose of Study:** Uganda is currently suffering from a severe deficit in healthcare workers, with only 81 medical personnel for every 100,000 people. The rural areas are disproportionately impacted by this shortage. In an effort to address this shortage, Uganda has recently turned its attention toward task shifting, which is the transfer of certain responsibilities from a doctor or nurse to a healthcare worker with less training. A cornerstone of this concept is the training of Village Health Workers (VHW’s). This project took place in Kiboga, a rural district in central Uganda with a population of 300,000 and a mere five physicians responsible for direct patient care. A VHW training program was conducted in collaboration with the NGO Global Youth Partnership for Africa (GYPA).
Methods Used: A group of 19 volunteers attended courses four hours per week for a total of six weeks. Lectures were based on David Werner’s “Where There is No Doctor” and students were supplied with a copy of this book and a first aid kit. The author interviewed district officials to ascertain what skills the village health workers needed, and created and taught classroom sessions in collaboration with a GYP A volunteer physician. A pocket guide was designed by the author and distributed to the students. Additional copies of the pocket guide were provided to previous village health worker graduates and to the program coordinator for use in future courses.

Summary of Results: 18 of the 19 volunteers successfully completed the six-week training course. The students were enthusiastic about the training, as they felt it would prepare them to take an active role in influencing the health of their community. Residents throughout Kidoga who were interviewed stated that the implementation of village health teams has contributed to improved health outcomes and will continue to have a positive impact on the community. GYP A will continue to organize the course and train volunteers.

Conclusions: Training VHW’s can help combat the healthcare worker shortage and leads to improved health literacy throughout the community. Further analysis is needed in order to ascertain whether training and utilizing VHW’s results in measurable improvements in health outcomes. Additional work is necessary in order to increase collaboration between the district and the NGO’s teaching the courses.

180 REDUCING TYPHOID INFECTIONS IN ANTSIRABE, MADAGASCAR THROUGH THE PROMOTION OF HANDWASHING AND HOUSEHOLD WATER PURIFICATION

Embrick E University of Washington School of Medicine, Seattle, WA.

Purpose of Study: Typhoid fever is very common in Madagascar. At Andranomadiso Lutheran Hospital (HLA), located in the central highland town of Antsirabe, it was one of the most common reasons for hospitalization. During the months of December - July, 2010, the rates of hospitalization for typhoid fever increased dramatically. Approximately 1/3rd of pediatric inpatients between June 14th and July 10th had been diagnosed with the disease. Since typhoid is transmitted through fecally-contaminated food and water, an education program was created to promote hand washing and point-of-use water treatment in an effort to decrease the spread of the disease.

Methods Used: Typhoid was identified as a major health issue in Antsirabe during a community health assessment. An educational program was created based on information gathered from the scientific literature and discussions with local healthcare providers, health-related NGOs, and the families of typhoid patients. A presentation was given, discussing ceramic filtration, chlorination, solar disinfection, and boiling as water purification methods, and a local NGO was identified which produces low-cost water chlorination solution for household use. The presentation also reviewed methods of safe water storage, as well as standard hand washing technique using soap. A Malagasy-language, picture-based handout covering key points was created and distributed.

Summary of Results: The presentation was given to a group of approximately 150 people at a Lutheran Church conference on the outskirts of Antsirabe. One hundred brochures were distributed to attendees afterward. The presentation was about twenty minutes long and was given in French and translated into Malagasy for the congregation.

Two-hundred brochures were left for three pediatric nurses, who agreed to discuss typhoid prevention with their patients and hand out the brochures.

Conclusions: This project met the goals of helping to increase awareness of typhoid fever and educating the population about simple affordable methods to prevent the disease. Additional educational efforts are warranted in order to reinforce these messages and promote preventative behaviors in a larger portion of the local population.
as a framework for other health-promotion activities. Future community health projects should focus on creatively encouraging increased parental participation.

183 REDUCING INTESTINAL PARASITIC INFECTIONS IN RURAL PERU THROUGH COMMUNITY EDUCATION

Pudwill L  University of Washington School of Medicine, Seattle, WA.

Purpose of Study: Helminth infections are the second-leading cause of illness in Yantalo, a village in the tropical Amazon of northern Peru, for 2006–2010. The population is susceptible to parasitic infections due to the unsafe water, poor sanitation, crowded conditions, low education levels, poverty, agricultural activity, and subtropical climate. The aim of this project was to reduce intestinal parasitic infections in Yantalo through sustainable community-based education.

Methods Used: High school students were taught about parasite life cycles, health consequences, and prevention. Student volunteers then developed and gave presentations about hygiene and parasites to younger children. A presentation was given to a group of adults and hand-washing activities were done with kindergarten students. Educational materials were prepared for future use by educators, health workers, and volunteers with the Yantalo Foundation. The education coincided with the village’s first anti-parasite medication distribution campaign, sponsored by the Ministry of Health. The work was done with the help of volunteers from the Yantalo Foundation.

Summary of Results: A total of 214 high school students received education about parasite prevention. Six groups of high school students gave presentations to 248 elementary students in twelve classes. Twenty adults attended the lecture about parasite prevention and received a handout. Fifty kindergarten students participated in the hand-washing sessions. All educational materials and information were given to the Foundation for future use in the community. The school nurse agreed to supervise high school students in teaching younger children. The clinic physician was assisted in the distribution of Albendazole to school children, which will be repeated every three months.

Conclusions: The project increased awareness of hygiene, water treatment, and parasite prevention among school children through education that promoted community involvement. The education will be sustainable with cooperation of local students, the Yantalo Foundation, and the school nurse. It will be important to complement education with periodic distribution of anti-parasite medication. Additional adult education and water treatment measures are also necessary to reduce the prevalence of parasitic infections in Yantalo.

184 WHAT NEW KNOWLEDGE DO STUDENTS GAIN FROM INTERNATIONAL HEALTH WORK?

To E University of British Columbia, Vancouver, BC, Canada.

Purpose of Study: To determine if students gain new knowledge in global health work after participation in a student-driven international project.

Methods Used: The 17 UBC students that participated in the Global Health Initiative (GHI) projects were surveyed. Students were asked to rate their pre and post project knowledge in four areas: cross-cultural communication, project development, project sustainability, and community collaboration/local empowerment. Mean relative percentage change in each learning area was calculated. The survey also collected qualitative information on project learning outcomes by asking “What were the most valuable lessons you learned while working overseas?”.

Summary of Results: Quantitative Results: Students consistently reported that they increased their knowledge in each of the measured learning areas. Mean relative increase in each area: cross-cultural communication 44%, project development 119%, project sustainability 111%, and community collaboration/local empowerment 105%. Qualitative Results: Even though there was diversity in participant responses, common lessons and themes emerged. These include the value of appreciating cultural norms and work ethics, relationship building with locals, interdisciplinary collaboration, effective communication practices, flexibility in project implementation, thorough pre-departure preparation (project planning and cultural awareness), and development of locally-sustainable projects.

Conclusions: Through partaking in GHI projects, students consistently demonstrated significant increases in their understanding of international health project development based on the four learning objectives measured in this study. Students also have garnered many other lessons in international health from participation in GHI as reported in the qualitative results.

185 THE TREATMENT AND PREVENTION OF DIARRHEA AND DEHYDRATION IN RURAL MONGOLA

Wallace R  University of Washington School of Medicine, Seattle, WA.

Purpose of Study: Diarrhea is pervasive in the rural Darhad Valley of Mongolia due to contamination of surface waters coupled with inadequate sanitation measures. With no access to oral rehydration packets and little knowledge concerning rehydration therapy in the community, individuals with diarrhea, especially children, are at risk for dehydration and death. Therefore, an educational program was established to instruct community members about the causes, treatment, and prevention of diarrhea and dehydration.

Methods Used: Based on a review of recent literature a presentation was designed to address diarrhea and dehydration in bagh (township) 1 in the Darhad Valley. With the organizational help of community leaders, the presentation was given to multiple sites within the region including an annual community festival called Nadaam that attracted much of the population. To complement the presentation, a muslim poster was made that utilized pictures and Mongolian text. Additionally, to educate local physicians, a laminated pamphlet was made with BioRegions International, an NGO with 12 years experience in the area, addressing dehydration and rehydration therapy. Lastly, a presentation was made for the Darhad Valley Kindergarten class regarding hand washing and other basic sanitation measures.

Summary of Results: The diarrhea/dehydration presentations were given to over 50 members of the community; approximately half of the 80 families in bagh 1 were present. Furthermore, the poster and the notes for the presentation have been left with BioRegions International for future use. The dehydration pamphlets were distributed to over twenty doctors in the region during an annual seminar. The handouts were well received and have been observed hanging on display for the community in several clinics. Lastly, around 25 children and some of the parents attended the Kindergarten presentation.

Conclusions: The project successfully met the goals of increasing awareness among the community concerning the causes of diarrhea, the importance of hygiene, ways to identify dehydration in infants and adults, and the treatment of dehydration using homemade oral rehydration salts. Additional efforts to combat diarrhea are needed, such as improving the quality of surface waters, promoting zinc tablets, and instituting rotavirus vaccination programs.

186 PEDIATRIC MALARIA TREATMENT DELAYS ACROSS SOCIOECONOMIC LEVELS IN SUBURBAN ACCRA, GHANA

Wong KR  Yale University School of Medicine, New Haven, CT; 2University of California at San Diego, La Jolla, CA and 2University of Ghana School of Public Health, Accra, Ghana.

Purpose of Study: The study reported here examines how a Ghanaian family’s socioeconomic status (SES) affects the delay-time before a child with Plasmodium falciparum malaria is brought to a government hospital. It was hypothesized that a delay of appropriate clinical treatment results from a family’s apprehension of inability to pay the hospital fee.

Methods Used: In this ethnographic study, parents of pediatric patients with blood smear-confirmed malaria (N=15) were interviewed through both quantitative questionnaires and narratives at the Legon Hospital Pediatrics Ward. The questionnaires ascertained the dependent variable (time lag during which the child languished at home), as well as the independent variable of SES, measured by: 1) monthly income range, 2) health insurance, and 3) years of household education. Over the course of four months, 170 hours of dialogue and observation supplemented the numerical data.

Summary of Results: There was actually no statistical association between delay-time and family financial standing, nor a correlation between delay-time and enrollment in Ghana’s recently-established National Health Insurance Scheme. Parents of all backgrounds postpone clinical treatment even
after the onset of clear malaria symptoms in order to conserve their time; the waiting lines at hospitals are often several hours long and the illness is endemic in West Africa. Yet, this study does confirm that lower-SES households less effectively prevent malaria. Only half of families in the lowest income group utilized insecticide-treated mosquito nets, while all six families in the highest income group use the precaution.

**Conclusions:** In this township, the study suggests that low-SES families do not disproportionately delay clinical treatment. Especially when raising multiple children, parents of all SES groups delay at home and self-treat with herbal teas or street-bought (and often counterfeit) chloroquine and artesunate anti-malarials. This has its risks. Delayed clinical care and the rapid deterioration of originally-uncomplicated *P. falciparum* cases contrib-ute to 20,000 pediatric malaria fatalities in Ghana annually.

187 FACTORS AFFECTING PERCEIVED HEALTH OF LATINA CAREGIVERS IN EAST LOS ANGELES

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**Purpose of Study:** To examine the effect of perceived burden on the health of female Mexican and Mexican-American caregivers who give unpaid assistance to elderly relatives. Currently, eight million Latino adults provide informal, unpaid care to adult relatives, and the population of Latino caregivers is likely to continue to grow with the growth of the older Latino population. In order to better support these caregivers, it is important to understand their caregiving experiences.

**Methods Used:** We analyzed data from a convenience sample of caregivers (N = 96) on different domains of caregiving, including perceived physical and emotional intensity, number of ADLs/IADLs, and cultural reasons for caregiving. We conducted univariate, bivariate, and multivariate analyses to understand the caregiver population demographics, characteristics of the caregiving situation, attitudes toward caregiving, and how these factors affect caregiver self-perceived health. Analyses were done using PASW Statistics GradPack Version 18.0.

**Summary of Results:** Better caregiver health was associated with higher education, younger caregiver age, fewer forms of support provided to the care recipient, the care recipient not having dementia or mental illness, and lower perceived physical intensity of care. However, only perceived physical intensity of care was significant in our final regression model.

**Conclusions:** These results suggest that subjective measures of caregiver burden like perceived intensity are stronger predictors of Latino caregivers’ health than are objective measures of the amount of care provided.

Western Student Medical Research Forum

**Student Session II - Community Health**
8:30 AM
Friday, January 28, 2011

188 DEVELOPING PHYSICAL FITNESS PROGRAMS FOR SENIOR ADULTS IN MONTPELIER, ID

Aebischer TD UW School of Medicine, Seattle, WA.

**Purpose of Study:** Diabetes, hypertension, coronary heart disease, and other Chronic Health Conditions (CHCs), affect over 100 million Americans, with 88% of those over 65 having at least one or more CHC. Moderate physical activity provides a 30% reduction in incidence of CHCs. This project focused on establishing a model for designing and initiating a senior adult focused physical fitness program that encourages initial participation and long-term program adherence, a model to be used in Montpelier and other similar rural communities.

**Methods Used:** Surveys involving area healthcare providers and leaders identified the absence of fitness programs. A literature review identified benefits and best practices for designing and initiating senior oriented fitness programs. The local newspaper provided project awareness. Area seniors were gathered for a discussion group focusing on their ideas regarding attractors and detractors related to physical fitness. An educational seminar was held covering benefits of physical activity and providing specific exercises addressing identified physical concerns. Local leaders were engaged to pursue further program development. Cooperation was made with the local hospital’s Physical Therapy department.

**Summary of Results:** Initial focus groups participants in the initial program design and addressing their concerns at the forefront was essential to success. Identified infrastructure concerns relating to sidewalks, crosswalks, and facilities, were directed to the city council. Proposals were made to involve area youth programs in cleaning a local walking trail. Interest and perceived benefits of the program were demonstrated by a 224% increase in attendance from discussion group (49) to seminar attendance (110). Forty “practical exercise” packets were distributed. A report summarizing the research and senior concerns was distributed to local city and healthcare leaders to guide future program development.

**Conclusions:** The benefits of increasing physical activity for seniors are significant. The success rate of senior fitness programs is greatly improved by establishing a community-wide approach. Programs are best initiated after surveying for concerns of the participants, tailoring the program to address those concerns, and then educating participants prior to program initiation. Community interest and education will be enhanced.

189 AN ALTERNATIVE SAFETY NET TO THE EMERGENCY ROOM: A FREE MEDICAL CLINIC FOR THE HOMELESS IN SPOKANE, WASHINGTON

Inaba CS University of Washington School of Medicine, Seattle, WA.

**Purpose of Study:** Community free clinics offer healthcare services to a growing disenfranchised population that might otherwise use the emergency department for costly treatment of nonurgent medical issues. Using as a model the House of Charity (HOC) Outreach Clinic in Spokane, Washington, this study seeks to explore how a local free clinic can prevent unnecessary ER visits by providing free outpatient care to the underserved.

**Methods Used:** Patients seeking free medical care at the HOC were surveyed by questionnaire and chart review. Data collected include where patients would seek care if the HOC were unavailable, clinic and ER usage, health status, housing status, insurance coverage, sociodemographics, chronic illnesses, and reasons for visit and treatments received at the HOC.

**Summary of Results:** Preliminary results based on 26 surveys collected between June and August 2010 indicated that 56% of respondents would wait to receive treatment at the HOC free clinic instead of using the emergency department (28%) if the HOC were temporarily unavailable. 50% versus 31% indicated that they use the HOC clinic instead of the ER, respectively, as their primary source of medical care. 65% had no insurance and 50% had been homeless for over six months. Reasons for visit included primarily medication refills for chronic medical conditions.

**Conclusions:** The HOC Outreach Clinic serves as a primary source of medical care for disenfranchised individuals who might otherwise seek expensive ER treatment for chronic health issues that can be addressed in the outpatient setting. Funding used to cover the high cost of nonurgent hospital treatment might be better applied toward the support of free clinics that provide similar treatment at lesser cost.

190 SOCIODEMOGRAPHIC PREDICTORS OF TRAVEL DISTANCE TO A FREE CLINIC IN LOS ANGELES

Kim MJ¹, Ra H¹, Han YK¹, Jo A², ¹David Geffen School of Medicine at UCLA, Los Angeles, CA; ²David Geffen School of Medicine at UCLA, Los Angeles, CA and ²College of Letters and Science, UCLA, Los Angeles, CA.

**Purpose of Study:** Patients often seek health services from free clinics, sometimes traveling inconvenient distances to do so. We sought to understand the relationship between travel distance and various sociodemographic factors among patients accessing the UCLA-Korean Resource Center Community Health Center (UK-CHC).

**Methods Used:** Chart reviews were conducted on 145 patients seen at UK-CHC from August 2008 to March 2010. Clinic staff calculated individ-ual patients’ travel distance from place of residence to the clinic using Google Maps. Using STATA 9.1 software, we examined the relationship between distance traveled and the following variables: age, gender, insurance status, employment status, language preference, education, and proportion of life spent in the U.S.

**Summary of Results:** On average, patients traveled 11.4 miles to reach the clinic (range: 0–73.8 miles). The average length of U.S. residence was
Among the variables examined, lack of health insurance was the strongest predictor of the distance of travel patients in seeking health services at UK-CHC. This suggests the importance of taking travel distance into consideration when referring patients to other sites that can provide more comprehensive health services. Additionally, our results point to the pressing need for the expansion of health insurance to those who lack it.

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COLLABORATION IN SUBSTANCE ABUSE PREVENTION
Marvinsmith BD University of Washington School of Medicine, Seattle, WA.
Purpose of Study: Within the Confederated Salish and Kootenai Tribes (CSKT) of Montana, there is a significant need for substance abuse prevention (SAP) programs. Tribal Health and Human Services (THHS) is a comprehensive network of health care providers available to the CSKT community. Despite a variety of social programs managed by the Tribes, no youth SAP program exists. The purpose of this project was to provide new information and foster collaboration in SAP among the care providers of the tribal community.
Methods Used: Consultation and observation with the primary care providers and behavioral health staff members of THHS demonstrated the need for SAP in the community. All providers confirmed little collaboration between primary care and behavioral health. A literature review was performed to garner new information on SAP in tribal communities, and a presentation on these strategies was created. All studies examined were published within the past two years (since 2008). The presentation was given at a regularly scheduled meeting for the clinicians of THHS. The behavioral health staff was invited to attend.
Summary of Results: The literature revealed new efforts in SAP in tribal communities. The most successful were in Community-Based Participatory Research (CBPR). Prevention strategies, evidence-based practice guidelines, and a detailed explanation of CBPR were presented. A list of research and academic institutions focused on SAP work with tribal communities was included. A reference list of strategies reviewed, including point of contact, was provided. Ten staff members attended the presentation, including representatives from clinicians, behavioral health, and administration. A twenty-minute discussion among the providers followed the presentation. All attendees received a handout. The director of behavioral health requested an electronic copy of the presentation.
Conclusions: The providers of THHS are a crucial rallying point in the development of a youth SAP program due to their interaction with patients as well as recognition from the relevant authorities within the Tribes. This educational presentation provided a forum for inter-professional collaboration as well as a basis for developing an indispensable program.

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ADDRESSING POSTPARTUM TESTING OF GESTATIONAL DIABETES MELLITUS IN OTHELO, WA
Morgan K University of Washington School of Medicine, Seattle, WA.
Purpose of Study: The incidence of gestational diabetes mellitus (GDM), or diabetes affecting pregnant women, is on the rise in the US. The Columbia Basin Health Association including the Othello Family Clinic sees a large population of pregnant women, the majority of which are Hispanic. Minority populations have a higher risk of developing postpartum diabetes if they have been diagnosed with GDM. Yet there is no system in place to remind providers to perform postpartum retesting and as a result many women are not retested. The purpose of this project was to increase provider and staff awareness of postpartum GDM screening guidelines and to discuss ways to increase the number of women retested for GDM at their postpartum visit.
Methods Used: Discussions with staff and observation of patient encounters were utilized to determine the health issues in Othello and the patient group that was to be the focus of the project (recently postpartum women diagnosed with GDM). Clinic quality managers provided clinic statistics on the number of women diagnosed with GDM in 2009, the number of these women retested postpartum, and the number of GDM women that developed postpartum diabetes. A literature review was performed to find the current guidelines for postpartum GDM testing and to find studies in which solutions to low postpartum testing were assessed. A presentation was given at the monthly High Risk OB meeting to increase the awareness of postpartum GDM testing guidelines and the clinic’s current state of retesting.
Summary of Results: A presentation on the postpartum testing of GDM was given to a group of twenty-three CBHA providers, hospital nurses, maternal services staff, and case managers at a monthly High Risk OB meeting. The presentation ended with a discussion of the obstacles to retesting and the future steps needed to increase postpartum testing numbers. A possible system for generating reminder recalls to be sent to both providers and patients was discussed.
Conclusions: Providers and staff understand the need to improve postpartum testing of GDM, especially with the Hispanic population they serve. Obstacles to retesting include provider forgetfulness and lack of postpartum follow up care. By increasing awareness of the current retesting status and the current guidelines for postpartum testing of GDM, we can increase the quality of postpartum care of women with GDM.

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CREATION OF A SUPPORT GROUP FOR FAMILIES OF DRUG AND ALCOHOL ABUSERS
Naderi R University of Washington, Seattle, WA.
Purpose of Study: Alcoholism and drug abuse are prevalent through all age groups in the geographically isolated community of Forks, WA. Forks and the surrounding Native American reservations experience high rates of poverty and unemployment as well as inadequate activities for teenaged populations. Though rehabilitation services are available for the substance abusers, there are very few resources available for family and close friends of abusers. The objective of this community health project is to initiate dialogue among the population for the establishment of a support group for family members of substance abusers.
Methods Used: A cross-disciplinary group of healthcare providers was interviewed to identify and verify the need for a family recovery resource. Educational materials from the only available family support program, Al-Anon, were compiled and reproduced for more widespread use. A literature review validated the significance of family support groups in the recovery of a substance abuser. An in-depth analysis of the nationally predominant twelve-step recovery programs was performed. A community meeting was organized for families and friends of drug and alcohol abusers, to discuss the possibility of a new family support system. A publicity campaign was initiated to distribute flyers among local businesses and at chemical dependency centers on Indian reservations. It was arranged for Families Anonymous meetings to be held at least for a month following this meeting.
Summary of Results: Health care providers in the area, especially those working with patients on pain management regimens, supported the effort by informing patients and families about the meeting. 15 people attended the meeting and received handouts from Al-Anon, Families Anonymous, and other 12-step family support groups. There was an open discussion about the community’s response to the support group. Research findings were presented, and guest speakers reinforced the effectiveness of family support groups in the recovery of the addict.
Conclusions: People learned more about Al-Anon and other resources available in the area. Attendees were enthusiastic about the initiation of a Families Anonymous chapter. There was discussion of the creation of an Al-ateen chapter for children living in homes with alcoholics. In summation, the campaign was well embraced by the community.

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PROMOTING CHILDREN’S ORAL HEALTH IN SHELTON, WASHINGTON
Parker M University of Washington, Seattle, WA.
Purpose of Study: One year olds in Washington are five times as likely to have cavities than average American one-year-olds, and other age groups show a higher rate as well. This problem is apparent in Shelton, where, like many of Washington’s rural towns, the water supply is not fluoridated.
Cavities are the most prevalent childhood health problem, and are preventable with good hygiene. Pediatricians can play a major role in the prevention of dental disease by educating parents about oral hygiene and dental visits for children and babies. The purpose of this project was to increase families' knowledge about preventing dental decay.

Methods Used: Clinical observations and conversations with clinicians helped identify causes of childhood caries. A literature review was conducted in order to find educational materials for families, as well as recommendations for counseling parents about oral hygiene and dental care. A family oral health education table was set up at a community fair, and included interactive materials and supplies from the Department of Health. A brief presentation was given to the providers at Oakland Bay Pediatrics about the effectiveness of oral health counseling by pediatricians, and recommended educational points. Oral health posters in English and Spanish were ordered and installed in exam rooms.

Summary of Results: Twenty-five children participated at the community fair education table. Several parents said that they did not know that children should go to the dentist at age 1, and were given educational pamphlets. The providers at Oakland Bay were very receptive to the information presented, and were encouraged by the evidence for the effect of parent counseling on children's rates of cavities. The providers agreed that more materials were needed in exam rooms, and are considering putting a reminder in the EMR to counsel about oral health.

Conclusions: Childhood tooth decay is a complex problem that should be targeted through public health policy, education, and social programs promoting access to quality health care and healthy foods. Parents need help learning about existing resources and what they can do to prevent dental decay in their young children. The providers at Oakland Bay are concerned about the high rate of dental disease in their patient population, and are increasing their knowledge and resources to address this problem.

195 IMPROVING IMMUNIZATION ADHERENCE IN THE COMMUNITY OF SHOSHONE, IDAHO

Rohrbach M University of Washington School of Medicine, Seattle, WA.

Purpose of Study: Reports from America's Health Rankings indicate that Idaho ranked 49th in the nation regarding immunization coverage for children ages 19 to 35 months in 2009. Idaho recorded a 65.9 percent coverage, falling nearly 20 percent below the nation's top state. The focus of this intervention was to identify barriers of adherence and initiate time-efficient strategies for improving immunization rates within the rural community of Shoshone, Idaho.

Methods Used: To identify the healthcare needs in the community, efforts involved questioning social workers, health educators and clinicians. After identifying an area of concern, a literature review led to the assembly of a vaccine information packet (VIP) designed to address the barriers of adherence identified by recent studies. The most common barriers investigated discovered included: parents' unanswered concerns; a lack of education; a lack of direction; poor access to care; and various socioeconomic factors. The initial intervention site in Shoshone utilized an established event known as the "lunch in the park" program, which provides free lunches for children, to serve as the introduction of VIP use. Additional VIPs were also supplied to the nurse heading immunizations at the Shoshone Family Medical Center in anticipation of future use, both in clinical and public settings.

Summary of Results: During the intervention, parental attendance approached 30 individuals and the inadequate vaccination rates in Idaho were brought to the attention of more than a dozen parents. Parents' questions and concerns were addressed and nine VIPs were distributed, were brought to the attention of more than a dozen parents. Parents' questions and concerns were addressed and nine VIPs were distributed, and were encouraged by the evidence for the effect of parent counseling on children's rates of cavities. The providers at Oakland Bay are concerned about the high rate of dental disease in their patient population, and are increasing their knowledge and resources to address this problem.

196 PSYCHOSOCIAL MEDIATORS TO PHYSICAL ACTIVITY DURING THE PERINATAL PERIOD: A SYSTEMATIC REVIEW OF THE LITERATURE

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Purpose of Study: Less than half of US women reported regular exercise during pregnancy and 46 percent of normal weight women, 46 percent of obese women, and 59 percent of overweight women gain in excess of the recommended gestational weight advised by the Institute of Medicine. This evidence suggests that a sedentary lifestyle contributes to excessive gestational weight gain, which identifies a need for effective intervention strategies that target mediators to physical activity (PA) in order to increase activity levels in perinatal women. The purpose of this study is to critically review prospective and intervention studies identifying potential mediators to PA during the perinatal period.

Methods Used: PubMed, MEDLINE, CINAHIL, and PsychINFO were searched for prospective studies that examined potential mediators to PA, as well as any intervention studies that aimed to increased PA levels during pregnancy up to two years postpartum. Fourteen prospective studies and 7 intervention studies were selected that met the inclusion criteria of this review. Articles were reviewed and discussed according to the population, study design, mediators examined, measured outcomes, results, conclusions and limitations of the studies.

Summary of Results: The prospective studies identified several significant correlates of PA during the perinatal period including exercise and barrier self-efficacy, social support, prepregnancy exercise behavior, safety concerns, and perceived stress. Three intervention studies significantly increased PA levels, three did not achieve significant increases in activity, and one study performed an intervention to alter the potential mediators to PA, but did not measure activity levels.

Conclusions: The most commonly reported significant predictors of PA during the perinatal period in this review were self-efficacy and prepregnancy exercise behavior. Several intervention studies followed theoretical frameworks and targeted various mediators to PA. However, very few mediators were directly measured to determine the efficacy of the interventions in altering the mediators and PA levels. Further research is needed to establish significant mediators in the perinatal period, as well as effectively alter these mediators in intervention programs.
Conclusions: ACE screening for pregnant women was implemented at Olympic Primary Care, the main providers of prenatal care in the area. This project builds on established methods in the literature and work that had been done by county public health leaders; bridging a gap between the efforts of JCPH and OPC. Pregnant women who have the highest ACE scores can be identified and referred to appropriate mental health services to help stop intergenerational transmission of trauma.

Western Student Medical Research Forum
Student Session III - Adolescent Medicine, Pediatrics, and Neonatal
8:30 AM Friday, January 28, 2011

198 WOMEN INFANTS AND CHILDREN (WIC) POLICY CHANGE: EFFECTS ON FRUIT AND VEGETABLE CONSUMPTION AMONG LOW-INCOME CHILDREN NEW MEXICO
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Purpose of Study: There is growing recognition of the increased need for consumption of currently suboptimal levels of fruits and vegetables by children. Intake is known to protect against chronic disease but low-income children are at risk for decreased intake. Since the 1960’s WIC has offered supplemental nutrition packages. The IOM recently reviewed WIC’s original packages and found the need for improvement. One recommendation was to increase funding for fruit and vegetables. As of October 2009 under the new packages, participants will receive monthly vouchers for purchase of fruits and vegetables. We wanted to investigate if the WIC policy change allows participants to increase fruit and vegetable consumption as measured by servings/day and if participants meet USDA guidelines for fruit and vegetable intake.

Methods Used: Our study is an ancillary study to the Child Health Initiative for Lifelong Eating and Exercise project, a NIH funded study focused on obesity prevention and intervention. The participants were children ages 3–5 from the reference arm of the intervention study. To examine the effects of policy change we added questions to the parent interview to assess participation in WIC both pre/post policy change. To assess consumption we used a 24-h dietary recall and food frequency questionnaire. A binary indicator of whether reported intake was > USDA guidelines (X servings for vegetables, Y servings for fruit) was calculated. Summary means and standard deviations were calculated for intake variables and relative frequencies were used to summarize the intake goal variables.

Summary of Results: We identified 130 eligible participants during the pre-policy period and 88 for the post-policy. Pre-policy group reported mean fruit servings 1.57, and mean vegetable servings 1.34. Post-policy group reported mean fruit servings 2.19±1.02, and mean vegetable servings 1.71±1.57. Relative frequency of servings meeting USDA guidelines goal for fruit intake pre-policy was 51% and post-policy change was 55% and for vegetable intake 8% and 15% respectively.

Conclusions: The WIC policy change showed minimal increase in fruit and vegetable consumption among low-income children. Children in the study did not meet USDA guidelines for number of servings/day.

199 CHARACTERIZING THE TRIGGERS OF PEDIATRIC ASTHMA IN A RURAL ENVIRONMENT
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Purpose of Study: Asthma is an important pediatric health concern in the US, affecting approximately one in 12 children. In the primarily Latino agricultural community of Yakima Valley, Washington, pediatric asthma has been established as a top health concern by surveys of the community and health care workers. While asthma triggers have been well characterized in urban settings, there has been little focus on rural pediatric asthma, particularly among immigrant farm working families. This study sought to characterize asthma in a cohort of 41 children who participate in the Yakima Valley Farm Workers Clinic Asthma Project. We hypothesized that the role of ambient environmental exposures and established indoor triggers in a rural community might differ from observations in urban inner-city children with asthma.

Methods Used: Bilingual interviewers conducted a questionnaire on residential, agricultural, and other ambient exposures. We conducted skin prick testing for inhalant allergens, exhaled nitric oxide measurements, and spirometric measurements on all subjects.

Summary of Results: The most frequently reported triggers of asthma included: respiratory infection (92.7%); exercise (80.5%); pollen (68.3%); household dust (68.3%) and cold weather (68.3%). Among agricultural factors, participants reported triggering due to cut grass (41.5%); crop sprays (34.1%); grain dust (29.3%); livestock (26.8%); and hops fields (17.1%). Participants reported occupational exposures, with 35.0% reporting an adult in the household exposed to dusts, gases, fumes, chemicals, or strong odors, and 73.7% reporting that the child is regularly exposed when accompanying a parent to work. In total, 17.1% said that work exposures contribute to the child’s asthma. Questions were asked to determine environmental exposures near to the home: 39.0% reported close proximity (< ½ mile) to crop farms; 22.0% to farms raising animals; 41.5% to major roads with heavy traffic; and 46.3% to dusty roads. Skin prick testing of 22 allergens in the region demonstrated that 75.6% of subjects are atopic and 24.4% are non-atopic.

Conclusions: This study suggests that children in a rural, agricultural setting may face unique environmental triggers of asthma. Intervention strategies derived from urban-based studies may require modification to best serve this population.

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37.1% of high school students report having tried cigarettes. 11.7% of high school students smoked three or more days in the last month and 25% used smokeless tobacco. This project aimed to increase kids’ interest in sports participation, specifically basketball, as a means of promoting healthy habits and preventing tobacco use.

**Methods Used:** Buffalo’s public health needs were informally assessed through discussions with various agencies and community members. With tobacco use a recurring concern, the Buffalo 2009 Youth Risk Behavior Survey was obtained and a critical review of the literature performed. This showed sports participation correlates with decreased tobacco use and sports teams are prime vehicles for curricula promoting healthy lifestyles. The project partnered with the local YMCA to utilize gym space, marketing materials, and scholarships. Anti-tobacco curriculum ideas were solicited from tobacco cessation leaders, respiratory therapists, and online resources. A basketball camp itinerary was created with professional support.

**Summary of Results:** A three-day basketball camp with daily tobacco education activities engaged 15 boys and girls ages 6 through 12. Tobacco curriculum included peer led activities, hands on experiments, an interactive talk with a juvenile drug probation officer, and a presentation by a teen-age nicotine addict. After active participation, feedback from campers indicated they received the message being shared. All campers articulated their pledge to be tobacco free on a camp poster. Parents reported campers came home and shared their newfound knowledge and brochures. Six participants reported this was their first sports camp and all asked when the next one would be. These inquiries were suggestive of future interest in sports participation.

**Conclusions:** Community anti-tobacco campaigns previously existed in Buffalo. None of these, however, have focused on sports involvement as a means of prevention. This project elucidated to community leaders the viability of this format for transmitting the anti-tobacco message. Children need an outlet for their energy and an introduction to sports supports a lifetime of healthy habit development, camaraderie, and positive peer support networks.

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**202 VIOLENCE IN THE COMMUNITY: A COMPREHENSIVE CURRICULUM TO PRODUCE YOUTH PEER-ADVOCATES FOR VICTIMS OF VIOLENCE**

Hunziker A University of Washington, Seattle, WA

**Purpose of Study:** Providers at the Rainier Park Medical Clinic identified violence as a significant health risk in the community. The literature indicates that inter-city youth, aged 12–15, will encounter violence personally or secondarily in their lives. The curriculum to produce youth peer-advocates in the Rainier Valley neighborhood of Seattle, Washington was developed to increase these youths’ ability to recognize violence, to prevent it, and to access community resources to treat it.

**Methods Used:** Youth members from the Rainier Valley Boys & Girls Club participated in the 5-day, 10-hour course. The curriculum incorporated information about violence toward self and others from the professional literature. Education was discussion-based to help students recognize the emotional and physical health implications of violence. Discussions led by community leaders included definitions of violence, how to identify violence, the health effects of violence on a personal and community level, how to access evidence-based literature electronic databases, how to access community resources, how to prevent violence, how to communicate with victims of violence and how to direct them to the appropriate resources for intervention and treatment.

**Summary of Results:** Four of the five youth in the program demonstrated their understanding of violence, how to recognize it’s effects, how to use personal coping skills, how they might intervene, and how to access resources. These students earned Proficiency in Peer-Advocacy certificates. All of the youth received service learning hours to apply to their high school graduation requirements and gift cards donated by the community partners. Copies of the curriculum were requested by community partners in order to repeat the peer-advocacy training.

**Conclusions:** This age group is interested and can learn in an interactive, fast-paced community based program. The incentive of gaining service hours and gift cards was important for registering participants but did not appear to impact student involvement. Partnering with the community was key to success and offered a forum for collaboration around youth violence.

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**203 INCREASING ACCESS TO ADOLESCENT REPRODUCTIVE HEALTH SERVICES ON PRINCE OF WALES ISLAND, ALASKA**

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**Purpose of Study:** Adolescents on Prince of Wales Island (POW) face multiple barriers to accessing reproductive health services. Barriers include great distances between island communities, a perceived lack of confidentiality and absence of comprehensive sexual education in schools. Native teens are more likely to engage in sexual activity and less likely to use contraceptive methods than non-Native teens. As a result, Natives experience a disproportionately high rate of STDs. Alaska Natives make up 18% of the state’s population, yet they account for 46% of Chlamydia cases. This intervention aims to reduce STD rates through education and increased access to preventive services.

**Methods Used:** Interviews were conducted with health care providers, tribal leaders and teens in order to identify barriers to adolescent services. Confidentiality concerns, geographic barriers and lack of education were identified as factors adversely affecting adolescent health outcomes. A literature review was conducted to determine intra-ethnic health risks and develop culturally-appropriate health services. Teen clinics were held in the Native villages of Klawock and Hydaburg, offering education, contraceptive services and STD screening. A POW Youth Resource Guide was developed to increase awareness and utilization of existing non-profit and tribal programs.

**Summary of Results:** Teen clinics were held in the Tlingit village of Klawock and the Haida village of Hydaburg, increasing access to reproductive health services. Teen Resource Guides were distributed at various sites around the island.

**Conclusions:** Health care providers and community leaders of POW recognize the need for comprehensive sexual education and preventive services. However, some community members still remain resistant to such efforts. Residents may respond more positively if interventions come from within their village, rather than from outside health care providers. It is recommended that local area health clinics work with tribal leaders and community members to identify possible educators within the village. It is also suggested that schools incorporate comprehensive sexual education into their curriculum, rather than solely abstinence-based programs in order to promote open dialogue about sexual activity and preventive practices.

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**204 ALCOHOL ABUSE PREVENTION AMONG ADOLESCENTS IN COTTONWOOD, ID**

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**Purpose of Study:** Alcohol is the most commonly used drug among rural adolescents in the United States. In Cottonwood, a small farming community, 30% of the population is under 18 years old. Early initiation of alcohol consumption greatly increases the probability of alcohol-related problems later in life, thus the alcohol abuse prevention program was created to target adolescents. The goal was to provide an interactive discussion session with students to encourage the development of critical thinking and social coping skills as well as provide education about the physical effects and dangers of alcohol abuse.

**Methods Used:** The need for alcohol abuse prevention was determined after questioning community members and St. Mary’s Clinic staff. A review of the literature revealed effective methods of adolescent alcohol and substance abuse education, including how to design an interactive session that encouraged the development of refusal skills. Sports physical nights held in Cottonwood, Nezperce, and Kamiah by the St. Mary’s Clinic were utilized to achieve contact with a target population of 6–12th graders. Each adolescent participated in small-group or one-on-one discussions about alcohol abuse. Discussions included tactics for handling peer pressure, the adolescent’s own experiences with alcohol, and education about the physical effects of alcohol consumption on health and athletic ability. Fatal vision goggles were obtained from the Moscow Police Department to help create an interactive environment. A brochure was developed to reinforce the discussion and answer additional questions.

**Summary of Results:** Approximately 138 adolescents participated in the alcohol abuse prevention discussions at the sports physical nights in Cottonwood, Nezperce, and Kamiah. Many of the students had not previously...
considered the session material and subsequently benefited both from discussing peer pressure situations and from learning more about the physical effects of alcohol.

Conclusions: An interactive discussion was a helpful format for alcohol abuse prevention education among adolescents. It encouraged the students to consider the physical effects of alcohol consumption and to practice refusal skills. The fatal vision goggles were an effective method of initiating discussion. The prevention program could be retained and even expanded upon for future education.

205 LIVER EXPRESSION OF IGF-1 IS NOT DECREASED IN PRETERM LAMBS VENTILATED FOR 3 DAYS AND WEANED FROM VENTILATION

Block C, Dahl M, Von Der Ahe N, McCoy MJ, Wang Z, Dong L, McKnight R, Null D, Yoder BA, Lane RH, Albertine K University of Utah, Salt Lake City, UT.

Purpose of Study: Growth is modulated by insulin-like growth factor-1 (IGF-1). Serum levels of IGF-1 are low in preterm infants with neonatal CLD. Hepatic expression of IGF-1 determines serum levels. We showed that 21d of mechanical ventilation for 3d will lead to long-term decrease in IGF-1 and its upstream signaling molecules Janus kinase 2 (JAK2) and signal transducers and activators of transcription 5b (STAT5b).

Methods Used: Preterm lambs (~128d gestation; term ~150d; n=4), treated with antenatal steroids and postnatal surfactant, were managed by MV for 3d, weaned using high-frequency nasal ventilation for 3d, and then weaned from ventilation and recovered for 10 wk (equivalent to ~2 y postnatal life in humans). Liver tissue was analyzed by quantitative real time RT-PCR (normalized GADPH mRNA expression) and immunoblot (normalized MemCode). Control lambs were delivered at term and lived 8 wk (n=4).

Summary of Results: At the end of the 11wk study period, the weight of preterm weaned lambs and control lambs was the same (21:6 vs 22:6 Kg, respectively). Liver tissue from weaned preterm lambs and control lambs had the same expression of IGF-1 mRNA (0.7±0.1 vs 0.6±0.1, respectively) and STAT5b mRNA (0.3±0.1 vs 0.3±0.01, respectively). Likewise, weaned preterm lambs and control lambs had the same relative abundance of STAT5b protein (10:8 vs 10:3, respectively) and JAK2 protein (77±6 vs 66±11, respectively).

Conclusions: Preterm birth followed by 3d of MV and subsequent weaning from ventilation support did not cause sustained growth delay or liver expression of IGF-1 and its upstream signaling molecules. Despite this sparing effect on the liver of preterm weaned preterm lambs, the lungs of the same preterm weaned lambs had sustained delay in alveolar formation that is characteristic of neonatal CLD. (J Invest Med 57:223, 2010). (HL62875, HL56401, HD41075).

206 IL-17 PRODUCTION BY CORD BLOOD MONONUCLEAR CELLS IS DIMINISHED COMPARED TO ADULTS

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Purpose of Study: Human neonates are uniquely susceptible to severe and overwhelming bacterial and fungal infections. Defective neonatal polymorphonuclear leukocyte (PMN) activation and movement contribute to this increased susceptibility to infection. T helper 1 (Th1) lymphocytes produce interleukin 17 (IL-17), which is thought to act on fibroblasts and endothelial cells to recruit neutrophils into local areas of microbial invasion. Previously, we have demonstrated that cord blood mixed mononuclear cells have defective production of the Th1 cytokines IL-18 and IFN-γ.

Here we examined cord blood mixed mononuclear cell production of IL-17, a cytokine critical in the host response to bacterial and fungal infections, and compared the results with that of mononuclear cells from adults.

Methods Used: Whole blood was collected from healthy adults and umbilical cord blood from healthy term deliveries. Mixed mononuclear cells (MMCs) were isolated on Ficoll and stimulated with phytohemagglutinin (PHA), a potent mitogen, and then incubated for 24 hours in tissue culture medium. IL-17 production was measured using a new, in-house developed assay utilizing Luminox multianalyte technology.

Summary of Results: Mononuclear cells from newborn infants (0.10 pg/mL +/- 0.1) produced significantly less IL17 than mononuclear cells from adults (309 +/- 2.94 SEM pg/mL, p<0.02).

Conclusions: IL17 has a profound effect on the immune response to bacterial and fungal infections. For instance, IL17 production is profoundly deficient in autosomal dominant Hyper IgE or Job syndrome in which patients suffer repeated bacterial and candida infections. The present report is the first of a deficiency in IL17 production by neonatal mononuclear cells, which likely contributes significantly to the increased susceptibility of human infants to microbial infections.

207 GLUCOSE TRANSPORTER mRNA EXPRESSION IN PLACENTAS OF GROWTH-RESTRICTED FETUSES

Kim JE1, Cho J1, Shin B2, Devasakar S3, Janzen C 1,2, David Geffen School of Medicine at UCLA, Los Angeles, CA and 3David Geffen School of Medicine at UCLA, Los Angeles, CA.

Purpose of Study: Intrauterine growth restriction (IUGR) is the inability of a fetus to achieve proper growth. IUGR is associated with increased perinatal morbidity and mortality. Glucose is an essential nutrient for fetal growth and is transported from mother-to-fetus across the placenta. Various facilitated glucose transporters (GLUTs) are present in the placenta, including the isoforms GLUT1, GLUT3, and GLUT4. Recent mouse studies demonstrated that homozygous GLUT3 null mutations resulted in early pregnancy loss, while GLUT3 heterozygotes exhibited fetal growth restriction. The objective of this study was to determine if mRNA expression of GLUT1, GLUT3, and GLUT4 in human placenta changes with IUGR.

Methods Used: In this prospective study, human placenta were collected at time of delivery. GLUT1, GLUT3, and GLUT4 mRNA expression in the maternal and fetal regions of each placenta was determined by quantitative real-time PCR. Results were quantified by the comparative CT method and statistical analysis was conducted using the Student’s t-test.

Summary of Results: Placentas were collected from control groups (n=3, birth weight percentile 65.3±28.0) and IUGR groups (n=7, birth weight percentile 4.7±3.1). qRT-PCR results showed no significant difference in mRNA expression levels of GLUT1, GLUT3, or GLUT4.

Conclusions: In this study, significant differences were not found in the mRNA expression of glucose transporters in human placentas affected by IUGR. However, there appeared to be a trend towards increased GLUT1 and GLUT3 expression with IUGR. Additional studies with increased sample sizes are necessary to determine if the observed differences are significant.

208 PATIENT/FAMILY CHARACTERISTICS IN AMERICAN INDIAN CHILD AND ADOLESCENT QUALITY OF CARE RESEARCH

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Purpose of Study: Across the country, both rural and urban American Indian (AI) children and adolescents are at a higher risk than other U.S. ethnic groups for developing mental health problems such as depression, substance abuse, domestic violence, and suicide, and have a disparately high need for mental health services. New tribal healthcare service systems, as well as state and federal agencies (such as Medicaid and the IHS), could highly benefit from a systematic assessment of the quality of the mental health care they provide to Alts. Perhaps the best known framework is that proposed by Donabedian, which organizes the assessment of care into an assessment of its structure, process, and outcome. Additionally, health policy makers, providers, and consumers have recognized that culturally competent care addresses and eliminates racial/ethnic disparities in health care. We propose to introduce a fourth domain, “patient/family characteristics,” to Donabedian’s framework, and to investigate how this domain relates to the other domains of assessment, as well as to cultural appropriateness of care.
Methods Used: Data extracted from medical records of three research sites (with a subsample verified by an independent rater as a quality control mechanism) and interviews with administrators, clinicians, parents, and youth will be analyzed with the aid of qualitative analysis programs such as NVivo, looking for clinician, patient, and family perspectives of treatment in regards to the four examined domains of quality of care.

Summary of Results: Collaboration with representatives from the partner sites and preliminary analysis of data extracted from medical records has defined patient/family characteristics to include sociodemographics, family structure, specific behavioral health difficulties, attitudes towards treatment, and barriers or support to care. Further research will analyze the relationship of these characteristics to structure, process, and outcome.

Conclusions: We hypothesize that the relationship between patient/family characteristics and structure, process, and outcome of mental health services will provide a useful model for subsequent studies in more culturally sensitive AI quality of care research.

Adolescent Medicine and General Pediatrics

Concurrent Session 1:00 PM
Friday, January 28, 2011

209 BICYCLE AND ATV HELMET SAFETY IN MCCALL, IDAHO
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Purpose of Study: McCall is a rural town of about 2,600 people nestled against the Payette National Forest. McCall’s picturesque scenery and access to the outdoors has made it a tourist destination. Because of its proximity to skiing, hiking, biking, and ATV riding, many McCall residents, as well as their children, are outdoor enthusiasts. While helmet use decreases head injury risk in bicycle and ATV crashes by 88% and 64%, respectively, none of McCall’s sporting good stores currently offer helmet safety courses. The purpose of this project was to create an interactive ATV and bicycle helmet program for 6th grade students at Payette Lakes Middle School that addressed barriers to helmet use, basic brain anatomy and deficits seen with brain damage, and proper ATV and bicycle helmet fit.

Methods Used: A literature review was performed to determine prevalence of ATV and bicycle injuries, helmet use, and possible educational interventions. After consulting with the chairman of the school board and the Payette Lakes Middle School health teacher, a bicycle and ATV helmet safety program was developed and presented to 6th grade students. Students were polled about their bike and ATV helmet practices. An activity was performed to simulate falling off a bike or being thrown from an ATV using hard boiled eggs, to represent the brain, wrapped with materials to represent the meninges and skull. Half the eggs were equipped with plastic helmets, and students compared egg damage with and without helmet use. Students were guided through the steps of properly fitting ATV and bicycle helmets and then asked to fix incorrectly placed helmets.

Summary of Results: Sixty-five 6th grade students participated in the bicycle and ATV helmet safety program. Students gave feedback on what they learned with “bike helmets provide 88% protection from head injury” as the most common response. A flyer on proper ATV and bicycle helmet fit was sent home with students to help them educate their parents. The curriculum was given to the 6th grade teachers and the Payette Lakes Medical Clinic to continue the program next year.

Conclusions: Because helmet use is low in middle school kids, an intervention to effect change had to be interactive and meaningful. By connecting brain function with brain damage in ATV and bicycle crashes, the students gained a better understanding of the purpose of helmets.

210 MATERNAL RISKS, COMMUNITY VIOLENCE EXPOSURE AND SELF-REPORTED ASTHMA AMONG CHILDREN IN FOSTER CARE
Hellyer J, Culhane SE, Garrido EF, Petrenko CL, Tausig HN University of Colorado Denver, Aurora, CO.

Purpose of Study: The occurrence of pediatric asthma has been associated with exposure to chronic stress. The goal of this study was to examine whether stress and asthma were associated in a high risk sample. The study examined the relationship between maternal and community risk factors and asthma in a sample of maltreated children placed in foster care.

Methods Used: Interviews were conducted with 355 maltreated children (9–11 years old) who had been court-ordered into foster care within the past year. Measures included youth self-report of asthma and community violence exposure and an index of maternal risk (criminal history, incarceration, alcohol and controlled substance use, domestic violence perpetration, domestic violence victim, mental illness, inadequate housing, history of foster care placement and history of maltreatment) based on data abstracted from child welfare records.

Summary of Results: After controlling for age, gender and ethnicity, maternal risk significantly predicted the presence of asthma (OR = 1.26, 95% CI = 1.04–1.52, p=0.02) over and above the effects of community violence exposure, but community violence exposure did not predict asthma over and above the effects of maternal risk.

Conclusions: Maternal risk factors were significantly associated with the presence of asthma in a maltreated, foster care population. This connection has important implications for clinicians who oversee the care of maltreated children as it may be an indication of which children in this already high-risk group have a greater propensity for physical health issues.

211 INTRATUBULAR GERM CELL NEOPLASIA IN THE PEDIATRIC POPULATION: A CASE REPORT
DeGirolamo K1,2, Masterson J1,2 1University of British Columbia, Vancouver, BC, Canada and 2BC Children’s Hospital, Vancouver, BC, Canada.

Case Report: Testicular cancer, specifically germ cell tumors, are the most common malignancy in young men and are curable in up to 95% of cases, but the incidence is on the rise. (1) The key event in the transformation to malignancy is hypothesized to occur in early adulthood and perhaps the carcinogenic pathway for germ cell tumors begins in fetal life due to maternal estrogen or exposure to environmental estrogens. (2) Intratubular Germ Cell Neoplasia (ITGCN), previously known as Carcinoma in Situ of the testis, is a premalignant condition that can progress to testicular cancer and is associated with testicular germ cell tumors. CIS was first discovered after Skakkebaek biopsied testicles of fertile men who later developed testicular germ cell tumors. (2) These cells are thought to be more susceptible to mutations from changing hormone levels and thus germ cell tumors typically occur after puberty or in patients with sexual development disorders, such as feminization of the testis. (1) This is a rare occurrence in prepubertal children and it would be ideal to know the prognostic implications. We present a case report of a 4-month-old male with undifferentiated ITGCN, and a co-morbid mature teratoma. To the best of our knowledge this is the first case documented of this kind and demonstrates how little is known about this condition and how this specific patient was managed surgically and clinically.


212 LEARN PEDIATRICS: CREATING A WEB-BASED MULTIMEDIA RESOURCE FOR MEDICAL STUDENTS LEARNING PEDIATRIC CLINICAL SKILLS

Purpose of Study: Few non-profit online resources currently exist for teaching medical students pediatric clinical skills. Computer-based education is effective at teaching physical examination in addition to being cost-effective. The objective is to create a free, online resource containing written material, videos and other multimedia formats to assist medical students with learning pediatric clinical skills and approaches to common pediatric presentations. The long-term goal is to establish and maintain an open domain, non-profit website that will enhance the learning of medical students nationally and internationally.
Methods Used: The website, www.learnpediatrics.com, consists of ten pediatric modules: general, respiratory, cardiology, gastroenterology, newborn, genitourinary, neurology, musculoskeletal, hematology/oncology, and endocrinology. Seven of these modules will contain physical exam teaching videos. Medical students write content on a voluntary basis and receive credit for their contributions. Pediatric residents then edit the articles for content and clarity. Video scripts are written by pediatric residents, in consultation with attending staff. All actors involved in the video are volunteers. The University of British Columbia (UBC) Media Production records the narrations, films the clinical teaching videos and performs final edits with a pediatric resident or staff physician. Final written and multimedia materials are posted to the site by students employed by the faculty in work-study arrangements.

Summary of Results: Learn Pediatrics has been successfully transferred to the UBC domain. Completed videos including respiratory, newborn, cardiology and neurology have been posted. The scripts are written for both gastroenterology and genitourinary. All videos and scripts have been edited by content experts. Seventy content topics out of 125 have been written by students and edited by pediatric residents. There is ongoing involvement to complete writing of the content topics.

Conclusions: Learn Pediatrics is a free, web-based resource for medical students learning pediatric clinical skills and is envisioned to become a valuable resource for medical students not only at UBC, but worldwide. Future directions include self-assessment tools and evaluation of the website.

213 SMOKING PREVENTION IN ADOLESCENT GIRLS
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Purpose of Study: Montana—the land of the cowboy—has high rates of tobacco use. Marias Medical Center in Shelby, Montana—a town of approximately 3,500 residents—sees adolescents who feel cultural pressures to smoke cigarettes almost daily. Marlboro’s 2007 “Camel No. 9” advertising campaign suggests that adolescent girls are actively being targeted for cigarette advertising. The purpose of this project was to promote evidence-based, age and gender appropriate smoking prevention strategies.

Methods Used: Discussions with family doctors, nurses, Toole County public health department employees, and observing clinic visits were methods used to identify non-smoking adolescents as the community project focus. A grassroots approach was taken to reach local youth, including the submission of a letter to the local paper and a day spent at the Shelby youth swim meet encouraging swimmers to make a tobacco-free pledge. To develop local awareness of the harms of smoking, efforts were combined with the Toole Country public health department at their open house. Youth-friendly “props” were used to facilitate meaningful discussions with pre-teens. A literature review was performed for validation of the recommendation of nosmokingroom.org, an educational “game,” and guidance in choosing methods and content of communication with the target group.

Summary of Results: A letter to readers of the Shelby Promoter, the local weekly newspaper, encouraged local adolescents seeking tobacco information to take advantage of local physicians and internet resources such as nosmokingroom.org, an anti-tobacco, interactive website for girls ages 8–12 designed by pediatricians. At a youth swim meet, over thirty adolescents from Shelby and surrounding towns each pledged to remain tobacco-free by signing a shirt. T-shirts, frisbees, and water bottles featuring anti-smoking messages were given to swimmers. Further tobacco information was provided to residents at the health department’s open house.

Conclusions: Low evidence-based age and gender appropriate strategies are needed to prevent smoking in adolescents. Interactive methods such as collecting pledge signatures and game-like educational tools online add to the tools of smoking prevention. However, more evidence is needed to support educational gaming as an effective method of smoking prevention in adolescents before the strategy can be applied more globally.

214 BRIGHTER SMILES: A COMPARISON OF GROWTH IN RURAL UGANDAN CHILDREN TO WHO STANDARDS
Zhang M1, Ashaba B2, Berg T1, Choi W, Kasangaki A2, Musinguzi N2, Meredith C1, Nambatya B2, Nyairo S2, Cannon W1, Macnab A1 University of British Columbia, Vancouver, BC, Canada and 2Makerere University, Kampala, Uganda.

Purpose of Study: Patterns of growth amongst a nation’s children most accurately reflect the nutritional and health status of its population and predict the health of future generations.

Methods Used: Children enrolled in “Brighter Smiles”, a collaborative health-promotion program, were evaluated for parameters of growth. Height, weight, BMI and health data via questionnaire were obtained for children at a primary school in rural Uganda to compare their status to the WHO Child Growth Standard (2006). The WHO Standard uses multiethnic sampling (populations from 6 countries) to capture the genetic variability among continents and generate a single international growth standard. This standard is intended to underscore that differences in nurture, rather than nature, determine disparities in physical growth, and provide a basis for appropriate healthcare policy and intervention.

Summary of Results: 233 children (105 males, 128 females), aged between 5–13 years, were evaluated. Amongst the girls 91% were below the 50th centile for height and 90% for weight. Similarly, amongst the boys 89% and 88% were below the 50th centile for height and weight respectively. A high proportion of these children come from single parent or child-headed families (this community was the epicentre for the AIDS epidemic) and poverty, borderline nutrition, malaria, and parasitic infections are prevalent. Prior research here has also documented that cultural and religious beliefs lead to restricted intake of essential foods, and both low food quality and infectious diseases commonly exacerbate malnutrition.

Conclusions: The finding that such a high percentage of our cohort’s children had height and weight below the 50th centile most probably reflects the impact of local social circumstances, sub-optimal nutrition and infectious disease, and indicates the value and potential for the WHO standards to identify where children’s growth patterns would benefit from interventions to promote appropriate nurture, and improve health and growth potential.

215 DISPARITY IN HEALTH LITERACY BETWEEN ENGLISH SPEAKING AND SPANISH SPEAKING PARENTS OF HOSPITALIZED CHILDREN
McDermott J1,2, Joseph M1,2, Poynter R1 Children’s Hospital Central California, Madera, CA and 2UCSF Fresno Medical Education Program, Fresno, CA.

Purpose of Study: Health literacy is defined as “the degree to which individuals have the capacity to obtain, process, and understand basic health information and services needed to make appropriate health decisions”, according to Healthy People 2010. Low socioeconomic status, lower education levels, and English as a second language are factors that correlate with lower health literacy in adults. However, few have studied disparities in parent health literacy when comparing groups by primary language, an important factor that could adversely affect child health outcomes. We hypothesize a significant difference exists between health literacy rates of English speaking and Spanish speaking parents in a hospital setting.

Methods Used: The study is being conducted in a tertiary children’s hospital in the Central Valley of California. We aim to study 200 parents, 100 English speaking and 100 Spanish speaking. Parents of patients admitted on the inpatient pediatric medical services are being recruited. Health literacy scores are assessed using the “New Vital Sign” tool, a 6 item survey validated in English and Spanish. The same person is administering the surveys to all subjects; a certified Spanish translator is used to administer the survey to Spanish speaking parents. All subjects also complete a brief questionnaire to obtain demographics. Statistical analysis is done using Chi-Square with Fisher’s Exact Test.

Summary of Results: Results of 43 completed surveys from 21 (49%) Spanish-speaking parents and 22 (51%) English speaking parents have been analyzed. Analysis of our initial data shows the possibility of limited literacy in 11 (52%) in Spanish speaking parents compared to 2 (9%) in English speaking parents (p=0.003). Data collection is ongoing and will be completed within two months. Final results will be discussed at the meeting.

Conclusions: Preliminary analysis reveals significantly lower health literacy in Spanish speaking compared to English speaking parents. We expect the same trend to continue in the remaining study population. With the information gained from this study, we plan to educate our medical staff and support services in identifying parents at risk for low health literacy and providing linguistically appropriate resources.
216 KEY ELEMENTS FOR SUCCESSFUL GLOBAL HEALTH EDUCATION ELECTIVES: LESSONS FROM “BRIGHTER SMILES AFRICA”
Zhang M1, Berg T1, Ashaba B2, Berg M2, Choi W1, Meredith C1, Musinguzi N2, Nambatya F2, Nyairo S2, Cannon W1, Kasangaki A1, Macnab A1
1University of British Columbia, Vancouver, BC, Canada and 2Makerere University, Kampala, Uganda.

Purpose of Study: Global health electives have the potential to foster opportunities for service learning and create a platform to educate students on the key determinants of health, and cultural competencies and logistics necessary for successful program delivery. To be effective and relevant to the host country such electives require structure.

Methods Used: We describe the key elements (identified via annual evaluation) central to success of a five year global-health partnership (Brighter Smiles) between the University of British Columbia and Makerere University in Uganda.

Summary of Results: SERVICE DELIVERY - School-based health promotion is an effective model endorsed by WHO. Giving interactive workshops provides knowledge and skills for healthy practices and promotes changes in school health and injury. Control were children with no intervention on a broad range of health and social issues. COMMUNITY-BASED LEARNING - Clinical opportunities and collaborative research/evaluation provide new knowledge. Students learn the priorities and realities of medical care, prevention and social and cultural practices, establish the effectiveness of service delivery programs, and provide feedback and future direction to participating individuals and communities. COLLABORATION - Partnership of teams from different universities with local community leaders during project delivery and workshops generates invaluable dialogue and learning opportunities. Insights into realities and opportunities are gained, and essential skills learned re team-work, communication, and cultural sensitivity. MENTORSHIP - Passion and experience shared by local and visiting faculty is infectious and invaluable. This is an essential component to ensure safe, culturally-relevant and sustainable student experiences.

Conclusions: Global health electives based on the ‘Brighter Smiles’ template should prove to be relevant, and offer effective and appropriate learning for university students.

217 BODY MASS INDEX AND ACUTE INJURY RISK IN CHILDREN AGE 5-17 YEARS
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Purpose of Study: Obesity is an epidemic that affects many Canadian children. Between 1981 and 2001 the prevalence of overweight and obese children in Canada increased by 200–300%(1). In 2004, 18.1% of Canadian children age 2–17 were overweight and 8.2% were obese(1). Obese children have prolonged recovery times and increased morbidity and mortality following an acute injury(2). The relationship between obesity and risk of acute injury has not yet been established but is essential for proper weight loss counseling of youth. Our study investigates the risk of acute injury associated with increasing body mass index (BMI) in children.

Methods Used: This is a case based, case control study with patients’ age 5 to 17 years who presented to BC Children’s Hospital Emergency Department from July 2009 to December 2010. Cases were defined as children who presented in the emergency department with an injury. Controls were children who presented with a complaint who had not sustained an injury in the previous 12 months. Height and weight were measured and BMI categories (underweight, normal, overweight and obese) were assigned according to the CDC classification per age and sex. Descriptive statistics were used to report distribution of participants’ characteristics (BMI, sex, age, physical activity, socioeconomic status). Multivariate logistical regressions estimated the odds of injury occurrence by BMI category adjusted for confounders.

Summary of Results: To date we have screened 500 patients and 394 were enrolled. Although the proportion of male subjects (45%) and BMI category adjusted for confounders.


218 CONCUSSION EVALUATION METHODS AMONG WASHINGTON STATE HIGH SCHOOL FOOTBALL COACHES AND ATHLETIC TRainers
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1University of Washington School of Medicine, Seattle, WA; 2University of Washington Medical Center, Seattle, WA; 3University of Washington Medical Center, Seattle, WA and 4University of Washington Medical Center, Seattle, WA.

Purpose of Study: To determine concussion assessment methods among Washington State high school football coaches and athletic trainers.

Methods Used: A Catalyst WebQ survey link was emailed to varsity head football coaches and athletic trainers from 106/298 Washington State school districts. The survey was voluntary and anonymous. Survey questions inquired about participants’ concussion management training, familiarity with the Zackery Lystedt Law, and standardized tools used for concussion assessment both on-the-field and for follow-up.

Summary of Results: Twenty-eight of 59 respondents (47%) used the Standardized Concussion Assessment Tool 2 (SCAT2) for on-the-field assessment; urban respondents were statistically significantly more likely to use SCAT2 (p=0.05). Neurocognitive testing (NCT) was used by 19/59 (32%) respondents. This was statistically significantly used more commonly by those in urban districts (p=0.001). A neuropsychologist interprets test results for 11/19 (57.9%). There is no statistically significant correlation between years of experience and use of the SCAT2, but those with more than 10 years experience were less likely to use NCT (p=0.01). All participants reported being familiar with the Zackery Lystedt Law, but only 44.1% reported that the law has changed their concussion management.

Conclusions: There are statistically significant differences between SCAT2 and NCT use in urban and rural districts, as well as NCT usage amongst respondents with varying years of experience. Further understanding and identification of barriers limiting identification and management of concussions in high school athletes is crucial to ensure proper return to play and to prevent serious permanent injury. Additional education is necessary to ensure that athletic trainers and coaches are aware of current recommendations by the International Concussion Conference for the evaluation and management of concussions.

219 ATHLETIC PARTICIPATION AND SUBSTANCE USE IN UNIVERSITY STUDENTS
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Purpose of Study: Athletic participation may protect against substance use in high school students; however, the data is conflicting at the collegiate level. We examined the associations between athletic participation and substance use in university students, and the substance use patterns in team-based and individual-based competitive sports.

Methods Used: US university students (18–26yrs/661M,973F) were recruited through a social networking website to complete an online questionnaire on athletics and health. Competitive athletes (CA) participated in a competitive sport within the past year; recreational athletes (RA) exercised independently and not as part of a team; sedentary students (SS) did neither. CA were further divided into team-based and individual-based sports. Prevalence of alcohol use, binge drinking, cigarette smoking, other tobacco use, marijuana use, and other drug use were compared between groups using chi-squared analyses.

Summary of Results: There were 969 CA (429M,540F;402 team-based, 565 individual-based), 502 RA (157M,344F), and 166 SS (75M,89F). Alcohol was the dominant substance used, with CA and RA drinking and binge drinking more than SS (alcohol: 86.8% vs 88.9% vs 76.3%, p<0.001; binge drinking: 55.5% vs 54.9% vs 39.1%, p<0.001), with the greatest binge drinking among female RA (53.6%) and male CA (63.0%). There were no
An experimental model of abdominal aortic aneurysm

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Purpose of Study: The cytoprotective enzyme, heme oxygenase-1 (HO-1) is expressed in several vascular cell types, including macrophages. We hypothesized that HO-1 attenuates macrophage activation and inhibits abdominal aortic aneurysm (AAA) formation.

Methods Used: Wildtype (WT, HO-1−/−, n=14) or HO-1 heterozygous (Het, HO-1+−, n=13) FVB mice were subjected to aortic elastase perfusion to induce experimental AAAs. AAA progression was monitored weekly by ultrasonography. At D7 and D28 post-AA perfusion, mice were euthanized for histological analyses. Expression of inflammatory cytokines and chemokines was assayed by qRT-PCR in thioglycollate-elicited macrophages from WT and Het mice (n=5 for each group).

Summary of Results: Abdominal aortic diameters were significantly increased in Het compared to WT mice after elastase perfusion. WT mice exhibited a mean aortic diameter increase of 27.9±2.1% at D7 and 50.5±5.3% at D28, whereas, Het mice exhibited a mean increase of 45.0±2.4% (P=0.01) at D7 and 78.7±8.5% (P=0.001) at D28 relative to untreated Het mice. In addition, thioglycollate-elicited peritoneal macrophages from Het mice exhibited increased expression of the proinflammatory cytokines MCP1, TNFa, IL1β, and IL6 (4.7±2.0, 3.1 and 2.3-fold, P<0.05, respectively). Meanwhile, mRNA levels of anti-inflammatory cytokines IL10 and TGFβ1 were reduced in Het mice (0.47± and 0.61-fold, P<0.05, respectively).

Conclusions: In conclusion, induction of HO-1 activity significantly reduces AAA formation in the elastase model of experimental AAA disease. The underlying mechanism appears in part to be mediated by a reduction of the local activation of macrophages in the aneurinal site.
223 ZOTAROLIMUS DRUG ELUTING STENTS IN PERCUTANEOUS REVASULARIZATION COMPARED TO 1ST GENERATION DES IN HIGH RISK PATIENTS WITH LEFT MAIN CORONARY ARTERY DISEASE

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Purpose of Study: Percutaneous coronary intervention (PCI) in patients with unprotected left main (UPLM) coronary artery disease continues to evolve following the results of the SYNTAX trial and other studies demonstrating effective and safe outcomes for patients in the PCI cohort. However, there are limited data on how second generation drug eluting stents (DES) will affect outcomes in high risk patients undergoing PCI compared to first generation DES for UPLM.

Methods Used: We retrospectively reviewed 50 patients with UPLM who underwent PCI with Zotarolimus DES (ZDES) and compared them with 20 patients also with UPLM who underwent PCI with a Sirolimus or Paclitaxel DES (SPDES) during the same time period and followed them for 12 months. IVUS guidance was used in all cases. The primary end-point were major adverse cardiac events (MACE), history of diabetes death and any secondary endpoints were: Target vessel revascularization (TVR), ARC probable stent thrombosis (ST) and contrast use amount as a surrogate for deliverability.

Summary of Results: Among patients undergoing PCI with DES for UPLM, the major cardiac event (MACE) rate at 12 months was 6 vs 25% for SPDES, (p < 0.05). This was accompanied by zero TVR in the ZDES group. There was a 10% TVR in the SPDES group (p<0.05). Neither stent groups had any ARC prob ST. The EuroScore was 7.8 ± 2.2 among all patients, there was no difference between ZDES or SPDES groups. In the ZDES group the contrast used amount was 216.4 ± 101 ml vs 413.4 ± 160 ml in the SPDES group, (p < 0.002).

Conclusions: In high risk patients with PCI for UPLM, use of Zotarolimus DES resulted in a lower 12 month MACE and TVR compared to 1st generation DES. The improved deliverability of the ZDES platform appears to have a clinical impact, not only in outcomes but also in amount of contrast use. Our retrospective review suggests that platform deliverability is an independent predictor of clinical outcomes.

224 IMPACT OF RECENT DEVICE PROCEDURES ON THE INCIDENCE OF CARDIAC DEVICE INFECTION UNDERGOING EXTRACTION

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Purpose of Study: The increased demand of device implants and upgrades based on current guidelines increase the potential for device infections (DI). Each type of procedure (new implant, device upgrade, device change) may potentially impact the development of DI. The aim of this study is to identify if having a cardiac device procedure in the previous year increases the chances of DI compared with patients with no procedures in the previous year. As a secondary end point we investigated if any type of procedure carries a higher risk of DI.

Methods Used: Retrospective analysis of the device extraction procedures (DE) performed at our institutions between January 2000 and December 2009 was conducted. The following data was collected: age of the patient, left ventricular ejection fraction (LVEF), history of diabetes, COPD or Creatinine values > 2 (CR) at the time of DE, type of DI that resulted in DE, type of procedure, if any, performed in the previous year to the DE. Categorical variables were compared with a Chi square method. Data was expressed in mean ±SD for continues variables and N (%) for categorical variables.

Summary of Results: 72 patients underwent DE due to DI. Age 69 ±12.5 years, males 64 (89%), LVEF 58 ± 36%; diabetes 17 (23.9%); COPD 6 (8.5%); CR 6 (8.5%); 42 patients (58.3%) had solely pocket infection and 30 (41.7%) endocarditis. Of the entire cohort, 43 (59.7%) had a procedure performed in the previous year compared with 29(40.3%) with no procedure done (p<0.001). Of the patients who underwent a procedure the most prevalent was device upgrade 21 (29.2%). This showed to be significantly more common than device change (13 (18.1%); p=0.01) and new implants (9 (12.5%); p=0.03). There was no difference between device change and new implants (p=NS).

Conclusions: Device procedures within the previous year carry a significant risk of device system infection. Among such procedures, device upgrade has the highest prevalence, and there is no difference between new implants and device changes. Prospective data will be necessary to confirm our findings.

225 CARDIOMYOPATHY FACTORS IN DUCHENNE MUSCULAR DYSTROPHY

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Purpose of Study: Duchenne Muscular Dystrophy (DMD) is characterized by progressive deterioration of both skeletal and cardiac muscle. This X-linked disorder is caused by mutations in dystrophin, one of the largest genes identified. Cardiomyopathy (CM) is one of the leading causes of death in DMD. CM treatment options are available and therefore early detection is important. Identifying genetic markers associated with early onset CM could predict which patients require early intervention. Steroids have become a widely accepted treatment to preserve skeletal muscle strength for DMD patients, and previous studies have suggested a cardioprotective role for steroids. The two objectives of this DMD study were to investigate: 1) genetic markers of CM and 2) the effect of steroids on CM onset.

Methods Used: Data were collected through the Muscular Dystrophy Surveillance, Tracking and Research Network (MD STARnet), a population-based surveillance program funded by the Centers for Disease Control and Prevention with five participating sites (Arizona, Colorado, Georgia, Iowa, and western New York). Echocardiographic (echo), steroid usage, and genetic data on DMD patients born from 1982-2001 were analyzed. CM was defined via echo parameters of systolic function; Shortening Fraction <28% and/or Ejection Fraction <55%.

Summary of Results: The mean age of CM onset was 14.9 years (n=191). The mean age of steroid initiation was 7.6 years and averaged 61.8 months of treatment (n=90). The age of CM onset had a significant positive correlation (r = .45, p < .001) with the number of months treated with steroids. Cases with a dystrophin point mutation had an onset of CM 22 months earlier compared to cases with dystrophin deletions or duplications (n = .027).

Conclusions: The significant findings of this MD STARnet study were: 1) dystrophin point mutations are associated with earlier onset of CM and 2) steroid treatment delays the onset of CM in DMD.

226 THE EFFECT OF IVUS GUIDANCE ON CLINICAL OUTCOMES IN PERCUTANEOUS REVASULARIZATION COMPARED TO CORONARY ARTERY BYPASS GRAFTING AMONG HIGH RISK PATIENTS WITH LEFT MAIN CORONARY ARTERY DISEASE

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Purpose of Study: Percutaneous coronary intervention (PCI) in patients with unprotected left main (UPLM) coronary artery disease continues to evolve following the results of the SYNTAX trial and other studies demonstrating effective and safe outcomes for intermediate risk patients undergoing PCI with drug eluting stents (DES). However, there are limited data on how intravascular ultrasound (IVUS) guidance combined with DES will affect outcomes in high risk patients undergoing PCI UPLM.

Methods Used: We retrospectively reviewed 119 patients with UPLM who underwent PCI with DES and mandatory IVUS guidance against 50 patients with UPLM who underwent CABG surgery during a 3 year period and followed them for 12 months. The patients were age and gender matched, it was not possible to perform a propensity risk evaluation, since the CABG patients were of low to intermediate risks. The primary endpoints were: Major adverse cardiac events = Myocardial infarction (MI), cardiac death and target vessel revascularization (TVR). The secondary endpoints were: TVR and graft occlusion/arC probable stent thrombosis (ST). Logistic regression analysis was used to model predictors of MACE.
Among patients undergoing PCI vs CABG for UPLM, the major cardiac event (MACE) rate at 12 months was (11.8 vs 12.6%, p = NS). The TVR rate at 12 months was (3.3 vs 4.2%, p = NS). There was no graft occlusion and/or ST by either groups. There was a significant difference in EuroScore in the PCI vs CABG cohort (8.4 ± 2.2 vs 3.7 ± 1.2, p < 0.05) demonstrating a lower risk group of patients who underwent CABG revascularization. Presence of EuroScore > 6 is associated with an MACE odds ratio (OR) of 7.9 (p < 0.005) in this study population.

Conclusions: Despite a higher EuroScore, which was significantly predictive of MACE, patients undergoing PCI with IVUS guidance for UPLM had a similar clinical event rate compared to low-intermediate risk patients undergoing CABG at 12 months. The use of DES and IVUS guidance possibly improved the efficacy and safety outcomes. Our retrospective review lends credence to a future randomized trial incorporating both components in the design.

227 CHEST PAIN AND ST ELEVATION: THE BRUGADA ALTERNATIVE (CASE REPORT)
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Case Report: In the context of ST elevation without evidence of coronary artery disease but family history of sudden cardiac death, Brugada Syndrome must be considered. A 56-year-old male with a past medical history of depression and tobacco abuse but no cardiac history presented at the Veteran’s Hospital Emergency room with a chief complaint of chest pain. The patient reported that the pain was pressure-like in quality and radiated to his left arm. He described palpitations, shortness of breath and a feeling of “something was wrong.” The patient was in his yard when the pain began: it lasted 1–2 minutes during the first episode and approximately 5 minutes during the second episode.

In the ambulance the patient had ST elevation per monitors. The patient was given aspirin. The on call team was alerted. In the ED, an ECG was performed showing ST elevations in leads V1 and V2. Despite his objective findings, the patient was in no acute distress with stable vital signs. He was taken directly for a left heart catheterization. The patient was found to have normal coronaries and ejection fraction. The patient was admitted to the hospital and started on standard medical therapy. Because of a combination of negative troponins, completely clear coronary arteries and the transient nature of his ST elevations without coronary artery spasm, the patient was suspected to have Brugada syndrome. He was discharged and sent to an electrophysiologist where an ICD was placed for prevention of cardiac death.

Brugada syndrome, first described in 1989, is characterized by particular subsets of ST elevation in the context of a distinct clinical history. The ST elevation found in Brugada Syndrome is thought to be the result of a genetic abnormality of the sodium channels of the epicardium. Brugada is associated with sudden cardiac death, unexplained syncope, sudden ventricular and atrial arrhythmias. The only proven treatment for patients with symptomatic Brugada syndrome or Brugada pattern with inducible tachycardia is ICD placement. In patients with unexplained arrhythmias and chest pain, every clinician should add Brugada to the running differential in his/her head alongside Prinzmetal’s Angina, pericarditis and electrolyte imbalances.

228 TAKOTSUBO CARDIOMYOPATHY OCCURRING 14 DAYS AFTER CEREBRAL ANEURYSM RUPTURE
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Case Report: Takotsubo cardiomyopathy can present as a life threatening cardiac event that involves left ventricular apical akinesis, mimicking acute coronary syndrome (ACS) in patients with an emotional precipitant. It has also been noted to be associated with subarachnoid hemorrhages and can present as severe left ventricular dysfunction with cardiogenic shock in this patient population. The pathogenesis and temporal relationship between these acute neurological events and Takotsubo cardiomyopathy is not well understood. We present a 62 year old female with no previous history of coronary artery disease or coronary risk factors who developed cardiogenic shock two weeks after being admitted with a subarachnoid hemorrhage. The echocardiogram showed classic features of Takotsubo cardiomyopathy, including apical ballooning with basal hyperkinesis. This case is notable for the very late presentation of this cardiac syndrome after the offending neurological event. To our knowledge, this is the first reported case of Takotsubo cardiomyopathy presenting so late after a subarachnoid hemorrhage.

229 ASPERGILLUS ENDOCARDITIS OF THE LEFT VENTRICLE IN A RENAL TRANSPLANT PATIENT: A CASE REPORT
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Case Report: Aspergillus causes an opportunistic infection with a high rate of dissemination and poor overall outcome especially in immunocompromised patients. In transplant recipients, intense immunosuppressive therapy to prevent allograft rejection poses a major risk factor for Aspergillosis. In transplant recipients, Aspergillus can affect virtually any organ with mortality from endocarditis approaching 70%, despite cardiac surgery and antifungal therapy. We present the case of a 65 year old male who presented to the hospital after syncope and left sided weakness. Head CT demonstrated left frontal lobe infarct and echocardiogram showed a left ventricle multiple-lobe mass which was mobile and attached to the posterior basal wall of the endocardium by a stalk. Histopathology of the resected mass confirmed Aspergillus endocarditis. Hospital admission was further complicated by disseminated infection including endophthalmitis, complete heart block, meningitis and respiratory failure despite resection of the mass and aggressive antifungal medical therapy. Valve replacement or resection should be carried out in all patients. The value of combination therapy with antifungal agents is uncertain, however consideration should be given to use a second agent in addition to liposomal amphotericin.

230 ADULT CONGENITAL PULMONARY STENOSIS. CASE REPORT AND LITERATURE REVIEW
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Case Report: While pulmonary valve stenosis (PS) is a relatively common congenital heart condition, it is typically diagnosed and treated in the first few years of life. We present a relatively rare case of a 21 year old female with severe PS who underwent successful balloon pulmonic valvuloplasty (BPV). This 21 y/o Mexican female complained of long-standing shortness of breath and cyanosis. On presentation she was in no distress with BP 142/92, HR 64, and room o2 92 Sat 65% (80% on GLNC). There was mild cyanosis of lips and digital clubbing. Cardiac auscultation revealed a 4/6 harsh late-peaking systolic murmur at the LUSB, with attenuation of P2. Lungs sounds were normal. No lower extremity edema. CXR revealed cardiomegaly. TTE revealed severe right ventricular hypertrophy (RVH) with moderate dilatation, moderate pulmonary valve leaflet thickening, and mean pulmonic valve gradient of 34 mm Hg. A patent foramen ovale (PFO) was present. She was treated with beta blocker(BB) and referred for cardiac catheterization and BPV where the hemodynamic findings were confirmed and shunt study revealed a right to left shunt with Qp 2.7 and Qs 5.5 L/min. Successful BPV was performed with resultant gradient of 21 mm Hg. Adult congenital PS is relatively an uncommon disease. Clinical presentation varies from mild exertional dyspnea to signs and symptoms of right heart failure depending on the severity of obstruction and the degree of myocardial compensation. Our patient was unique in that she presented very late despite severe stenosis and symptoms with right to left shunting through a PFO. Indication for BPV is similar to surgical valvotomy; namely peak to peak gradient greater than 50 mmHg. Depending on the degree of RVH, there is a risk of hyper dynamic contractility and subsequent dynamic RV outflow obstruction once the obstruction is relieved, a complication called suicide right ventricle. This may be ameliorated by pre-procedure BB therapy. In conclusion, severe congenital PS is rarely seen in adults especially in the US as most cases are corrected early in life. Adult BPV appears to be equally effective in adults as children and is a viable alternative to surgical valvotomy in most patients. Reserved for those with gradients greater than 50mmHg, complications are few. Suicide RV is one such complication that required special attention.
FILTER-GROWN CACO-2 INTESTINAL EPITHELIAL MONOLAYERS WERE USED AS AN IN-VITRO MODEL SYSTEM TO STUDY THE EFFECT OF IL-6 ON INTESTINAL TIGHT JUNCTION PERMEABILITY.

METHODS USED:

1) Trans-epithelial resistance (TER); 2) Western blot analysis; 3) Intestinal permeability.

RESULTS:

1) LPS treatment at physiologically relevant concentrations (0.1–1 ng/ml) caused a time and dose-dependent decrease in TER. 2) Conversely, LPS treatment resulted in a time-dependent increase in permeability to paracellular marker inulin. 3) The LPS-induced increase in TER was mediated by an increase in MLCK protein expression. 4) Silencing MLCK by siRNA transfection prevented the LPS-induced increase in TER. 5) Silencing MLCK expression by siRNA transfection prevented the LPS-induced increase in TER.

CONCLUSIONS:

In conclusion, our data demonstrated that LPS caused an increase in intestinal TJ permeability and that was mediated by an increase in MLCK protein expression.
p38 (small interfering RNA which is a small double-stranded RNA molecule used to silence gene expression of a specific protein). siRNA-induced depletion of p38 kinase prevented the IL-1B-induced drop in Caco-2 TER. 5) Activation of p38 pathway caused a down-stream activation of nuclear transcription factor (ATF-2). Inhibiting ATF-2 by siRNA-induced knock down prevented the IL1B-induced drop in Caco-2 TER.

Conclusions: In conclusion, this study demonstrated that IL-1B-induced increase in intestinal TJ permeability is mediated by activation of p38 signaling pathway leading to the activation of ATF2 nuclear transcription factor.

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WILSON DISEASE: ABNORMAL METHIONINE METABOLISM IN PATHOGENESIS OF LIVER DAMAGE
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Purpose of Study: Wilson disease (WD) is a recessive disorder of copper accumulation with established genetic background but unclear pathogenesis. Aberrant hepatic methionine metabolism plays a role in the pathogenesis of liver injury by regulating homocysteine (Hcy) and S-adenosylhomocysteine (SAH), which induce endoplasmic reticulum (ER) stress and consequent induction of lipogenic pathways. We hypothesized that abnormal methionine metabolism is involved in the pathogenesis of hepatic steatosis in WD.

Methods Used: Livers were removed from Jackson toxic milk mice (tx-j), a model of WD, and C3H control mice fed the same diet at 16, 20, and 24 weeks of age. Profiles of selected hepatic methionine metabolites in the tx-j mice were compared to those of the wild type mice at the three ages and were correlated with histopathology, triglyceride accumulation, and activation of ER stress, apoptosis, and lipogenesis markers.

Summary of Results: At 20 and 24 weeks, the tx-j mice showed greater hepatic triglyceride levels and higher cytoplasmic lipid droplets in hepatocytes compared to the controls. SAH hydrolase (SAHH) mRNA expression, protein levels, and activity were significantly lower in the tx-j mice at all ages and correlated negatively with hepatic copper levels, which were significantly higher in all tx-j mouse age groups. The transcript and protein levels of the ER stress marker glucose related protein 78 (GRP78), the apoptosis marker growth arrest DNA damage inducible gene 153 (GADD 153), and the lipogenesis transcription factor sterol regulatory element binding protein 1c (SREBP-1c) were increased at all age points in the tx-j mice. Plasma HCY levels were elevated at 20 weeks and correlated with hepatic triglyceride levels.

Conclusions: Reduced hepatic SAHH expression was associated with increased SAH and subsequently with increased plasma Hcy as well as the activation of ER stress lipogenic pathway gene expression, which were paralleled by the progression of hepatic damage. These findings support the hypothesis that aberrant methionine metabolism plays an important role in liver damage pathogenesis in WD.

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PREVALENCE OF PEDIATRIC INFLAMMATORY BOWEL DISEASE AMONG HISPANICS IN AN URBAN ACADEMIC CENTER IN THE SOUTHWEST UNITED STATES
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Purpose of Study: Inflammatory bowel diseases (IBD), ulcerative colitis (UC) and Crohn disease (CD), affect approximately 2 million Americans. While it has been historically thought to be a disease affecting Caucasians it is now apparent that other ethnic groups are also affected. The Hispanic population is increasing in the United States; therefore, this study was done to determine the prevalence of IBD among ethnicities in an urban pediatric gastroenterology clinic in the southwestern United States.

Methods Used: A retrospective medical record review was performed on 1424 pediatric patients between the ages of 1 and 18 years of age who were seen at an urban academic pediatric gastroenterology center in Las Vegas during the years 2004–2010. The race and ethnicity of all inpatient and outpatient cases were identified. Groups included African American non Hispanic (AA), Caucasian non Hispanic (C), Hispanic (H), Asian/other (O) or unknown. In addition, any patient with the diagnosis of CD, UC, proctitis, nonspecific colitis, IBD, or colitis were evaluated for radiographic, endoscopic, histologic and clinical course consistent with the diagnosis of IBD.

The prevalence of IBD among race/ethnic groups was compared using a two sample t-test. A p value of < 0.05 was defined as being statistically significant.

Summary of Results: The patient race/ethnicity distribution of all records reviewed was as follows: 11.6% AA, 42.8% C, 39.2% H, 3.2% O. Unknown, in comparison, the Clark County School District has a distribution of 41% H, 12% AA, 42% C, and 5% O. Forty-three of the 1424 patients were diagnosed with IBD, 29 CD and 14 UC. The prevalence of IBD by race was 8/558 H (1.4%), 8/165 AA (4.8%), and 2/610 C (0.4%). The prevalence of IBD between C (4.4%) and AA (4.8%) was similar (p=0.82). However, the prevalence of IBD was significantly higher in each of these races compared to Hispanics (C vs. H, p=0.0027, AA vs. H, p=0.0088).

Conclusions: In this pediatric patient population, Hispanics have decreased prevalence of IBD compared to Caucasians and African Americans. Although genetics contribute to the development of IBD, the role of environmental and dietary factors cannot be overlooked.

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TREATMENT OF CHRONIC HEPATITIS B PATIENTS ACCORDING TO CURRENT GUIDELINES: A COMMUNITY-BASED STUDY
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Purpose of Study: Prior studies have found that a significant proportion of chronic hepatitis B (CHB) patients do not receive antiviral therapy (AVT). Our goals were to characterize CHB patients by treatment eligibility and actual treatment status by current published guidelines and to examine reasons for the lack of AVT in patients who are treatment eligible.

Methods Used: We conducted a retrospective study of patients who were first evaluated for CHB at one of two community gastroenterology clinics between April 2007 and February 2009. Using criteria published by the American Association for the Study of Liver Diseases (AASLD) in 2009 and a panel of U.S. hepatologists (US Panel) in 2008, treatment eligibility was determined for patients using clinical and laboratory data from the first six months of presentation. Treatment status was followed in the patients who were found to be treatment eligible and reasons for non-treatment were identified.

Summary of Results: A total of 612 consecutive CHB patients were included in the analysis. Mean age was 44±13 years, 54% were male, and almost all were Asian (99%). About half of the patients (51%) were treatment eligible by US Panel guidelines. Of these patients, 47% additionally met AASLD guidelines. The latter were more likely to be younger, hepatitis B e antigen positive, and had higher median alanine aminotransferase level (ALT) and HBV DNA levels. Overall, treatment was initiated in 50% of eligible patients: 29% of US Panel-eligible-only patients and 72% of AASLD-eligible-patients. On multivariate analysis, independent predictors for actual treatment initiation were higher ALT in AASLD-eligible-patients and higher ALT and older age in US Panel-eligible-only patients. The leading reason for non-treatment was further observation as desired by the physicians.

Conclusions: Approximately half of treatment eligible patients did not receive antiviral therapy for CHB as recommended by either AASLD or US Panel guidelines. Substantial undertreatment of CHB is observed especially in patients eligible only by US Panel guideline and should be further examined.

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ROLE OF ANTIMICROBIAL PEPTIDES IN ESOPHAGITIS
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Purpose of Study: Esophagitis is caused by exposure of the epithelia to allergens (eosinophilic esophagitis-EoE) or acid (gastroesophageal reflux disease-GERD) but the exact pathogenic mechanisms of these diseases are unknown. Recent data supports a potential role for microbial flora in the initiation or perpetuation of esophagitis. We hypothesize that dysregulated innate defense molecules (defensins) of esophageal epithelia contribute to
A 7 day old term male infant presented to the Emergency Room with jaundice, lethargy, increased work of breathing and a tense abdomen in severe hypovolemic shock. He was intubated, fluid resuscitated and transferred to the ICU with jaundice, lethargy, increased work of breathing and a tense abdomen in severe hypovolemic shock. He was intubated, fluid resuscitated and received a blood transfusion. On admission his hemoglobin was 27. An abdominal ultrasound demonstrated a large right hepatic subcapsular hematoma, massive hemoperitoneum and numerous additional focal echogenic tumors of the liver. Spontaneous rupture of these tumors is extremely rare and carries a very high mortality rate.

**Case Report:** A 7 day old term male infant presented to the Emergency Room with jaundice, lethargy, increased work of breathing and a tense abdomen in severe hypovolemic shock. He was intubated, fluid resuscitated and received a blood transfusion. On admission his hemoglobin was 27. An abdominal ultrasound demonstrated a large right hepatic subcapsular hematoma, massive hemoperitoneum and numerous additional focal echogenic tumors of the liver. Spontaneous rupture of these tumors is extremely rare and carries a very high mortality rate.

**Purpose of Study:** To delineate the intra cellular signaling pathway that mediates endotoxin induced increase in intestinal permeability using filter grown caco-2 monolayer as an in vitro model system.

**Methods Used:**
1. Filter grown Caco-2 intestinal epithelial monolayer were used as an in vitro intestinal epithelial model system.
2. Intestinal permeability was assessed by measuring Trans epithelial resistance (TER).
3. Western blot analysis.
4. ELISA induced assay was assessed to determine the activation of ELK-1 (transcription factor).

**Summary of Results:**
1. LPS at physiologically relevant concentrations of 0.3 ng/ml caused a time dependent drop in TER.
2. LPS at physiological concentrations of 0.3 ng/ml caused a time dependent increase in P38 kinase activation as determined by P38 kinase phosphorylation.
3. Inhibition of P38 kinase using an inhibitor prevented the LPS induced drop in TER.
4. LPS treatment resulted in time dependent activation of P38 kinase ELK-1.
5. Inhibiting ELK-1 expression by siRNA prevented the LPS induced drop in Caco-2 Trans epithelial resistance.

**Conclusions:** LPS at physiological concentration caused an increase in Caco-2 Tight Junction permeability. LPS induced increase in intestinal permeability was mediated by P38 kinase activation of ELK-1 transcription factor.

**240 EPIDEMIOLOGY OF BILIARY ATRESIA IN NEW MEXICO**

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**Purpose of Study:** Biliary Atresia is a disease that is defined as a complete obstruction of the lumen of the extrahepatic biliary tree and it is the most common cause of death from liver disease in early childhood. The purpose of our study was to investigate the possible existence of seasonal, temporal, community size or regional influences in the occurrence of Biliary Atresia in New Mexico.

**Methods Used:** All patients with the diagnosis of Biliary Atresia who were born in New Mexico in the years 2003–2007 were included in this study. The data was collected from the University of New Mexico as well as Presbyterian Hospital, which are the only facilities in New Mexico that can make the diagnosis. Each patient’s season and year of birth, regional location, and community (urban vs. rural) were evaluated in this analysis. Statistical analysis was done utilizing the Chi Square Goodness of Fit model.

**Summary of Results:** We identified 14 patients who met the inclusion criteria. Our data did not show any significance in seasonality, community or regional location of birth. While the years as a whole did not show any statistical significance, the year 2004 did show a significant increase with a p-value <.05.

**Conclusions:** We conclude that there are not statistically significant seasonal, community or regional based variations in the incidence of Biliary Atresia in New Mexico. Also, while there is no significance over the time period as a whole, 2004 was shown to have a significant increase in the cases of Biliary Atresia. Our data can be used to further elucidate the diagnosis of Biliary Atresia and its possible contributing factors.

**241 SPONTANEOUS RUPTURE OF HEPATIC HEMANGIOMA IN A NEONATE - SURVIVAL WITH NON-OPERATIVE MANAGEMENT**

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**Background:** Hepatic hemangioma is the most common benign pediatric tumor of the liver. Spontaneous rupture of these tumors is extremely rare and carries a very high mortality rate.

**Case Report:** A 7 day old term male infant presented to the Emergency Room with jaundice, lethargy, increased work of breathing and a tense abdomen in severe hypovolemic shock. He was intubated, fluid resuscitated and received a blood transfusion. On admission his hemoglobin was 27. An abdominal ultrasound demonstrated a large right hepatic subcapsular hematoma, massive hemoperitoneum and numerous additional focal echogenic tumors. This study aimed to measure defensin expression in patients with esophagitis and in esophageal epithelial cells of an in vitro model of eosinophilic inflammation.

**Methods Used:** Defensin (hBD1 and 3) expression from esophageal biopsies (Esophagitis, GERD and normal) was measured by qRT-PCR. Epithelia hBD1 and 3 expression of an in vitro model of esophagitis [esophageal epithelia-HET-1A cell line and human peripheral eosinophils-IL-5 (100pg/ml) and GMCSF (100pg/ml)] were measured via qRT-PCR.

**Summary of Results:** Esophageal biopsies from Esophagitis patients have decreased expression of hBD1 compared to normal (0.19±0.22 vs 1.0±0.61, respectively; p=0.019). Esophagitis patients treated with topical steroids have similar hBD1 expression levels compared to normal (0.65±0.80 vs 1.0±0.61 respectively; p=0.56). HBD3 expression in biopsies from Esophagitis patients is significantly decreased compared to normal (0.15±0.21 vs 1.0±0.65, respectively; p=0.03). HBD3 expression in Esophagitis patients treated with topical steroids trends but is not significantly reduced compared to normal (0.21±0.14 vs 1.0±0.65 respectively; p=0.06). HBD3 expression in biopsies from GERD and GERD patients treated with proton pump inhibitors is significantly decreased compared to normal (0.10±0.07 vs 1.0±0.65 and 0.17±0.22 vs 1.0±0.65; p=0.03 and p=0.03, respectively). HET cell hBD1 and 3 expression are significantly reduced by IL-5 and GMCSF compared to HET cells cultured in media alone (0.71±0.12 vs 1.0±0.11 and 0.39±0.14 vs 0.95±0.24; p=0.03 and p=0.01, respectively). HET cells cultured with IL-5, GMCSF, and eosinophils have a trend towards decreased hBD1 expression compared to controls without eosinophils (0.77±0.08 vs 1.24±0.22; p=0.07, respectively).

**Conclusions:** Defensin expression is decreased in esophagitis. We speculate that dysregulated defensin expression contributes to esophagitis.
lesions in his liver. An abdominal CT scan confirmed these findings and further characterized the lesions to be most consistent with hepatic hemangioma. The patient was placed on high dose IV steroids and repeat ultrasound performed 7 days later found the hemangioma to have resolved and the subcapsular lesion persisted. Given that life-threatening hemorrhage had already occurred, it was felt the risk for recurrent bleeding was substantial and he underwent selective right hepatic arterial embolization which supplied the ruptured lesion 17 days after admission. He was discharged home on 3 mg twice daily of prednisolone which was tapered to off over 3 months. At 19 months he is healthy and developing normally. The last ultrasound demonstrated multiple lesions still present, but decreased in conspicuity.

**Conclusion:** We present a rare case in which a patient with spontaneous rupture of a hepatic hemangioma survived and is thriving today with no immediate surgical intervention. To our knowledge this is the first reported neonate to have a ruptured hepatic hemangioma and survive by managing with resuscitation, steroids and delayed embolization.

**Health Care Research II**

**Concurrent Session**

**1:30 PM**

**Friday, January 28, 2011**

**242 EVALUATING DESIGN ELEMENTS IN COMMONLY VISITED HYPERTENSION PATIENT EDUCATION WEBSITES**

Stratton SR, Dotson A, Kim S *University of Washington, Seattle, WA.*

**Purpose of Study:** The quality of the health education sites returned by hypertension-related Internet search queries is of increasing importance. Specific web design and user interactive features have been shown to support learning and retention. This study reviewed systematically the content presentation and web design features of sites commonly visited by patients searching for information about hypertension.

**Methods Used:** Using Google AdWord’s Keyword Tool, we identified 60 commonly used search phrases for hypertension and their monthly search volume. Based on projections of click-through traffic, we generated a list of 39 unique hypertension patient health education sites with at least 150,000 estimated visits per month. Using the results of previous studies examining website design features, we developed a site design quality score (SDQS) to grade sites based on the presence or absence of 12 desirable design elements, 11 interactive features, and 13 content transparency and integrity characteristics. The potential score was 0–36.

**Summary of Results:** Monthly search volume for selected phrases ranged between 673,000 and 2,240,000. Websites had a median design element score of 4/12, median interactivity score of 3/11, and median transparency and integrity score of 5/13. The overall median SDQS was 14, range 4–26. The median Flesh-Kincaid reading grade level was 8.6, range 3.4–14. Health-centered non-profits, governmental, and educational sites (n=9) had a median SDQS of 17 while commercial sites (n=22) had a median SDQS of 11.5. There was a poor association between SDQS and estimated site traffic from Google (r=0.117).

**Conclusions:** Search volume for hypertension is high, and patients use a large variety of search terms. There is great variation in the design quality of patient health education websites for hypertension. All websites examined have significant room for improvement in design, interactivity, readability, and/or transparency as well as in the use of multimedia and user self-assessments. Non-profit, educational, and governmental sites had a higher median SDQS score than commercial sites. Google’s search algorithm does not strongly select for hypertension patient education sites that have higher SDQS scores.

**243 ONSITE CLINICS IN SIX WORKPLACES: MEASURES OF SUCCESS**


**Purpose of Study:** Workplace clinics (WPCs) provide onsite care for minor illnesses, can assist primary clinicians (PCs) in disease management, and can deliver occupational health services. There are few reports of utilization patterns and financial benefits of WPCs. However, we measured near-term WPC outcomes at a pipe manufacturer (PM), town hall (TH), lumberyard (LY), canning facility (CF), metal fabricator (MF), and electric insulator manufacturer (EIM). Co-pays were $3 per visit at TH and zero at other WPCs.

**Methods Used:** We placed a part-time mid-level clinician (MLC) at each WPC and recorded visit numbers, diagnoses and referrals in each, and estimated cost savings to employers. We used visit complexity as a surrogate for the cost which would have been incurred, had the service been rendered offsite in Charlotte, NC.

**Summary of Results:** Utilization was high (Table). Leading reasons for visits included: hypertension and dyslipidemia (each 12%), respiratory and/or allergy symptoms (11%), and obesity (9%). Hence, most visits were for conditions handled by the MLC. Referrals were made in 24% of visits, nearly half (11%) to the worker’s PC. Over-the-counter medicines were advised in 60% of visits, and agents with the lowest co-pay were prescribed in another 30% of visits. Estimated cost savings from the WPC ranged from $249 to $874 per worker per year.

**Conclusions:** Employers realized substantial savings from WPCs, ranging from $103 to $230 for each hour of MLC service. However, our analysis does not identify the costs billed by PCs, and hence could overstate the actual savings to an unknown extent.

**Demographic and Savings of Onsite Clinics at 6 Worksites**

<table>
<thead>
<tr>
<th>Worksites</th>
<th>PM</th>
<th>TH</th>
<th>MF</th>
<th>LY</th>
<th>CF</th>
<th>EIM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of employees</td>
<td>1,212</td>
<td>996</td>
<td>287</td>
<td>240</td>
<td>125</td>
<td>125</td>
</tr>
<tr>
<td>% using Onsite Clinic</td>
<td>90%</td>
<td>65%</td>
<td>47%</td>
<td>70%</td>
<td>54%</td>
<td>45%</td>
</tr>
<tr>
<td>Visits per year</td>
<td>3,205</td>
<td>1,716</td>
<td>1,116</td>
<td>619</td>
<td>466</td>
<td>330</td>
</tr>
<tr>
<td>Per cent Referrals</td>
<td>24</td>
<td>10</td>
<td>33</td>
<td>11</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Savings, $/per visit</td>
<td>302</td>
<td>421</td>
<td>101</td>
<td>69</td>
<td>109</td>
<td>41</td>
</tr>
<tr>
<td>Savings, $/worker per year</td>
<td>249</td>
<td>422</td>
<td>352</td>
<td>288</td>
<td>874</td>
<td>326</td>
</tr>
<tr>
<td>Savings, $/per MLC hour</td>
<td>103</td>
<td>226</td>
<td>97</td>
<td>320</td>
<td>296</td>
<td>212</td>
</tr>
</tbody>
</table>

**244 BUILDING CAPACITY TO REDUCE DIABETES DISPARITIES: TRAINING COMMUNITY HEALTH WORKERS**

Bouchonville M, Colleran K, Kipp B. *University of New Mexico HSC, Albuquerque, NM and University of New Mexico HSC, Albuquerque, NM.*

**Purpose of Study:** Community health workers (CHWs) participate in many aspects of health, through commitment to their communities by overcoming barriers to care: language, resources, culture, and trust. CHWs are ideally situated to help tackle the diabetes epidemic. Unfortunately, CHW’s face barriers to obtaining training including: time, cost, and travel. Using the ECHO model, we developed a distance training program utilizing case based learning, didactics and resource sharing. We hypothesize that participation in the program will increase CHW attitudes toward treatment, knowledge, and confidence in working with diabetics.

**Methods Used:** Twenty-three subjects were enrolled in the first training. Subjects completed baseline attitude, knowledge (DkT), and confidence surveys. They attended a three day skills training session, followed by weekly participation in tele/video conferences for 6-months. Each session consisted of a didactic, case presentations by participants which were discussed by the UNM multidisciplinary diabetes team and participants, Q&A and resource sharing. Participants completed surveys at the end of the training. Wilcoxon Signed Rank statistics were used to compare pre and post test results. Regression analyses were also done.

**Summary of Results:** Twenty-one subjects completed the program. There were significant improvements in the DkT, 57% correct at baseline versus 71% correct at the end of the study p=0.0002. Confidence in clinical skills increased significantly from 3.3 at baseline to 4.4 (on a scale of 1–5) p=0.0001. Confidence in non clinical skills including education and advocacy increase from 3.62 to 4.29, p=0.0002. Participants demonstrated improved attitudes on the seriousness of diabetes (4.1–4.39, p=0.04). Regression analysis demonstrated significant correlation between attitudes of the seriousness of diabetes and the psychological impact of diabetes, the role of patient autonomy, and participant confidence in clinical skills, r = 0.55, p=0.01, r=0.57, p=0.007, r=0.46, p=0.36, respectively.

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Conclusions: CHW participation in the ECHO distance training program resulted in significant increases in knowledge, confidence, and attitudes in diabetes. Future studies are ongoing to determine if the training has a impact on patient outcomes.

245 AVOIDING THE IMPRESSION OF IMPROPRIETY: UNDERSTANDING COMMON ERROR TYPES IN PUBLICATIONS CITED BY RESIDENCY APPLICANTS

Simmons HR¹, Kim S², Zins AM², Amies-Oelschlager AE², University of Washington, Seattle, WA and ³University of Washington School of Medicine, Seattle, WA.

Purpose of Study: Multiple studies have demonstrated that many publications reported by applicants to residency are unverifiable when searched. We sought to investigate what type of errors medical students may make in reporting a publication that may preclude the ability for a listed publication to be verified.

Methods Used: We performed a retrospective review of the ERAS applications submitted to the University of Washington OB/GYN residency for the 2008 and 2009 matches. We searched reported peer reviewed journal articles, abstracts, published and unpublished (submitted/accepted) submitted in PubMed and exhaustive list of online resources. If still unfound, journal editors were contacted to inquire about the article in question. Errors were categorized as: 1) deliberate (insertion of name or article never submitted), 2) overstatement (incorrectly publishing a publication as peer reviewed, listing articles where they were not an author, changing the ranked author list, or citing publications without authors), 3) minor (deleting all other authors, title change, wrong journal publication name, date, volume, wrong Pub Med ID number, or failure to list the journal name), and 4) unverifiable (publication unable to be found despite exhaustive search).

Summary of Results: Of the 937 applicants that 546 applicants (58.2%) listed a total of 2,251 publication entries (mean = 4.12). Of these, 354 applicants listed 999 peer reviewed journal articles/abstracts, of which 751 were reported published and 248 reported unpublished. 150 (42.6%) of applicants who reported a peer reviewed publications had at least one error identified or a publication which could not be found. In the 999 publications, there were 10 deliberate errors (1.0%), 161 overstatement errors (16.1%), and 54 minor errors (5.4%). 157 (15.7%) publications or journals were unverifiable. All publications with a Pub Med ID number listed were able to be verified.

Conclusions: Over one third of peer reviewed publications listed by medical students applying in OB-GYN are erroneous in some manner. Students should be aware of these errors in order to avoid the appearance of impropriety, real or perceived.

246 HEALTH NEEDS AND HEALTHCARE SYSTEM RESPONSES AMONG VICTIMS OF GENDER-BASED VIOLENCE IN AFGHANISTAN

Stokes S, Miller E. UC Davis School of Medicine, Sacramento, CA.

Purpose of Study: Gender-based violence remains a challenge to promoting women’s health in Afghanistan. This study aims to describe the range of health needs and heterogeneity of healthcare experiences among Afghan women exposed to gender-based violence.

Methods Used: In-depth, anonymous face-to-face interviews were conducted with 22 Afghan women ages 18 and older currently receiving services through a Kabul-based non-governmental organization providing shelter to victims of human rights abuses. When permitted, the interviews were audiotaped and transcribed. Otherwise, detailed notes were taken during the interviews. All interviews were coded for key themes related to health and abuse utilizing a content analysis approach.

Summary of Results: The median age of respondents was 19 years (range 18–26 years). Over half of women reported histories of physical abuse by their husband (60%, n=13), 46% by their husband’s family (n=10) and 41% by members of their own family (n=9). 82% also reported receiving threats of honor killings (n=19). Almost all women endorsed current symptoms of depression (91%, n=20), and eleven reported attempting suicide in response to abuse experienced. Nearly all women had received medical care for symptoms associated with their abuse (n=19). Nine women were treated for severe injuries sustained from physical abuse, nine were hospitalized for attempting suicide and two were required to undergo vulvodynia examinations by a physician. Only one woman reported that her healthcare provider attempted to report her abuse to authorities. All women receiving medical care for symptoms associated with their abuse being offered any mental health counseling (n=19), and none were informed by their healthcare providers about supportive services for victims of gender-based violence.

Conclusions: The interviews suggest that Afghan women receiving medical care for health complications associated with gender-based violence may not be properly identified by healthcare providers and may not be receiving counseling or supportive services through healthcare providers. Further studies on healthcare responses to victims of gender-based violence are needed. Training in assessment for abuse during medical evaluations may assist healthcare providers in Afghanistan to better recognize victims of gender-based violence and to connect women to support services.

247 SMOKING CESSATION DURING PREGNANCY INCREASES MOTIVATION TO BREASTFEED

Merritt TA, Armstrong Loma Linda University, Loma Linda, CA.

Purpose of Study: In the second and third trimesters we showed the video “When You Smoke Your Baby Smokes” to 96 high risk women which highlighted the adverse effects of tobacco smoke on their fetus and newborn.

Methods Used: The videos and follow up questionnaires were followed by motivational interviews to the gravida or recently delivered mothers.

Summary of Results: Of 96 pregnant mothers, 92% of 25 self identified as currently smoking (or smoking within the last year) were enrolled in a tobacco treatment program which provides an incentive-based, 8 week program with monitoring using salivary cotinine. Fifteen (60%) remained tobacco free for 9 weeks and at the time of delivery. Among the 40% who failed to remain tobacco free, viewing the video prompted mothers to quit for at least one week. 21 mothers (83%) chose to breastfeed during the postpartum period.

Conclusions: After delivery (mean Gestational Age mean 35.2±/–3.1 weeks) the impact of the program prompted 25% of mothers who smoked early in pregnancy to change from a state of contemplation to preparation and 65% to change from contemplation to action. Mothers also disclosed that viewing the video increased their desire and willingness to breastfeed their infant.

248 PATIENT COMPLIANCE IN A CLINICAL TRIAL OF AN HERBAL THERAPY FOR TYPE-2 DIABETES

Brueckner E, Martin JT, Nguyen DH, Mendoza N, Nguyen B, Espino R, Hoettedumlao J, Semenova Y. Western University of Health Sciences, Pomona, CA.

Purpose of Study: Patient compliance is the most critical part of the treatment of chronic diseases. Unfortunately, with diseases like Type-2 Diabetes, where disease onset is insidious, patients demonstrate poor compliance until it is too late. Compliance in diabetics taking mainstream glycemic-control drugs is well studied; however, compliance in diabetics taking herbal therapy has not been investigated. Our study examines compliance among treatment groups over the duration of our clinical trial.

Methods Used: 107 Type-2 Diabetic subjects are enrolled in a 6 month, double-blind, placebo controlled trial. Criteria for participation include having a HAlc value between 7.0 and 9.0, being age 20–75, having normal renal and hepatic function, and having undergone no insulin therapy or diabetic-regimen drug or dosage changes for at least 3 months before and throughout the trial. Subjects take 1 capsule of the drug or the placebo by mouth, once daily for 6 months. On a monthly basis, participants meet with study coordinators to retrieve more capsules, return remaining capsules and
their daily glucose log. The capsules returned are counted, and these values are used to assess compliance.

Summary of Results: The fraction of subjects that returned more capsules than expected (indicating that they missed doses) significantly increased over the six month trial period. The average number of capsules returned by the experimental and control groups did not differ significantly (T = 1.39, DF = 43, P = .17). However, the variance in number of capsules returned was significantly lower in the experimental group (F = 1.94, P = .049).

Conclusions: An explanation for the difference in variance between the control and experimental group may be related to the daily monitoring of the glucose levels that the subjects performed. If our herbal therapy has a blood glucose lowering effect, the subjects will notice the changes in their daily glucose readings. This awareness among the experimental group provides an incentive to maintain their initial level of compliance, and explains the relatively small variance. The control group lacks this hypothesized incentive, and therefore is less likely to maintain a steady compliance levels throughout the study, leading to relatively large variance.

249 BIOMECHANICAL CHARACTERIZATION OF THE CARTILAGINOUS ENDFACE IN THE INFANT SPINE

Farmer RP, Paletta R, Ferguson V, Burger E. University of Colorado, School of Medicine, Aurora, CO.

Purpose of Study: The purpose of this study is to improve our understanding of the interface between mineralized and non-mineralized biological tissues. This is of great importance to ensure successful prosthetic implant fixation and to improve our understanding of the etiology of spinal disc degeneration and developmental disorders. In the spine, bone and cartilage meet at the osteochondral interface - a region that experiences high shear forces in normal use. We seek to characterize the gradient of properties from bone to cartilage within this interface using scanning electron microscopy, in both backscattered (BSE) and secondary electron (SEM) modes, to visualize the 2-D and 3-D structures of the cartilaginous endplate region in the human infant spine.

Methods Used: Within this interface, anchoring of the intervertebral disc (IVD) to the adjacent vertebral bodies by collagen fibrils will be assessed via immunohistochemistry staining for collagen types I, II, IV, and X. BSE imaging methods were developed using mature, ovine lumbar spine samples that were histologically embedded in poly(methylmethacrylate) (PMMA) prior to sectioning and imaging. Additional BSE imaging has also been performed on a human fetal (37 weeks gestational age) lumbar spine L3-L5. A second ovine lumbar vertebra is currently being in order to image the mineralized surface of the cartilaginous endplate.

Summary of Results: The infant cartilage endplate (CE) showed a highly porous mineralized cartilage region that was connected to, but distinct from, the underlying subchondral bone (SCB) and is an area of rounded pores that likely contained chondrocytes. While the adult ovine tissue possessed similar characteristics, the mineralized CE was significantly more compact and of greater thickness and was thicker in the AF area than in the NP area. This thinning is likely an adaptation to greater hydrostatic forces within the NP region. In addition, the CE overlying the annulus contained tidemarks - a histological feature that is typically associated with thickening of the calcified region in articular cartilage and may indicate degenerative changes.

Conclusions: This work forms the basis for two separate studies of both human and ovine spinal tissues to understand how the mineralized region is altered with age in healthy subjects.

250 VALIDITY AND RELIABILITY OF THE JOINT COMMISSION SUBJECT IDENTIFICATION CRITERIA FOR ASSESSING THE QUALITY OF CHILDREN’S INPATIENT ASTHMA CARE

West S, Nkoy F, Fassl B, Stone B, Halbern S, Maloney C. University of Utah, Salt Lake City, UT.

Purpose of Study: The Joint Commission (JC) requires free standing children’s hospitals to report compliance with asthma quality core measures for children hospitalized with acute asthma. Identification of children to be included in JC reporting is based on the primary ICD-9 discharge diagnosis code. This approach excludes children with asthma not meeting this specific criterion. Objectives of this study are to 1) determine the sensitivity and specificity of JC ascertainment of asthma discharges and 2) to describe characteristics and outcomes of all asthma discharges stratified by JC ascertainment criteria.

Methods Used: This was a retrospective cross-sectional study of patients 2-18 years admitted to a tertiary care children’s hospital between 1-08/07-08. Using administrative data, we identified all patients discharged with an asthma ICD-9 diagnosis code (493.xx). We then classified patients as: True Asthma (children whose symptoms and treatment were clinically consistent with asthma based on chart review by 2 physicians), JC Criteria (primary ICD-9 diagnosis of asthma and missed Asthma children with true asthma not identified by JC criteria). Administrative data was used to retrieve and compare patient demographics and hospitalization outcomes.

Summary of Results: Of 476 patients with a 493.xx ICD-9 diagnosis code, 235 were classified as True Asthma. 184 of these matched JC Criteria, and the remaining 51 were classified as Missed Asthma. Reliability of true asthma ascertainment between the 2 physicians was 100%. JC criteria sensitivity was 78% and specificity 99%. Patient demographic characteristics and readmission rates were similar among the groups.

However, patients with asthma not identified by JC criteria were more likely to be admitted with concurrent respiratory infections (p = 0.001), had significantly longer hospital stays (p = 0.002) and higher cost (p = 0.001).

Conclusions: The JC criteria are moderately sensitive but highly specific at identifying true asthma discharges. A quarter of patients with true asthma were missed. Quality of asthma care may not be adequately represented by utilizing JC identification criteria. Future studies should compare compliance with CAC measures between asthma patients that are and are not identified using JC criteria.

251 THE USE OF MULTIATTRIBUTE UTILITY THEORY IN THE SELECTION OF RADIATION THERAPY TREATMENTS

Burt A, Phillips MH, Civan Hartzler A. University of Washington School of Medicine, Seattle, WA; University of Washington Medical Center, Seattle, WA; and University of Washington Medical Center, Seattle, WA.

Purpose of Study: To determine the accuracy of utilities acquired from Time Trade Off (TTO) and Standard Gamble (SG) exercises for the purpose of individual decision-making and to evaluate the efficacy of multiattribute utility algorithms (MAU) in modeling patient preference.

Methods Used: Ten health states, representing hypothetical treatment outcomes, were selected from the 48 total possible health states. Five residents and physicians from the University of Washington Department of Radiation Oncology completed direct health state and direct profile rankings based on a visual analog scale. Utilities for the ten health states were elicited using TTO and SG. TTO scores were adjusted using certainty equivalents; SG scores were adjusted using prospect theory.

Summary of Results: TTO and SG were both able to accurately predict direct health state ranking (TTO p = 0.855, SG p = 0.875) and direct health profile ranking (TTO p = 0.866, SG p = 0.851). The MAU model based on TTO utilities yielded utilities similar to adjusted TTO utilities acquired from the interview (W = 14, p = 0.1836). The SG MAU model repeatedly underestimated corresponding interview utilities. Average error between the MAU model and the acquired utility was greater for TTO than SG (TTO average error = 0.096, SG average error = 0.059).

Conclusions: Both TTO or SG can effectively be employed to gather patient utilities; utilities from either exercise can be used to construct a MAU model. Adjustments to the MAU model to increase accuracy should be explored. Utility gathering is a successful technique for increasing the patients' role in health care decision-making. Future work will employ these techniques to elicit utilities from prostate cancer patients and subsequently utilized to rank treatment plans.

252 PROGNOSTIC VALUE OF MAXIMAL COMPARED TO 3-MINUTE VASCULAR OCCLUSION TEST IN PATIENTS WITH SEVERE SEPSIS OR SEPTIC SHOCK

Brisbane W, Shen J, Walters E, Nguyen H. Loma Linda University, Loma Linda, CA; Loma Linda University, Loma Linda, CA; Loma Linda University, Loma Linda, CA; Loma Linda University, Loma Linda, CA.

Purpose of Study: Severe sepsis and septic shock involve immune activation and inflammatory response at the vascular endothelium leading to microvascular injury. This diffuse endothelial disruption is responsible for
organ dysfunction, global tissue ischemia, and death. Measurement of tissue oxygen saturation (StO2) by near-infrared spectroscopy at the thenar eminence during a 3-minute vascular occlusion at the forearm has been shown to provide a marker of disease severity. In this study, we compared the prognostic value of StO2 after the 3-minute vascular occlusion test (VOT) to that of a maximal VOT.

**Methods Used:** A prospective observational study was performed in severe sepsis or septic shock patients presenting to the ED and admitted to the medical ICU. The lowest StO2 measurement (StO2min) at the thenar eminence (InSpectra 650 StO2, Hutchinson Technologies, MN) after a 3-min VOT and maximal VOT was obtained at hour 0, 1, 2, 3, 4, 5, 6, 12, 24, 36, 48, and 72. The 3-min VOT was performed with a tourniquet on the forearm inflated for 3 minutes. The maximal VOT was similarly performed but with the tourniquet inflated until StO2 reached a lowest steady state plateau.

**Summary of Results:** Sixteen patients were enrolled with age 65.9 ± 12.8 years and in-hospital mortality was 50%. APACHE II and lactate in survivors vs. non-survivors were 18.75 ± 8.1, 28.25 ± 10.2 (p = 0.06), and 3.9 ± 1.8 mmol/L vs. 8.3 ± 3.6 mmol/L (p = 0.01), respectively. Baseline StO2 and StO2min are illustrated in the Table.

**Conclusions:** StO2min after maximal vascular occlusion instead of only three minutes of occlusion is better at identifying mortality in severe sepsis and septic shock. The association of StO2min with oxygen transport mechanisms remains to be examined.

### Summary of Results

<table>
<thead>
<tr>
<th>Number of Measurements</th>
<th>StO2 Baseline (%)</th>
<th>3-min VOT StO2min (%)</th>
<th>Max VOT StO2min (%)</th>
<th>Time to max VOT StO2min (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survivors</td>
<td>85</td>
<td>74.1 ± 10.1</td>
<td>44.1 ± 15.4</td>
<td>25.6 ± 15.1</td>
</tr>
<tr>
<td>Non-survivors</td>
<td>73</td>
<td>71.7 ± 9.9</td>
<td>45.5 ± 11.6</td>
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</tr>
</tbody>
</table>

**F-Value**

n/a 0.13 0.52 <0.01 <0.01

**254 TELEPHONE FOLLOW-UP OF EMERGENCY DEPARTMENT PATIENTS: PATIENT CHARACTERISTICS AND CONTACT PERCENTAGES**

Oberfoell S1, Arora S2, Menchine M1, 1DGSOM, Los Angeles, CA and 2Keck School of Medicine at the University of Southern California, Los Angeles, CA

**Purpose of Study:** Emergency Physicians (EPs) fear being unable to contact patients for follow-up or to provide results of important diagnostic tests. Many EPs will not discharge patients until all diagnostic tests have returned, even if the likelihood of a consequential finding is low. This reluctance to discharge may result in longer wait times and Emergency Department (ED) crowding. We aim to quantify the percentage of patients who can be reached after leaving the ED. We hypothesize higher contact per-

**Methods Used:** Based on our literature review, we estimated control follow up percentages to be 75% and calculated that 626 total subjects would be needed to achieve 80% power to detect a 10% absolute difference between groups. We developed and field-tested a bilingual survey instrument on EP interventions. We conducted a randomized, controlled trial of a consultation model on a sample of ED patients. Research assistants approached patients, obtained informed consent, and enrolled subjects in the trial. Subjects were random-

**Conclusions:** Halfway to our desired total enrollment we are not seeing signaling in immune

**255 REGULATION BY ESTROGEN AND PROGESTERONE OF INTERFERON ALPHA SIGNALING IN HUMAN LEUKOCYTES**

Zheng J, Hughes G University of Washington School of Medicine, Seattle, WA

**Purpose of Study:** 90% of systemic lupus erythematosus (SLE) patients are female, and the incidence peaks during reproductive years when estrogen (Es) and progesterone (Pg) are at their highest. Studies show that Es increases the risk of SLE in both human and animal models, while Pg is protective. Interferon alpha (IFN-α) is a central pathogenic cytokine in SLE and can directly activate multiple immune cell types to favor autoimmunity. Though recent studies suggest a link between Es and IFN-α signaling in immune cells, the relationship is not well understood; and very little is known about the effects of Pg. To study this relationship between female reproductive hormones and lupus autoimmunity, we tested the hypothesis that, in human leukocytes, Es enhances IFN-α signaling while Pg suppresses it.

**Methods Used:** Peripheral blood mononuclear cells (PBMCs) were isolated from blood of healthy donors. Cells were cultured in media, ethanol vehicle, or physiologic concentrations of β-estradiol (Es), Pg, or a combination of both hormones, with or without IFN-α. Total RNA was isolated, and expression of IFN-α inducible genes (CXCL10, MX1, PRK, HIF11, ISG20) was measured by quantitative PCR and normalized to housekeeping gene 18S rRNA.
Summary of Results: Consistent with our hypothesis, we observed that Es enhanced IFN-α signaling in human leukocytes; we did not observe an effect of Pg. Es treatment significantly increased IFN-α induced expression of CXCL10 (p=0.0273, two-tailed paired T-test). A similar trend was observed for MXI, PKK and IFIT1. Es alone did not increase expression of any of these genes. In addition, we observed that IFN-α signaling response to Es was bi-modal, because PBMCs from some donors consistently showed Es-sensitivity while others did not.

Conclusions: Our research shows for the first time that Es can enhance IFN-α induced gene expression in human leukocytes of healthy donors, possibly by regulating IFN-α signaling pathways. This suggests a novel mechanism by which Es increases a woman’s risk of developing SLE. Additional experiments will allow us to determine whether Es regulates all or a subset of IFN-α-inducible genes and what factors determine Es sensitivity, such as Es receptor expression. We plan to extend these findings to PBMCs of SLE patients to ask whether abnormal sensitivity or resistance to Es or Pg may contribute to disease.

256 EOSINOPHILIC VASCUITIS

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Case Report: Eosinophilic vasculitis is usually equated with Churg-Strauss syndrome, a rare systemic, anti-neutrophil cytoplasmic antibody-associated vasculitis. Churg-Strauss syndrome is most often found in patients with asthma, but the onset can occur simultaneously with the diagnosis of asthma. We present the case of a 20 year old male college wrestler, who initially presented with a complaint of dyspnea while wrestling. The patient was found to have recurrent pleural effusions for which he was referred to cardiothoracic surgery. During the second decortication procedure, nodularity and thickening were discovered, and a wedge resection of the lung was performed.

The histology was notable for a reactive pleuritis and necrotizing granulomas of the lung with eosinophilic necrosis, vasculitis and interstitial lymphoid infiltrates. Rheumatology was consulted for suspected Churg-Strauss syndrome. Laboratory studies demonstrated a peripheral eosinophilia of 10.2%. However, serum anti-neutrophil cytoplasmic antibodies, human immunodeficiency virus antibodies, and antinuclear antibodies were all negative. The initial fungal and parasitic stains were reported negative. A review of the pathology slides led to the question of the patient’s participation in a popular fad involving live crab ingestion, which he confirmed having done at a sushi bar four months prior to onset of symptoms. Serum antibody tests confirmed the diagnosis of Paragonimus westermani infection. P. westermani infections are extremely rare in the United States, but found more commonly in endemic areas of Asia. Pulmonary parasitic infections of P. westermani usually demonstrate necrotizing granulomas with an eosinophilic infiltrate; however, there is little evidence in the literature of this parasitic infection leading to a vasculitis. Our case illustrates a rare parasitic infection diagnosed as a Paragonimus-induced eosinophilic vasculitis of the lung.

Conclusions: These results demonstrate that the tumor suppressor and T-regulatory functions of Foxp3 are mediated through separate signaling pathways. Our findings may have important application in the design of autoimmune and cancer therapeutics targeting FOXP3.

258 THE IMMUNOMODULATORY ROLE OF HIGH DOSE INTRAVENOUS IMMUNOGLOBULIN IN A MURINE MODEL OF BILARY ATRESIA

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Purpose of Study: Biliary atresia (BA) is an inflammatory, sclerosing disease affecting the bile ducts of neonates causing choleostasis. While the incidence of BA is low, it is the most common indication for pediatric liver transplantation. Current understanding of the pathophysiology of BA suggests that a viral insult initiates bile duct injury followed by progressive autoimmune-mediated inflammation and fibro-obliteration of the bile ducts. Intravenous immunoglobulin (IVIg) is composed of polyclonal, polyspecific immunoglobulin and has demonstrated clinical benefit in several other autoimmune and inflammatory diseases. One mechanism of IVIg action is to increase Treg production, thereby decreasing autoimmune-mediated injury. The purpose of our study is to determine if markers of bile duct injury are diminished and Treg production is increased in IVIg-treated BA mice.

Methods Used: Neonatal BALB/c mice were injected with reovirus rotavirus (RRV) or Hanke’s Balanced Salt Solution (HBSS) 12-18 hours after birth. On day 7, 9, 11 BA mice were given 1g/kg high dose IVIg or albumin by intra-peritoneal injection. Survival in each group was monitored and, at 14 days post-injection, mice were sacrificed and blood, livers, and extrahepatic bile ducts were collected. Direct bilirubin assay was performed on serum from pools of 3-4 mice. Liver immune cells were isolated by Percoll density gradient and quantification of liver Tregs was performed by flow cytometry.

Summary of Results: IVIg did not improve survival in BA mice compared to albumin control. A significant decrease in direct bilirubin levels was observed in mice treated with IVIg (4.1±0.9 mg/dL) compared to albumin (10.2±1.5) (unpaired t-test, p= 0.01) and untreated BA controls (10.5±1.9; p=0.001). Increased Treg production was observed in IVIg-treated BA mice (10.6±1.2% Foxp3) compared to albumin (7.5±0.1; p=0.06) and untreated controls (5.9±1.4; p=0.07).

Conclusions: Immune therapy with high dose IVIg was associated with decreased bilirubin suggesting diminished bile duct injury. An increase in Tregs in IVIg-treated mice implies better control of autoimmunity and may be a possible mechanism of action for IVIg in BA.

259 HUMAN TRANSITIONAL B CELLS IN NORMAL AND AUTOIMMUNE PERIPHERAL BLOOD

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Purpose of Study: SLE and RA tissues have been shown to have higher levels of autoreactive B cells suggesting a defect in the negative selection process that occurs during the immature stages of B cell development. Mouse studies indicate that immature stages of B cell development can be subdivided into the transitional stages T1 and T2. When these transitional B cells are exposed to activation stimuli, the cells undergo receptor editing, anergy, or apoptosis in order to eliminate autoreactive cells. The TNF family members APRIL and BAFF have been shown to promote survival of these transitional subsets in the mouse. Current understanding of the pathophysiology of BA suggests that a viral insult initiates bile duct injury followed by progressive autoimmune-mediated inflammation and fibro-obliteration of the bile ducts. Intravenous immunoglobulin (IVIg) is composed of polyclonal, polyspecific immunoglobulin and has demonstrated clinical benefit in several other autoimmune and inflammatory diseases. One mechanism of IVIg action is to increase Treg production, thereby decreasing autoimmune-mediated injury. The purpose of our study is to determine if markers of bile duct injury are diminished and Treg production is increased in IVIg-treated BA mice.

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Conclusions: Immune therapy with high dose IVIg was associated with decreased bilirubin suggesting diminished bile duct injury. An increase in Tregs in IVIg-treated mice implies better control of autoimmunity and may be a possible mechanism of action for IVIg in BA.
Summary of Results: The use of markers CD21 and CD24 allowed us to identify T2, late transitional T2, and FM subsets in normal and autoimmune (SLE and RA) peripheral blood. A lower percentage of T2 cells were identified in the SLE and RA patient samples compared to normal blood. In SLE and RA samples, the receptors BAFF-R and TACI and the ligand APRIL were elevated on CD19+ IgM+B cells compared to normal peripheral blood. Conclusions: The use of CD21 and CD24 markers can distinguish between T1, T2, and FM B cell subsets. Fewer transitional subsets are present in autoimmune peripheral blood and represent a more activated phenotype as depicted by the higher expression of APRIL, BAFF-R, and TACI. The elevated levels of members of TNF family could contribute to the escape of autoreactive B cells during the negative selection process during the transitional stages of B cell development.

260 AUTOIMMUNITY AND ASBESTOS RELATED DISEASE IN AMPHIBOLE EXPOSED INDIVIDUALS OF LIBBY, MONTANA
Marchand LS1, Pfau J2 University of Washington, Seattle, WA and 2Idaho State University, Pocatello, ID.

Purpose of Study: Autoimmune disorders stemming from an over-reactive immune system are the cause of substantial disease and disability. Recent literature has proposed a link between asbestos exposure and various autoimmune responses. Exposure to asbestos continues to occur throughout the world making this issue a current health concern. Residents of the Libby, Montana area have experienced significant exposure to amphibole asbestos due to the mining of asbestos-contaminated vermiculite near the community. This population exhibits an auto-immune-like disorder that has yet to be well defined. Pleural and/or interstitial abnormalities are common findings in these patients as both malignant and non-malignant disease types. This study investigated the relationship between autoimmunity, asbestos related disease (ARD), and asbestos exposure.

Methods Used: Serum samples from Libby’s Center for Asbestos Related Disease were evaluated through indirect fluorescence testing, checking for anti-nuclear antibody (ANA) and cell based ELISA testing, looking for anti-mesothelial antibody (AMA).

Summary of Results: It was found that 62% of the Libby samples were ANA positive, a frequency nearly double what would be expected of a healthy population. On average it was determined that the odds of having pulmonary disease was over 9 times greater for individuals with positive ANA tests compared with individuals with negative ANA test results. The Libby population also exhibited a much higher level of AMA than did the control group. A total of 23 (19%) Libby subjects were found to have significantly high AMA levels and were established as AMA positive. Of these subjects nearly 75% (17) had pleural disease. This meant that on average the odds of having pleural disease is 4.72 times greater for individuals with positive AMA levels compared to subjects without positive AMA. In contrast there was no significant relationship between AMA positivity and interstitial disease. Sex and age were controlled for.

Conclusions: Data supports the possibility that AMA’s are contributing to ARD. Additional research may be needed to further establish this relationship, but determining the nature of this association with clinical disease progression might lead to an excellent target for therapeutic intervention and disease screening.

261 LYMPHOCYTE PROFILES FROM THE SOFT TISSUES OF METAL-ON-METAL HIPS
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Purpose of Study: Aseptic loosening in metal-on-metal hips is classified as being caused by wear debris or metal allergy. Metal sensitivity is associated with increased lymphocytic aggregates termed ALV and controls compared to wear. The purpose of this study was to characterize the immunological profiles of lymphocytes and other immune cells in tissues around failed M hips using IHC to characterize the nature of the immune response.

Methods Used: Tissue samples from 21 archived MM hips were fixed in formalin, paraffin embedded, sectioned and stained with H&E. The modes of failure included 11 metal allergy, 8 high wear-related pseudotumor, and 2 other cases for controls. IHC was performed using a variety of histological markers specific for immune cells. A 0-4 scoring system was used based on the percentage of cells stained in a HPF. Digital image analysis was based on recognition by color thresholding.

Summary of Results: 0-4 scoring of all cases showed more total T and T cell subtypes (CD4, CD8, CD8RO) in metal allergy cases than high wear cases or controls. Additionally, lymphocyte subpopulations in the metal allergy group showed increased levels of both T and B cells. Plasma cells were less present than T and B cells in metal allergy cases. Digital analysis produced values incongruent with manual scoring. These differences occurred mainly in samples that contained wear debris, necrosis, blood, and heterogeneous tissues.

Conclusions: Originally explained as delayed type hypersensitivity (DTH), soft tissue reactions containing a unique lymphocyte dominated histological profile were revealed to contain T cells, B cells, and plasma cells. The presence of B and plasma cells is inconsistent with DTH. Such heterogeneous lymphocytic populations, particularly in metal allergy, suggest an adaptive immune response. More specifically, the prevalence of memory T cells and activated T cells (CD45RO) suggest a T cell mediated adaptive response. An antibody dependent (Type II) mechanism occurring alongside the DTH reaction may explain the unexpected cells. Wear debris and constant metal ion exposure is associated with a T cell lymphopoeinia and may alter lymphocyte profiles. Further research requires elucidating the role of antibody processes in this response.

262 OUTCOMES AND COST-EFFECTIVENESS OF RESIDENT-FORMED PALPATION-GUIDED INTRAARTICULAR PROCEDURES IN A TEACHING HOSPITAL
Yaqub S1, Ashraf U2, Norton H1, Gibb J1, Sibbitt WL1, Bankhurst A1 1University of New Mexico Health Sciences Center, Albuquerque, NM and 2Texas Tech University Health Sciences Center, Amarillo, TX.

Purpose of Study: Patients are often apprehensive of undergoing an invasive procedure performed by inexperienced residents, even with attending supervision. We hypothesized that intraarticular procedures would be less painful and have better outcomes when performed by experienced attending proceduralists compared to resident and fellows in training.

Methods Used: 326 joints underwent arthrocentesis followed by corticosteroid injection using the one-needle two-syringe techniques performed by 1) experienced proceduralists, 2) residents with direct attending supervision, or 3) fellows with direct attending supervision. All procedures were performed with a mechanical syringe. Pain was measured with the 0–10 cm Visual Analogue Pain Scale (VAS) 1 prior to the procedure, 2) during needle placement (needle introduction, anesthesia, and arthrocentesis), 3) during injection of corticosteroid, 4) 2 week post-procedure, and 5) 6 months postprocedure. A responder was defined as an asymptomatic joint at 2 weeks (VAS=2 cm). Other outcomes included therapeutic duration (months), time to next injection, cost/patient/year, and cost/responder/year.

Summary of Results: As measured by the 10 cm VAS, procedural pain and injection pain for residents and fellows were not statistically different than attendings (p = 0.4 and 0.5, respectively). Similarly, pain at 2 weeks, pain at 6 months, percentage of responders, percentage of non-responders, therapeutic duration, time to next injection, cost/patient/year, and cost/responder/year were not different between either residents or fellows compared to experienced proceduralists (p>0.1 for all).

Conclusions: The procedural pain, outcomes, costs, and cost-effectiveness of resident and fellow performed intraarticular injection/aspiration procedures performed with attending supervision in a teaching hospital are identical in all measures of quality to those performed by experienced proceduralists. These outcome data are important to reassure patients and 3rd party payers that quality of care at teaching hospitals is high.

263 CHOLESTASIS AS AN ATYPICAL AND MISLEADING PRESENTATION OF KAWASAKI DISEASE: CASE REPORT AND LITERATURE REVIEW
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Purpose of Study: Kawasaki Disease (KD) is an acute self-limited vasculitis of childhood that classically is characterized by fever, bilateral non-exudative conjunctivitis, erythema of the lips and oral mucosa, extremity changes, rash, and cervical lymphadenopathy. Coronary artery aneurysms or ectasia develop in 15% to 25% of untreated children. We report a case of
a 4 ½ yr old with a very atypical presentation of KD involving cholestasis, and review the literature on this rare presentation of KD.

Methods Used: Records from this patient’s 12-day hospitalization were reviewed. A literature review was conducted using PubMed with key search terms “Kawasaki Disease” and “cholestasis” or “jaundice.”

Summary of Results: A previously healthy 4 ½ yr old presented with fever, emesis, abdominal pain, diffuse rash, and jaundice. Lab findings revealed conjugated hyperbilirubinemia. She was initially diagnosed with cholangitis; however, over the next few days the findings of classic KD progressively developed. Her KD diagnosis and initiation of therapy occurred within the optimal 10 day window from the onset of KD symptoms. After 2 doses of intravenous immunoglobulin and pulse steroid therapy, she demonstrated marked improvement. No coronary artery changes have been detected on echocardiography.

Two case reports from the literature had similar presentations. The first was a 6 yr old who had a delay in diagnosis due to his unusual presentation and developed diffuse dilatation of his coronary arteries, which improved with treatment. The second was a 10 yr old who also had a significant delay in diagnosis and for whom treatment was initiated after the 10 day window. He had a very complicated course consisting of multiple coronary artery aneurysms with severe multiple stenoses requiring surgical intervention.

Conclusions: Our case adds to the 2 similar reports published in the literature highlighting the importance of considering KD in a child who presents with cholestasis, abdominal pain, and fever of unknown etiology. Not considering this atypical presentation may lead to a late diagnosis, and loss of the 10-day time window for early intervention, thus increasing the risk of coronary artery abnormalities.

264 ULTRASOUND-GUIDED ARTHROCENTESIS OF THE KNEE USING NEW ASPIRATING SYRINGE TECHNOLOGIES

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Purpose of Study: Ultrasound image-guidance results in less traumatic, more complete arthrocentesis. The present randomized controlled trial investigated new aspirating syringe technologies for image-guided arthrocentesis of the knee.

Methods Used: 42 ultrasound-guided arthrocentesis procedures of the knee were randomized to new aspirating technologies: 1) 60 ml vacuum syringe, or 2) the 25 ml RPD (reciprocating procedure device) mechanical syringe. The one-needle two-syringe technique was used. Completeness of arthrocentesis was determined by sonography. Outcome measures included patient pain by the 10 cm Visual Analogue Pain Scale (VAS), synovial fluid volume, complications, and therapeutic outcome at 2 weeks. Needle control of the new technologies was measured in the linear displacement model and compared mechanical and vacuum syringes to conventional syringes.

Summary of Results: Both the mechanical syringe and the vacuum syringe controlled the needle better than a conventional syringe, reducing unintended forward penetration by 75% (3.6±0.5 mm) and 87% (12.0±4.2 mm), respectively (p=0.0001). Image-guided aspiration with both aspiration technologies permitted complete arthrocentesis with low levels of procedural pain (10 cm VAS: Vacuum Syringe: 2.9±3.1 cm, Mechanical Syringe: 3.1±2.5 cm, p<0.08) and significant fluid yield (Vacuum Syringe: VAS: 35±23 ml, Mechanical Syringe: 34±27 ml, p=0.9). The vacuum syringe permitted facile automatic aspiration of up to 60 mls; the mechanical syringe permitted 25 ml aspiration before a syringe exchange was required.

Conclusions: Ultrasound-guided arthrocentesis of the knee can be facilely achieved with new aspirating syringe technologies with improved needle control, enhanced patient safety, large volume aspiration, and complete joint decompression.

265 OUTCOMES AND COST-EFFECTIVENESS OF ULTRASOUND-GUIDED INVASIVE PROCEDURES PERFORMED BY RESIDENTS IN A TEACHING HOSPITAL

Ashraf U, Weis B, Smalligan RD, Gibb H, Norton H, Sibbitt WL,
Bankhurst A, Texas Tech University Health Sciences Center, Amarillo, TX and University of New Mexico Health Sciences Center, Albuquerque, NM.

Purpose of Study: Ultrasound-guided procedures are becoming increasingly important in invasive medicine, but skills in ultrasound require considerable experience. We hypothesized that ultrasound-guided procedures would be less painful and have better outcomes when performed by experienced proceduralists compared to those performed by residents and fellows in training.

Methods Used: 256 joints underwent ultrasound-guided arthrocentesis followed by corticosteroid injection using the one-needle two-syringe technique performed by 1) experienced proceduralists, 2) residents with attending supervision, or 3) fellows with attending supervision. All ultrasound-guided procedures were performed with a mechanical syringe. Pain was measured with the 0–10 cm Visual Analogue Pain Scale (VAS): 1) prior to the procedure, 2) during needle placement (needle introduction, anesthesia, and arthrocentesis), 3) during injection of corticosteroid, 4) 2 week post-procedure, and 5) 6 months post-procedure. A responder was defined as an asymptomatic joint at 2 weeks (VAS<2 cm). Other outcomes included therapeutic duration (months), time to next injection, cost/patient/year, and cost/responder/year.

Summary of Results: As measured by the 10 cm VAS, procedural pain and injection pain during ultrasound-guided procedures for residents and fellows were not statistically different than experienced proceduralists (p = 0.6 and 0.5, respectively). Similarly, pain at 2 weeks, pain at 6 months, percentage of responders, percentage of non-responders, therapeutic duration, time to next injection, cost/patient/year, and cost/responder/year were not different between either residents or fellows compared to experienced proceduralists (p>0.1 for all). Conclusions: Ultrasound-guided procedures performed by trainees were identical in all measures including procedural pain, outcomes, costs, and cost-effectiveness to those performed by experienced ultrasound proceduralists. Since ultrasound guidance is becoming increasingly important in invasive medicine, these equivalent outcome data are important to reassure patients and 3rd party payors that quality of ultrasound procedures at teaching hospitals is high.

Infectious Diseases
Concurrent Session
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266 IDENTIFICATION OF NEUTROPHILS AS A PREDOMINANT SOURCE OF IL-1ß PRODUCING CELLS IN THE EARLY INNATE IMMUNE RESPONSE TO STAPHYLOCOCCUS AUREUS CUTANEOUS INFECTION

Hebron FF, Cho J, Ramos RI, Miller LS UCLA David Geffen School of Medicine, Los Angeles, CA.

Purpose of Study: Staphylococcus aureus is a gram-positive bacterium responsible for a wide range of skin infections including superficial skin infections such as impetigo and more invasive skin infections such as cellulitis and folliculitis. It has been previously demonstrated that the IL-1ß-IL-1R pathway is critical for neutrophil recruitment to a site of S. aureus infection in the skin. However, the dynamics of IL-1ß production during the course of infection and the cellular sources of IL-1ß have not yet been defined.

Methods Used: The goal of this study was to determine the kinetics of IL-1ß gene expression in real-time during a S. aureus cutaneous infection by utilizing an IL-1ß-dsRed reporter mouse line and to identify the IL-1ß producing cells using confocal microscopy.

Summary of Results: IL-1ß gene induction could be detected in the infected-skin lesions of live mice in real-time using in vivo fluorescence imaging as soon as 8h post infection and peaked by 24h. The majority of these early IL-1ß-producing cells were detected with the neutrophil marker MPO while a small number of IL-1ß-producing cells were detected with the macrophage marker M20.

Conclusions: These findings identify what we believe to be a novel role for neutrophils as the predominant source of IL-1ß during the early innate immune response to cutaneous S. aureus infection.

267 INHIBITION OF HERPES SIMPLEX VIRUS (HSV) GLYCOPROTEIN’S gB, gD, gH-gL INDUCED CELL FUSION AND VIRAL SPREAD VIA HEPARAN MICROMIMICS

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Purpose of Study: Multinucleated giant cells (polykaryocytes) resulting from virus-induced cell fusion are a hallmark of herpes simplex virus-type-1 (HSV-1) infection. However, the role of HSV-1 3-O-sulfated heparan sulfate (3-OS HS) receptor during cell fusion is poorly understood. The goal of this study was to understand the role of heparan mimics in blocking the HSV-1 infection.

Methods Used: HSV-1 glycoprotein induced quantitative reporter gene (luciferase) based cell fusion assay was used. The counting of multinucleated giant cells (polykaryocytes) was achieved by using fluorescent microscopy. Effector Chinese hamster ovary (CHO-K1) cells expressing various combinations of HSV-1 glycoproteins were co-cultured with the 3-OST-3 expressing primary cultures of human corneal fibroblasts (CF), a natural target cell-type for HSV-1 infection. Pre-treatment of effector cells with heparinase enzyme (4U/ml) and heparan mimics (5.6 nM) were used to demonstrate the role of 3-OS HS during cell fusion.

Summary of Results: We demonstrated that cell-to-cell fusion and polykaryocytes formation required expression of four essential HSV-1 glycoprotein (gB, gD, gH-GL) and exhibited a very strong dependence on the expression of heparan sulfate on CF. Further enzymatic removal of HS from CF surface by heparanase-I treatment severely impaired the fusion reaction. Interestingly, the incubation of effector cells expressing HSV-1 glycoprotein’s with heparan sulfate mimics inhibited significantly both membrane fusion and polykaryocyte formation.

Conclusions: Our results indicate that 3-OS HS could play a crucial role in HSV-1 induced cell-to-cell fusion during corneal eye infection. Generation of specific inhibitors targeting against HSV-1 gD epitope that interacts with 3-OS HS would be beneficial in the development of the thabets to prevent viral spread in the corneal stroma. Here, we provide evidence for the physiological significance of 3-OS HS mediated cell-to-cell fusion.

268 THE ROLE OF FC-GAMMA RECEPTOR IIIB VARIATION IN CONDITIONING INTERFERON-GAMMA LEVELS IN SUB-SAHARAN AFRICAN CHILDREN WITH SEVERE MALARIAL ANEMIA

Chaudhary A, Perkins D University of New Mexico, Albuquerque, NM.

Purpose of Study: Plasmoid faciparum infections are associated with the development of severe malarial anemia (SMA), one of the leading causes of childhood morbidity and mortality in sub-Saharan Africa. Although, the genetic and molecular basis of SMA is only partially defined, severity of malarial disease is affected by dysregulation of host-derived inflammatory mediators. Fcy- Receptor IIIB (FcγRIIB) facilitates binding to the Fc portion of IgG1 and IgG3, which are important in modulating immune responses and in protection against P. falciparum in endemic areas. The granulocytic antigens NA1 and NA2 are the two recognized allelic forms of FcyRIIB. The overall aim of this study was to determine the association of FcγRIIB variants with severe malarial anemia and cytokine production in children presenting at a rural hospital in western Kenya with P. falciparum parasitemia.

Methods Used: Polymere chain reaction and Multiplex Assay were utilized to determine how variation in FcγRIIB conditions functional changes in cytokine production in children (n=383) with P. falciparum malaria.

Summary of Results: Stratification of children according to parasitism status revealed that the SMA group [hemoglobin (Hb) <6.0 g/dL] had lower levels of circulating IFN-γ than the non-SMA group (Hb>6.0 g/dL) (P=0.11). Multivariate analyses controlling for the confounding effects of age, gender, HIV-1 status, bacteremia, and sickle-cell trait showed that the heterozygous FcγRIIB NA1/NA2 genotype were protected from SMA (OR=0.755, [95% CI: 0.40-1.20]) relative to homoygous NA1 or NA2. Functional analyses showed that heterozygosity at FcγRIIB (NA1/NA2) was associated with significantly increased circulating IFN-γ levels relative to homzygous individuals (NA1; P=0.02).

Conclusions: Finding important genetic determinants of phenotypic outcomes in children with malaria, would enable clinicians to predict which children are at a higher risk for developing SMA, and may promote earlier treatment interventions to reduce mortality. Results from this study illustrate the need for further exploration to determine if suppressed levels of IFN-γ secretion due to variation in the FcγRIIB receptor could be ameliorated by exogenous administration of IFN-γ to reduce severe malaria in individuals with P. falciparum infection.

269 PROINFLAMMATORY IMMUNE RESPONSE AND PUERPERAL GROUP A STREPTOCOCCAL SEPSIS

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Purpose of Study: Group A streptococcus (GAS) is a serious and potentially fatal, source of postpartum infection. Bacterial virulence factors influence pathogenesis but do not effectively predict disease susceptibility or severity. The objective of this study was to determine if altered maternal innate immune response is associated with puerperal GAS sepsis.

Methods Used: Case-control analysis of innate immune response in women with a history of puerperal GAS sepsis. Cases had febrile illness and/or endometritis during the postpartum period and at least one positive culture. Cases were further stratified into mild vs. severe; severe cases had either 1) disease requiring surgical exploration/debridement, 2) admission to ICU, 3) toxic shock syndrome, or 4) hospitalization > 14 days. Cases were at least one year post-infection at the time of analysis. Controls were healthy subjects matched 1:1 by age, parity, and race. Immunoassays (Luminex) assessed cytokine, inflammatory marker production in stimulated peripheral blood mononuclear cells. Concentrations of 13 cytokines/inflammatory markers (IFNγ, IL1B, IL2, IL4, IL5, IL6, IL8, IL10, IL12, IL13, TNFα, IL2r, and soluble CD40L) were measured in response to heat-killed emm1 and emm28 GAS, the M-serotypes most closely associated with puerperal morbidity.

Summary of Results: Ten controls, 10 severe cases, and 6 mild cases were analyzed. In response to stimulation, CD40L, IL1B, IL2r, and IL6 production were significantly increased in both severe and mild cases compared to controls (CD40L mean response 131.7 vs. 69.2 pg/ml, p=0.001; IL1B mean response 1143.3 vs. 503.2 pg/ml, p=0.0001; IL2r mean response 13.7 vs. 6.4 pg/ml, p=0.001; IL6 mean response 4841.1 vs. 2534.0 pg/ml (p=0.01).

Conclusions: Puerperal GAS sepsis is associated with altered maternal innate immune responses. The markedly increased production of inflammatory cytokines may contribute to susceptibility and severity of puerperal GAS disease.

270 THE INCIDENCE OF BLOOD AND BODY FLUID EXPOSURE IN OUR TRAINEES

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Purpose of Study: The incidence of blood and body fluids exposure in trainees is not well documented. The purpose of this study was to document exposure rates in medical students and high risk specialty residents.

Methods Used: Third and fourth year medical students (MS), Surgery and Emergency Medicine residents (RS) were anonymously surveyed regarding percutaneous, cutaneous and mucous membrane exposures in the 6 months preceding survey administration.

Summary of Results: 770 surveys were completed. 37.7% (58/154) of RS and 15.4% (95/616) of MS were exposed. The incidence of exposure increased in a stepwise fashion with year of training. 31.2% involved a trauma patient. Exposure occurred most frequently while performing a procedure (60.6%) followed by observation of a procedure (5.7%) and blood draw (2.9%). The most frequent RS exposure was solid needlestick (31.3%), followed by hollow needlestick (20.9%) and splash in eyes (13.9%); for MS, splash in eyes (28.4%), was followed by solid needlestick (27.5%) and splash on skin (18.3%). Contributing factors were uncontrolled fluid splash (44.3%), patient movement (12.5%), fatigue (10.8%), unguarded sharps (9.7%) and unsafe instrument pass (6.8%). Only 27.3% of exposures were reported to occupational health. 41.5% of source patients underwent HIV and Hepatitis testing. Overall, 35.4% of incidents reported to occupational health services triggered the administration of prophylaxis. None reported known serocversion related to the exposure.

Conclusions: Body fluid exposure is common in trainees, increasing with level of training to greater than 25% in final year medical students and 70% in final year residents. Less than a third were reported to occupational health.
Identification of the reasons for non-reporting and potential target areas for the prevention of exposure is warranted.

271 EFFECTIVENESS OF ROUTINE METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS SCREENING
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Purpose of Study: Hospitalized patients are routinely screened for MRSA as a means to reduce hospital acquired MRSA. We sought to determine how reliable screening tests identify patients with proven MRSA infection.
Methods Used: We retrospectively reviewed charts of all patients aged 0–18 years hospitalized in a community based children's hospital between November 2008–November 2009. Patients with culture proven invasive MRSA infection were identified and their screening tests for MRSA were reviewed to determine any correlation. We assumed a null hypothesis that culture proven invasive MRSA results were independent of screening results and an alternative hypothesis that there was an association. Data analysis was performed by Fisher’s exact test using SPSS (18) with a p-value < 0.05. Our study was approved by the Institutional Review Boards of University of Nevada, Reno and Sunrise Hospital.
Summary of Results: 3453 patients were screened for MRSA during our study period. 3.45% (119/3453) had a positive test result. 150 patients were identified to have culture proven MRSA infection. Among those with culture proven MRSA infection, 42.1% (32/76) had a positive screening test result: nasal cultures accounted for 6.25% of positive cultures, 28.1% throat cultures, 12.5% urine cultures, 37.5% rectal, 6.25% axillary and 9.38% other sites. The null hypothesis was rejected in support of the alternative hypothesis.
Conclusions: A significant proportion of patients with invasive MRSA infection also had a positive MRSA screening test result. Routine screening for MRSA potentially could reduce Hospital acquired MRSA infection by identifying colonized patient who can then be isolated.

273 BOPA IS REQUIRED FOR PERSISTENCE OF BRUCELLA
Athuri V, Toosi R UC Davis, Davis, CA.
Purpose of Study: Brucella species cause brucellosis, a chronic disease characterized by undulating fever, in about 500,000 people every year. Survival of bacteria in macrophages and chronic persistence in the reticuloendothelial system (RES) of the host, two key features of Brucella pathogenesis, are dependent on the VirB type IV secretion system (T4SS). Previous studies have shown that the T4SS is involved in both trafficking of the Brucella-containing vacuole to an endoplasmic reticulum-derived niche and activation of genes involved in inflammation and immunity. However, the identity of the T4SS-secreted molecules contributing to these two phenotypes is unknown. A recent screen for genes co-regulated with the T4SS structural genes identified BopA as a protein with features of a potential effector. Genomic analysis revealed a second protein, designated BopB, which is similar to BopA at the amino acid level.
Methods Used: To determine whether BopA and/or BopB play a role in intracellular survival and persistence mutants lacking one or both of these genes (∆bopA, ∆bopB, and ∆bopA∆bopB) were constructed and characterized.
Summary of Results: The ∆bopA mutant was attenuated for survival in immortalized murine macrophages and exhibited reduced persistence in mice. In addition, T4SS dependent inflammassome activation, required for production of the cytokine IL-1 beta and IL-18 is partially dependent on BopA. The ∆bopB and ∆bopA∆bopB mutants are currently being characterized.
Conclusions: These results suggest that BopA contributes to survival of B. melitensis in macrophages, possibly by interacting with host cell proteins after cytosolic translocation.
specific regional prevalence of macrolide resistance mutations in T. pallidum is required before informed local decisions can be made concerning the use of azithromycin as an alternative to penicillin in the treatment of syphilis.

Morphogenesis and Malformations

Concurrent Session
1:00 PM
Friday, January 28, 2011

275 VIABILITY WITH GERM LINE HRAS G12V MUTATION IN COSTELLO SYNDROME IS DUE TO REDUCED MRNA EXPRESSION ASSOCIATED WITH DINUCLEOTIDE SUBSTITUTION

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Purpose of Study: Costello syndrome is most commonly due to p.G12S activating mutations in HRAS with relatively weak transforming activity. G12V has the highest transforming activity and greatest frequency in human cancers, but is rare in Costello syndrome. G12V has been hypothesized to result in early lethality and a more severe phenotype in most cases: mice with this mutation have high perinatal mortality. We report the molecular and phenotypic findings of a 12-year-old boy with an attenuated Costello phenotype with a germline p.G12V mutation resulting from a dinucleotide substitution, c.35_36delinsTG without evidence of mosaicism.

Methods Used: DNA and RNA extracted from peripheral blood; DNA also extracted from hair, cheek swab, and urine. Bidirectional sequencing for the coding regions and intron/exon boundaries of HRAS performed on DNA from blood and mutation confirmed in other samples. Random-primed cDNA prepared from RNA was used for analysis of HRAS expression.

Summary of Results: The boy had hypertrophic cardiomyopathy, mild pterygia, posteriorly angulated ears, pectus deformity, reflex, normal growth parameters, and mild developmental delay but full scale IQ of 76. No history of malignancy, ulceration or abnormalities of hair. HRAS sequencing identified a c.35_36delinsTG (p.G12V) mutation in all tissues sampled without evidence of mosaicism. A significant reduction in the level of the mutant allele was observed in cDNA.

Conclusions: Attenuated phenotypes have been reported with specific mutations in Costello syndrome but not with p.G12V. Although our patient had hypertrophic cardiomyopathy, the remaining phenotype was comparably attenuated. This is even more surprising given the association of the p.G12V mutant with higher stage and lethality in carcinomas. We demonstrate reduced levels of the mutant allele in cDNA and speculate this reduction results from altered mRNA processing. The other 2 individuals with Costello syndrome previously reported with a p.G12V mutation also had dinucleotide substitutions. We propose that the unusual dinucleotide substitutions result in viability due to reduced expression of the mutant alleles.

276 COLLAGEN SUBSTRATE SPECIFICITY OF PROLYL 3-HYDROXYLASES

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Purpose of Study: Current attention has focused on prolyl 3-hydroxylases (P3Hs), a class of enzymes responsible for 3-hydroxylation of certain prolyl residues in the collagen triple helix, forming 3-hydroxyproline (3Hyp). Mutations in the genes encoding P3H1 and associated proteins of its enzyme complex were found to cause recessive forms of severe osteogenesis imperfecta (OI). The goal of this study was to investigate the expression levels of the three P3H enzymes—P3H1, P3H2 and P3H3—in a cell line known to form 3Hyp at several sites in type II collagen, to better understand their different substrate specificities.

Methods Used: Using qPCR, expression of the three P3H enzyme genes was assayed in the rat chondrosarcoma cell line, RCS-LTC, and in normal adult rat cartilage. Similarly, the expression profiles of these genes, and other genes related to the functionality of the P3Hs, were compared qualitatively by RT-PCR and were compared to expression levels in the human osteosarcoma cell line, SAOS-2. Collagen produced by the RCS-LTC cells was analyzed for 3Hyp formation at known sites by mass-spectrometry.

Summary of Results: The RCS-LTC cells produced mRNA for all three enzymes, most prominently for P3H2, in contrast with normal adult rat cartilaginous and SAOS-2 cells which lacked mRNA expression for P3H2 and P3H3 respectively. Quantitative comparison of P3H mRNA expression between the RCS-LTC cells and rat cartilage yielded similar expression of P3H1, a ~2-fold increase in P3H2 and a ~0.5-fold increase in P3H3 expression in the RCS-LTC cells. Mass-spectrometric analysis of the RCS-LTC collagen showed near complete 3Hyp formation at Pro944, a secondary site previously found occupied in the type II collagen of vitreous, but not cartilage.

Conclusions: The high relative abundance of P3H2 mRNA expression coupled with high occupancy of Pro944 in the RCS-LTC matrix implicates P3H2 in the formation of 3Hyp at Pro944. Taken together with previous findings, it is possible that 3Hyp formation at Pro944 may be a signaling mechanism for the prevention of cleavage of the collagen N-propeptide, thereby maintaining the long, thin fibrils as seen in RCS-LTC cells and vitreous.

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277 PRENATAL DIAGNOSIS OF AMYOPLASIA CONGENITA

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Case Report: Amyoplasia congenita, the most common form of arthrogryposis multiplex congenital, is a sporadic disorder, as classically defined by Hall in 1983. It is characterized by symmetric limb involvement with fatty and fibrous tissue replacement of hypoplastic muscle, vascular facial skin lesions, and normal cognitive development. It is a static condition, and there is accumulated evidence that it can result from a variety of prenatal vascular insults to the ventral horn cells of the developing fetal spine during critical periods, as first suggested by Reid in 1986 (discussed in Gaitanis et al, 2010). Here we present the first published case diagnosed by fetal MRI. Fetal ultrasound at 19 weeks of gestation revealed flexion deformities of the upper and lower extremities and an absence of fetal limb movement. Chromosome analysis and FISH for common aneuploidies via amniocentesis were normal. Fetal MRI at 22 weeks of gestation confirmed ultrasonography findings, and revealed increased subcutaneous signal indicative of edema or increased fat in the muscle, and suggestive of muscle atrophy, in all four limbs. Gestation and delivery proceeded without complication. The patient was born at full term and noted to have a weak cry, normal gag reflex, and the typical physical features of amyoplasia congenita were identified. No pterygia were present. Postnatal brain and spine MRI revealed no specific anomalies of the CNS. The infant was discharged home on the second day of life.

Amyoplasia congenita is a distinct form of arthrogryposis with a good prognosis overall. We suggest that the characteristic fatty replacement and muscular atrophy in the limbs may be visualized on fetal MRI, and in the context of features consistent with arthrogryposis may allow prenatal diagnosis of this condition.

References:

278 MITOCHONDRIAL PATHOLOGY IN ANGELMAN SYNDROME

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Purpose of Study: Mitochondrial diseases share many clinical features with Angelman syndrome including developmental delay, seizures, gait ataxia, microcephaly, and GI symptoms. AS is caused by the loss of the maternally imprinted copy in the 15q112-q13 Angelman/Prader-Willi syndrome (AS/PWS) region. Approximately 70% of patients with AS have a cytogenetically detectable deletion in this region. We report a patient with AS who had mitochondrial pathology on muscle biopsy to further the understanding of pathophysiology of AS.

Methods Used: Microarray 105k CGH. Review of electronic chart & medical literature.

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Summary of Results: Our patient is an 8-year old male with developmental delay, microcephaly, seizures, ataxia, and constipation. Extensive biochemical evaluation, sequencing of multiple mitochondrial genes, and mitochondrial respiratory chain analysis were normal. After a muscle biopsy showed mitochondrial hyperplasia and ragged red fibers suggesting mitochondrial dysfunction, he was referred to our center for evaluation of possible mitochondrial disorder. Urine organic acids showed dicarboxylic acids and lactate, findings often seen in patients with mitochondrial disorders. An array CGH revealed a 4.85 Mb deletion from 15q11.2 to 15q13.1 encompassing the UBE3A gene, consistent with a diagnosis of AS.

Conclusions: Evidence of mitochondrial pathology on a muscle biopsy in a patient with AS suggests that UBE3A may regulate mitochondrial biogenesis and/or function. UBE3A is an ubiquitin ligase and ubiquination is important in maintaining the structural and functional integrity of the mitochondrion. Brains of mice with AS in which the maternal UBE3A allele is mutated have abnormal mitochondria that exhibit partial defect in mitochondrial respiratory chain (Su et al. 2009). We are also sequencing 524 nuclear mitochondrial genes in this patient as part of research study to determine if we can identify any potential modifying genes that may contribute to mitochondrial pathology in AS. Further studies are needed to determine whether some of the manifestations of AS may be explained by secondary mitochondrial pathology.

279 RECURRENT ACUTE PANCREATITIS, DISTINCTIVE FACIAL APPEARANCE, AND 3-HYDROXYISOBUTYRIC ACIDURIA: A PREVIOUSLY UNRECOGNIZED PRESENTATION OF PEARSON SYNDROME
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Purpose of Study: Pearson syndrome is the most severe of the mitochondrial DNA deletion syndromes and typically presents in infancy with transfusion-dependent anemia and variable pancreatic insufficiency. We report a patient with a novel presentation characterized by recurrent acute pancreatitis, distinctive facial appearance, and abnormal biochemical findings.

Methods Used: Retrospective chart review.

Summary of Results: The patient presented at 13 months of age with emesis, lethargy, poor oral intake, and dehydration, associated with failure to thrive and mild enlargement of the liver. Cognition was normal. Distinctive findings included a round face with full cheeks, and acute pancreatitis (abdominal pain, elevated lipase/amylase). Abdominal imaging revealed a pancreatic cyst (2 cm diameter) and mild liver enlargement. Brain MRI and MR spectroscopy were normal. At presentation she had anemia, mild leukopenia and thrombocytopenia, mild metabolic acidosis and normal liver enzymes. For the following months she was re-admitted eight times for recurrent acute pancreatitis (lipase up to 2,908). She had chronically elevated lactate (10±3.8 mM (n=56), normal 0.7–2.1 mM), elevated alanine, low arginine, lactate and pyruvic aciduria, and persistently elevated excretion of 3-hydroxy-isobutyric and 2-ethyl-3-hydroxypropionic acids suggestive of mitochondrial dysfunction. Renal tubulopathy was also present, with aminoaciduria, glycosuria and tubular acidosis. She progressed to severe intransitinal dystomyotilis (even with pancreatic enzyme supplements), liver failure, and transfusion-dependent pancytopenia. Bone marrow biopsy showed trilineage hematopoiesis with moderate dyserythropoiesis, vacuolization of erythroid and myeloid precursors without ringed sideroblasts. Mitochondrial DNA testing identified a large, heteroplasmic deletion consistent mtDNA deletion syndrome.

Conclusions: Pearson syndrome should be suspected in children with recurrent pancreatitis without a known cause, especially in the setting of multiorgan system involvement and suggestive biochemical testing.

280 A NEWLY DESCRIBED OVERGROWTH, MACROCEPHALY, DEVELOPMENTAL DISABILITY SYNDROME IS DUE TO A MICRODELETION OF 19P13.13 AND LOSS OF THE NFIX GENE
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Purpose of Study: The 19p13.13 microdeletion syndrome (M. Dolan, et al. Genetics in Medicine 2010, 12(8):503–511) is based on a series of cases with macrocephaly and overgrowth with 19p13.13 microdeletions of varying sizes, the smallest area of overlap being 311–340 kb and encompassing 16 genes. We present a patient with this phenotype and a smaller deletion within this same region. This narrows the critical region and suggests NFIX as a candidate gene responsible for this overgrowth syndrome.

Methods Used: A whole genome 1,800,000 single nucleotide polymorphism (SNP, Affymetrix, Inc.) chromosome microarray (LabCorp) was used on DNA derived from blood.

Summary of Results: An 8 year old male with autism, tall stature, macrocephaly and moderate-severe mental retardation presented for genetic assessment. He had pectus excavatum, constipation, and an undescended testis. The head circumference was 59 cm (>98th %ile) and height 140 cm (94th %ile). His eyelids were puffy, eyebrows were straight and his face was triangular. Palpebral fissures were downsloping. Skin on the palms was dry and loose. He was tactile defensive with stereotypic movements and poor eye contact. He spoke in 3 word phrases. SNP microarray identified a 188 kb deletion of 19p13.13 including NFIX (nuclear factor-1-X), a candidate gene in the original report. Maternal analysis with a region specific BAC FISH probe was normal. Paternal analysis is pending. NFIX is a transcription factor expressed in the developing human brain and skeleton. Mutations in NFIX have recently been reported in patients with Sotos-like and Marshall-Smith syndromes (V. Malan, et al. AJHG 2010 Aug 13;87(2): 189–98). Our patient’s clinical characteristics will be presented and compared with other patients with NFIX deletions and mutations from the literature.

Conclusions: This report refines the clinical and molecular characteristics of the 19p13.13 microdeletion syndrome and narrows the critical region to a 188 kb area that includes the NFIX gene. Mutations in NFIX have been recently reported in patients with other overgrowth syndromes. We conclude that the overgrowth macrocephaly phenotype associated with microdeletions of 19p13.13 is caused by loss of the NFIX gene.
282 LONG TERM FOLLOW-UP OF A 61-YEAR-OLD WOMAN WITH WOLF-HIRSCHHORN SYNDROME DUE TO AN UMBALANCED INHERITED 4P;13Q TRANSLocation

Leon EL, South S, Carey J University of Utah, Salt Lake City, UT.

Purpose of Study: Clinical description and delineation of the chromosomal alteration in the oldest known patient with Wolf-Hirschhorn syndrome (WHS). To advance knowledge of natural history.

Methods Used: Clinical observation and phenotype analysis, chromosome and CGH microarray analyses.

Summary of Results: The proposita was born to healthy nonconsanguineous parents, a 23-year-old G1P0-1 mother and 26-year-old father, of Caucasian descent. Family history is unremarkable. She was born postterm, via induced vaginal delivery, following an uncomplicated pregnancy. She was small for gestational age, with BW of 2.350 kg, and her length was 46 cm. Her neonatal course was remarkable for poor sucking. Her developmental milestones proceeded slowly, and by 18-months-old she was able to sit up with assistance. She has never been able to walk, feed herself or speak. Seizures started at about 11 months of age; first with grand mal convulsions and later in her childhood with minor motor seizures. She was diagnosed with agenesis of corpus callosum at 18 months. She now has intermittent episodes of seizures and she has been off seizure medication since she was 36. At her initial evaluation (26 years) her growth parameters were below the 3rd centile. Her facial features were typical for WHS. She had bilateral fifth finger clinodactyly, scoliosis, spasticity in four limbs, and decreased muscle bulk. Conventional G-banded cytogenetic studies detected a deletion in 4p. Her mother had a balanced translocation between 4p and 13q. CGH microarray analysis in order to characterize the size of the deletion/duplication is pending.

Conclusions: This is the oldest reported patient with WHS; she has an inherited unbalanced translocation. Previous studies indicate that approximately 15−45% of WHS cases are due to inherited translocations. The 4p/13q translocation is a rare translocation causing WHS.

283 NEUROLOGICAL FINDINGS IN TWO SIBLINGS WITH TAYBI-LINDER SYNDROME

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Case Report: In 1967 Taybi and Linder described two siblings, from consanguineous parents, with dwarfism, skeletal dysplasia, and brain malformations. The syndrome is now known as microcephalic osteodysplastic primordial dwarfism type I/II, or Taybi-Linder syndrome, and is characterized by microcephaly, growth retardation, and characteristic skeletal findings including: platyspondyly, short iliac wings, flat/irregular acetabular roofs, and short and flat long bones.

We present the neurologic and morphologic profiles of two siblings, female and male, born to non-consanguineous, Caucasian parents. The emphasis of the report is on the neurological profile of the disease, which includes brain malformations, intractable epilepsy, sensory deficits, profound developmental delay, and neuroendocrine dysfunction. We also present novel correlative neuroimaging (MRI) and electroencephalographic (EEG) findings.

Both siblings were born prematurely, with growth parameters at birth below the 25th centile for weight and length, and head circumference less than 10th centile. Facial dysmorphias included hypertelorism, down-sloping palpebral fissures, micrognathia, and globular nose and flames. Early skeletal radiography in both was consistent with Taybi-Linder syndrome. Karyotypes were normal and a targeted microarray in the sister was also normal. She had significant congenital heart disease, including VSD, PFO, and PDA. Her brother had microenops with hypospadias and bilateral cryptorchidism.

Both sibs had profound cognitive impairment, with blindness secondary to optic nerve hypoplasia and sensorineural hearing loss secondary to bilateral Mondini malformations. Neonatal onset seizures appeared in both, refractory to anticonvulsants and only partially responsive to ACTH. EEG’s demonstrated diffuse encephalopathy. Neuroendocrine abnormalities included central hypothyroidism, pseudo-hyperaldosteronism, and diabetes insipidus. MRIs of both sibs were notable for anomalies in cortical gyration, cerebellar hypoplasia, and hypoplasia of the corpus callosum.

The sister expired at age four years from complications of a viral illness. The brother is now 33 months and is tracheotomy and G-tube dependent, with limited development.

284 HEME OXYGENASE-1 DEFICIENCY IMPAIRS PLACENTAL VASCULAR FORMATION AND EMBRYONIC DEVELOPMENT

Zhao H, Azuma J, Kalish FS, Wong RJ, Stevenson DK Stanford University School of Medicine, Stanford, CA.

Purpose of Study: Placental vasculature formation is critical for establishing the fetomaternal interface and the development of healthy offspring. Heme oxygenase-1 (HO-1), the enzyme in heme degradation, plays a role in angiogenesis/vasculogenesis and is highly expressed in the placenta. Its deficiency is associated with several pregnancy disorders. Our objective was to study the effects of HO-1 deficiency on placental vasculature formation and embryonic development.

Methods Used: Placentas from crossbred HO-1 heterozygote (Het) mice were harvested at various gestational ages and genotyped by PCR. Changes in placental morphology were detected by HE staining. Uterine NK (uNK) cells, involved in early decidual vasculature development, were identified by DBA staining. Maternal vasculature was visualized from casted placentas and imaged by microCT. Expressions of decidual factors, including PIGF, VEGF, Flt1, Flk1, TGFβ, FNI, IL10, INOS and eNOS, were quantified by RT-PCR. All comparisons were made between age-matched Het and wild-type (WT) placentas.

Summary of Results: Compared to WT, Het matings overall yielded smaller fetuses and placentas. In Het placentas, spiral arteries were less dilated and junction zones were thinner by HE staining. 3D images revealed that Het placentas had thinner (2.1 vs 2.4mm) maternal vascular regions, less total vessel volume (8.3 vs 9.0mm3) and reduced vessel volume in the labyrinth (3.1 vs 4.4mm3). Het placentas also had more spiral artery branching, but with smaller diameters (0.30±0.05 vs. 0.41±0.04mm). Expression of both PIGF (placental growth factor) and VEGF (vascular endothelial growth factor), the two key vascular regulators, was significantly elevated in Het decidua (up to 2.7-fold and 10-fold, respectively). uNK cells in Het decidua appeared significantly smaller (21.8±4.7 vs. 26.3±3.9mm) and with more granularity.

Conclusions: HO-1 deficiency results in abnormal spiral artery dilation and remodeling, and poor labyrinth vessel development. The defects are likely due to irregular expression of vascular growth factors and improper structure of uNK cells. Therefore we speculate that HO-1 plays an important role in placental vasculature development and HO-1 deficiency may contribute to pregnancy complications, such as preeclampsia, spontaneous abortions and premature births.

285 PRENATAL EXPOSURE TO MATERNAL CIGARETTE SMOKING HAS A GREATER EFFECT ON FETAL GROWTH THAN NICOTINE ALONE

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Purpose of Study: We hypothesized that prenatal exposure to maternal cigarette smoking, but not nicotine alone, will cause IUUG in the offspring.

Methods Used: Pregnant Sprague-Dawley rats were exposed to MCS or nicotine alone (NA). MCS exposure (10 cigarettes per dam) occurred daily from day 11 (d11) of gestation until term (d21), excluding weekend days. NA dams received intraperitoneal osmotic pumps containing either a nicotine solution (1, 3 or 6 mg nicotine/kg/d) or saline on d13. All pups were delivered surgically and weighed on d21. Dams were weighed on d11 (MCS) or d13 (NA) and d21. Serum cotinine levels from dams were determined at time of delivery via ELISA.

Summary of Results: MCS pups averaged 21% smaller than unexposed pups (P<0.01), with males and females affected equally. Plasma cotinine levels in MCS dams were 235+/-83ng/ml (human reference range, 200-800ng/ml for active, daily smokers). Dams exposed to NA with similar plasma cotinine levels (245+/-80ng/ml) produced pups less growth restricted (5% males, 7% females; P<0.05) than MCS pups. Average initial weight of dams, litter sizes and placental weights at delivery were not different between control, MCS and NA animals. Average weight of MCS dams at delivery was 13% lower than controls (P<0.05).
Conclusions: These data suggest that prenatal MCS exposure has a greater effect on fetal growth and maternal weight gain than nicotine alone. *DTM and CF contributed equally to this work.

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MATERNAL TOBACCO SMOKE EXPOSURE AND INTRAUTERINE GROWTH RESTRICTION ALTER HEPATIC SREBP1A AND -2 LEVELS IN A GENDER SPECIFIC MANNER

Purpose of Study: Maternal tobacco smoke exposure (MTSE) and utero-placental insufficiency (UPI) cause intrauterine growth restriction (IUGR). Both MTSE induced IUGR (MTSE-IUGR) and UPI induced IUGR (UPI-IUGR) increase the risk for hypercholesterolemia in adult humans and rats. The major detrimental component of cholesterol is low density lipoprotein (LDL). Increased LDL production leads to significant adult morbidity and mortality. Morbidity and mortality from elevated LDL is more prevalent in males. Serum and hepatic LDL levels are regulated by sterol-responsive element binding protein 1a (SREBP1a) and -2 (SREBP2). Despite the importance of SREBP1a and -2 in the regulation of LDL throughout life, it is unknown whether MTSE-IUGR and UPI-IUGR alter SREBP1a and -2 mRNA and protein levels. We hypothesized that MTSE-IUGR and UPI-IUGR decreases SREBP1a and -2 mRNA and protein levels in males at birth with more substantial decreases seen in the MTSE-IUGR model.

Methods Used: To test this hypothesis we used both a MTSE-IUGR and a UPI-IUGR rat model to determine mRNA levels of SREBP1a and -2, and protein levels of SREBP2 at birth in both genders. MTSE-IUGR was achieved by cigarette smoke exposure from day 11 of pregnancy to term. UPI-IUGR was achieved by bilateral uterine artery ligation at day 19 of pregnancy.

Summary of Results: MTSE-IUGR decreased SREBP2 mRNA levels to 18% +/- 5% (p=0.058) in males, with no change in SREBP1a in either gender. UPI-IUGR decreased male SREBP1a mRNA to 64% +/- 8% (p=0.05), SREBP2 mRNA to 60% +/- 10% (p=0.06), and SREBP2 protein to 29% +/- 5% (p=0.01), with no change in females.

Conclusions: We conclude that MTSE-IUGR and UPI-IUGR decrease SREBP1a and -2 in a gender and model specific manner. Model specific alterations in SREBP1a mRNA levels suggest separate mechanisms through which each insult impacts gene expression. These mechanisms may include vasoconstriction from nicotine, hypoxyxia, or toxins from smoke exposure. We speculate that MTSE-IUGR and UPI-IUGR hepatic lipid profiles will demonstrate increased intrahepatic cholesterol in IUGR males.

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DISTINCT PHENOTYPE ASSOCIATED WITH INTERSTITIAL LONG ARM DELETION OF CHROMOSOME 4q13.3→q21.1
2 Golabi M, Hall B, Cotter P 1Sutter Pacific Medical Center, San Francisco, CA; 1University of Kentucky, Lexington, KY and 1Children’s Hospital of Oakland, Oakland, CA.

Purpose of Study: Report a new case of 4q13.3→q21.1, documentation two previously reported cases with identical features, and define the unique features associated with this deletion.

Methods Used: Oligonucleotide array CGH analysis was performed on our proband’s DNA. This test was comprised of 99,000+ DNA probes. The DECIPHER database documented clinical descriptions of two previously recorded cases with overlapping deletions.

Summary of Results: The proband, a female, was born at 37 weeks gestation to a 35-year-old mother. CVS showed a 46,XX karyotype. At birth the proband presented with significant IUGR and microcephaly. She was noted to have dysmorphic facial features, such as a bulbous nasal tip with a wide alae, abnormal helices, cleft palate, and small mandible. She had a short neck, broad chest, rhizomelic shortening of upper and lower extremities, short broad thumbs, distal digital fat pads of fingers and toes, and a plantar lipoma on her left heel. She had truncal hypotonia, delayed milestones, and sensorineural hearing loss. Tests showed a normal brain MRI, delayed bone-age, and elevated IGF 1 on two occasions.

Previous reports of patients with similar deletions: (1) A male with prenatal growth retardation and microcephaly with a deletion of 4q13.3→q21.2. (2) A female with prenatal onset of short stature, abnormal ears/hearing deficit, strabismus, abnormal palate, and delayed tooth age with a deletion of 4q1.3.2→q21.2. Although the proband’s deletion was smaller, she has a distinctive phenotype characterized by microcephaly, cleft palate, and sensorineural hearing loss. She also had a plantar lipoma, which is a rare finding. We suggest that a 4q deletion should be added to the list of conditions associated with plantar lipomas. Also of interest, she has significant short stature and delayed bone age despite a two-fold increase in IGF1 levels. Further evaluation will determine whether the proband might also have a mutation within the IGF1 receptor or whether one of the genes with the 4q deletion is involved in IGF1 regulation.

Conclusions: We propose the microdeletion of 4q13.3→q21.1 is associated with a distinct and recognizable phenotype.

Neonatology – General II
Concurrent Session
1:15 PM
Friday, January 28, 2011

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BATH-RELATED BURN IN THE NEONATE
Rajnada N, Sardesai S, Cayabyab RG Division of Neonatal Medicine, Keck School of Medicine,University of Southern California, Los Angeles, CA.

Case Report: We report a case of a 9-day old full term female infant with bath-related burns involving 70% total body surface area, managed with Meplex and Acticoat dressings in neonatal intensive care unit (NICU).

Introduction: Neonatal burns are rare and can be fatal. There is limited literature on the management of significant burns in this unique patient population.

Case Report: A 9-day old full term female neonate with bath-related burns was transferred to our Burn Unit and later to the NICU from an outside Emergency Room after nine hours of initial injury. She had sustained 70% total body surface area burns (7–10% third-degree burns and 60% second-degree burns) affecting the arms, legs, abdomen, back and maxillary region of the face. Early and aggressive fluid resuscitation was initiated. Upon arrival to the Burn Unit the wounds were covered with Meplex and Acticoat dressings. More severe burns were also kept moist using red-robin catheters to deliver saline underneath the dressings. The dressings allowed for nearly painless wound management. Multiple courses of antibiotics and anti-fungals were initiated secondary to blood cultures that grew Pseudomonas Aeruginosa and Candida Albicans. The patient was discharged after 80 days. Of note, at discharge the burns were well-healed with erythematous areas of skin and fibrotic changes noted on the chest, abdomen, back, lower extremities, buttocks and labial region. No surgical intervention or skin grafts were necessary. The patient was recently seen at the Burn Clinic. She was noted to have healed very well without scarring and had full range of motion of all extremities. Hypertrophic and erythematous areas were noted around the inner thighs and mon pubis. The patient will continue to be followed as an outpatient with the Burn Clinic.

Discussion: Because of the unique physiology in the neonatal period the management of thermal injuries in this population poses many challenges. The goals of our treatment included patient stabilization, preservation of cosmesis appearance, joint functionality and pain management. Acticoat is a suitable dressing for neonates who have sustained burn injury, with the advantage of minimal handling as the dressing need only be changed every 3–7 days.

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BOLUS VERSUS LONGER INFUSION OF ENTERAL FEEDINGS FOR PRETERM NEONATES
Corbin LL, Smith S OHSU, Portland, OR.

Purpose of Study: Developmental immaturity of the gastrointestinal tract precludes immediate full enteral feedings for many preterm infants. Previous studies suggest that preterm infants who receive slow infusion of enteral feedings have improved maturation of intestinal motility when compared to preterm neonates fed by rapid bolus. Our hypothesis was that infusion of gavage feedings over 120 minutes will lead to a 33% reduction in feeding intolerance, as defined by the proportion of total feedings withheld, when compared to bolus gavage feedings given over no more than 30 minutes.

Methods Used: This study is a single center, randomized controlled trial comparing two methods of enteral feeding for preterm neonates less than 32 weeks postmenstrual age and birth weight between 500 and 1500 grams.
Our study showed that infants receiving feeds over 2 hours had less feeding intolerance, as measured by the number of feeds withheld, than those infants who received bolus feedings. Further analysis will determine the number of days to achieve full feeds and to regain birth weight in this population.

**Summary of Results:** A total of 29 infants were recruited for the study, 15 bolus and 14 drip. Odds of a feed not being given were 67.8% lower (95% CI: 21.8–86.7% percent lower) for infants on drip feeds compared to the bolus feed (Wald X^2(1)=6.3, p=0.012). After controlling for gestational age (23–27 wks vs 28–30 wks), the odds of withholding a feed remain 68% (95% CI: 26–86%) lower for those on drip feeds compared to those on bolus feeds (Wald X^2(1)=7.08, p=0.008).

**Conclusions:** Our study showed that infants receiving feeds over 2 hours had less feeding intolerance, as measured by the number of feeds withheld, than those infants who received bolus feedings. Further analysis will determine the number of days to achieve full feeds and to regain birth weight in this population.

290 INCREASED AMINO ACIDS POTENTIATE GLUCOSE STIMULATED INSULIN SECRETION IN THE OVINE FETUS


**Purpose of Study:** Previous studies of intrauterine growth restriction restricted fetuses demonstrate reduced amino acid supply to the fetus. These fetuses are also characterized by decreased insulin secretion. It is unknown whether chronically increased fetal amino acid supply would stimulate insulin secretion, even in normally growing fetuses. We hypothesized that chronically increasing the fetal amino acid supply would increase glucose and arginine stimulated insulin secretion (GSIS, ASIS) in the ovine fetus.

**Methods Used:** Singleton ovine fetuses at 113–120 days gestation (term=148 days) were given an intravenous infusion of a complete mixture of amino acids (AA group, n=8) or saline (CON group, n=8) for 10–14 days. Fetal branch chain amino acids (BCAA), glucose, insulin, pH, blood gases, and hematocrit were measured during the infusion. On the final day, fetal Glucose and ASIS were measured using a square waveform hyperglycemic clamp and arginine bolus. Pancreatic insulin content was measured with ELISA.

**Summary of Results:** Fetal BCAA concentrations increased 50% in the AA group compared to CON (P<0.05). Glucose decreased over time in the AA group (22.7±1.5 baseline vs 17.9±0.4 mg/dl final day, P<0.01) but not in CON. pO2, O2 saturation, and O2 content tended to decrease during days 5–10 in the AA group and then returned to baseline. Hematocrit, pH, and insulin concentrations were not different between the groups during the infusion period. However, there was a pronounced increase in early phase insulin concentrations (15 min: 2.06±0.53 AA vs. 0.80±0.20 ng/mL CON, P<0.01) and this difference was sustained during the hyperglycemic clamp in the AA group (105 min: 1.38±0.23 AA vs. 0.70±0.14 mg/mL CON, P<0.001). ASIS appeared increased in the AA group but this was not statistically significant. Pancreatic insulin content was not different between groups during the infusion period. However, there was a pronounced increase in early phase insulin concentrations (15 min: 2.06±0.53 AA vs. 0.80±0.20 ng/mL CON, P<0.01) and this difference was sustained during the hyperglycemic clamp in the AA group (105 min: 1.38±0.23 AA vs. 0.70±0.14 mg/mL CON, P<0.001). ASIS appeared increased in the AA group but this was not statistically significant.

**Conclusions:** Chronically increased amino acid supply to the fetus potentiated insulin secretion in response to glucose. Because GSIS was potentiated more than ASIS, we speculate that amino acids upregulate glucose metabolism and generation of secondary messengers in the beta-cell.

291 INCREASED VITAMIN E INTAKE IS ASSOCIATED WITH HIGHER α-TOCOPHEROL CONCENTRATION IN THE MATERNAL CIRCULATION, BUT HIGHER α-CARBOXYETHYL HYDROXYCHROMANS CONCENTRATION IN THE FETAL CIRCULATION

Go M1, Didenco S1, Gillingham M1, Leonard S2, Traber M2, McEvoy C1

1 Oregon Health and Sciences University, Portland, OR and 2 Oregon State University, Corvallis, OR

**Purpose of Study:** Vitamin E may prevent oxidative stresses implicated in perinatal disease processes, but studies suggest fetal vitamin E stores are low despite maternal supplementation. We hypothesize that the placenta limits vitamin E transfer by increasing its metabolism, or the fetal liver actively metabolizes the vitamin, which would be reflected by the concentrations of vitamin E metabolites, carboxyethyl hydroxychromans (α- and γ-CEHC), in the fetal circulation. We measured α- and γ-CEHC levels in maternal and umbilical cord blood pairs and examined their relationships to circulating vitamin E (α- and γ-tocopherol) and maternal dietary vitamin E intake.

**Methods Used:** At least one fasting blood sample and a previous day’s 24-hr diet recall were done during pregnancy in healthy, non-smoking women. All samples were analyzed for α- and γ-tocopherol, α- and γ-CEHC and total lipid concentrations (n=17 pairs, all full term).

**Summary of Results:** Umbilical cord blood and maternal concentrations of α- CEHC (30.2 ± 28.9 vs. 50.4±52 nmol/L, P = 0.07) and γ-CEHC concentrations (104.5 ± 61.3 vs. 141.7±78.5 nmol/L; P = 0.08) were not significantly different, but the metabolite to tocopherol ratios were significantly higher in cord blood than in maternal blood (α-CEHCα-tocopherol = 5.7±5.5 vs. 1.6±1.9, P < 0.01 and γ-CEHC:γ-tocopherol = 389.6±291.5 vs. 86.8±64.4, P < 0.001). Maternal α-tocopherol/total lipid ratios were correlated with cord blood α-CEHC (r = 0.67, P < 0.003, and higher maternal vitamin E intakes were associated with the higher cord blood α-CEHC concentrations (r = 0.75, P < 0.003).

**Conclusions:** Although the results show similar CEHC concentrations in maternal and cord blood samples, the α-CEHCα-tocopherol ratios in the fetal circulation were different those in the maternal circulation. The higher intake of vitamin E during pregnancy resulted in elevated fetal metabolic levels, without increasing the fetal blood vitamin E concentration. Studies are needed to further define vitamin E metabolism in pregnancy and the function of vitamin E metabolites.

292 PROLONGED HYPOGLYCEMIA DECREASES INSULIN SECRETION, NOT β-CELL POPULATION IN OVINE FETUSES

Lavezzri JR, O’Meara M, Thorn SR, Brown LD, Hay WW, Rozance PJ University of Colorado School of Medicine, Aurora, CO.

**Purpose of Study:** Intrauterine growth restriction due to placental insufficiency (PI-UGR) is characterized by fetal hypoglycemia, hypoinsulinemia, hypoxia, decreased amino acids, increased catecholamines, nearly absent glucose stimulated insulin secretion (GSIS), and specific reduction of the β-cell population. We hypothesized that prolonged fetal hypoglycemia over the last 40% of gestation, independent of placental insufficiency, would replicate these defects.

**Methods Used:** Control fetuses (C; n=5) were compared to fetuses whose mothers received an 8 wk insulin infusion reducing maternal and fetal glucose 40% (HG; n=5). Four other HG fetuses (HG+I) received a direct insulin infusion with concurrent dextrose infusion to prevent fall in glucose for the final wk. Glucose, insulin, amino acids, O2, norepinephrine, GSIS, arginine stimulated insulin secretion (ASIS), pancreatic weight and fetal weight were measured at the end of the infusions. β-cell area was measured as the proportion of pancreatic sections which stained insulin. β-cell mass is the product of β-cell area and pancreatic weight.

**Summary of Results:** Glucose (22.8±1.7 C vs. 10.6±0.4 mg/dl; P<0.0001), and insulin (0.47±0.06 C vs. 0.18±0.03 mg/kg/ml; P<0.0005) were lower in HG fetuses. O2 and norepinephrine were similar. Tauine was increased (P<0.01), glutamate decreased (P<0.001), but all other amino acids were unchanged in HG fetuses. GSIS was present but decreased 45% in HG fetuses (P=0.05) and ASIS was preserved. β-cell area did not change (4.57±0.52 C vs. 4.66±1.21 HG %). The reduction in β-cell mass (30%) in HG fetuses was proportional to the decrease in pancreatic (37%) and fetal (40%) weights. By design in HG+I fetuses, insulin increased (0.89±0.19 mg/ml; P<0.005) and glucose did not change (9.2±0.8 mg/dl; GSIS was absent and ASIS decreased (P<0.0005) β-cell mass in HG+I maternal and pancreatic weight were not statistically different from HG fetuses.

**Conclusions:** Prolonged hypoglycemia decreased GSIS, though not as strikingly as seen in PI-UGR. Similarly, the β-cell population was not specifically decreased relative to fetal and pancreatic weight. These results contrast those of severe human IUGR and animal models of PI-UGR and define chronic experimental hypoglycemia as a model of IUGR with a unique β-cell phenotype.
293 EFFECTS OF A DIRECT FETAL AMINO ACID INFUSION ON OXGENATION AND ACID-BASE BALANCE IN FETAL SHEEP

Purpose of Study: Human maternal high protein supplementation during pregnancy increased the risk for small for gestational age birth. Maternal amino acid infusion in pregnant sheep results in competitive inhibition of amino acid transport across the placenta, increased fetal oxygen (O2) consumption, fetal hypoxia and acidosis. A direct fetal amino acid infusion, by-passing competitive inhibition of amino acid transport across the placenta, will increase fetal O2 consumption but preserve acid-base balance.

Methods Used: Singleton fetal sheep were intravenously infused with a complete amino acid mixture (AA, n=8) or saline (C, n=10) for an average of 12 days during late gestation. A mixed model ANOVA was performed to determine the effects of treatment group (AA or C) and day of infusion on fetal arterial branched chain amino acid (BCAA) concentrations; pH; blood gasses; hematocrit; plasma lactate, glucose, and insulin concentrations, and glucose/O2 quotient. On the final day of infusion, a metabolic study was performed to determine rates of umbilical blood flow, fetal O2 consumption, and fetal glucose and lactate uptake.

Results of Summary: Fetal [BCAA] were increased by 50% in the AA group vs. C group (P<0.005). Glucose decreased in the AA group only (22.6±1.5 vs. baseline 18.0±1.2 mg/dl final day, P<0.0005). Fetal arterial plasma insulin, pH, pCO2, hematocrit, hemoglobin-O2 saturation, and blood O2 content did not change. Fetal arterial blood pO2 decreased in AA from baseline (18.9±0.7 mmHg) on days 5 (16.5±1.4 mmHg, P<0.05) and 8 (16.3±1.3 mmHg, P<0.05) then returned to baseline. Fetal arterial plasma lactate concentrations increased in the AA group initially (20.9±1.3 baseline vs 4.4±1.3 mmol/L on day 7, P<0.005) then returned to baseline. The fetal glucose/O2 quotient decreased in AA (P<0.05) but not in C.

Fetal glucose uptake was lower in AA vs. C (2.52±0.36 vs 3.86±0.11mg/kg/min, P<0.05). Umbilical blood flow, lactate uptake, and O2 consumption rates did not change in AF fetuses.

Conclusions: A prolonged infusion of amino acids directly into fetal sheep did not increase O2 consumption and preserved acid-base balance. We speculate that decreased umbilical glucose uptake and glucose/O2 quotient in the AA group is due to increased fetal amino acid oxidation.

294 INTRAUTERINE GROWTH RESTRICTION (IUGR) INCREASES RETINAL EXPRESSION OF GENES INVOLVED IN THE PATHOGENESIS OF RETINOPATHY OF PREMATURITY (ROP) IN MALE RAT PUPS
Hale MA1, Jiang Y2, Yu X1, Callaway C1, Wang H1, Smith GT2, McKnight R1, Lane RH2, Hartnett M1, 2University of Utah, Salt Lake City, UT; 1University of Utah, Salt Lake City, UT.

Purpose of Study: IUGR increases the risk of ROP in human preterm infants. IUGR decreases human infant IGF-1 (insulin growth factor 1) serum levels, which is a predictor of ROP. IUGR also affects IGF-1 serum levels in rat pups. Despite the links between IUGR, ROP, and IGF-1, little information exists on whether IUGR affects retinal IGF-1 expression. Retinal IGF-1 expression is relevant because 1) IGF-1 is expressed throughout the retina; 2) IGF-1 mediates hypoxia inducible factor 1α (HIF-1α) expression; and 3) IGF-1 induces erythropoietin (Epo) expression through HIF dependent and independent mechanisms. We hypothesize that IUGR increases retinal IGF-1, HIF-1α, and Epo mRNA levels, as well as levels of their respective receptors, in newborn rat retinas.

Methods Used: To test this hypothesis, we induced IUGR in the rat pups through uteroplacental insufficiency. Pups were killed at 7 days of age (75% of retinal vascularization) (n = 6 each gender, control vs. IUGR). Retinal cDNA was synthesized, and real time RT-PCR was performed. Target genes include mRNA variants of IGF-1, IGF-1 receptor, HIF-1α, Epo, and Epo receptor.

Results of Summary: In newborn male rat pups, IUGR significantly increased retinal mRNA levels of 1) IGF-1 mRNA variants from both the proximal 1 and promotor 2 mRNA variants (P1: 172±24% of male controls; P2: 245±47% of male controls) (p < 0.03), and 2) Epo mRNA levels (144±17% of male controls) (p < 0.01). IUGR also increased HIF-1α and Epo receptor mRNA levels, though significance was only approached (p = 0.06).

Conclusions: We conclude that IUGR increases retinal mRNA levels in male newborn rats of genes associated with the pathogenesis of ROP. These findings are intriguing considering previous reports associating retinal para- crine expression of IGF-1 and Epo with the development of vascular retinopathy in adults. We speculate that postnatal environmental insults induce a neovascular retinopathy in IUGR male rat pups at a greater rate than controls or female IUGR rat pups.

295 THE INHIBITORY POTENCY OF LOW DOSES OF ZINC DEUTEROPORPHIRIN BIS GLYCOL ON HEME OXYGENASE ACTIVITY IN 3-DAY-OLD MICE
Katayama Y, Shaw N, Yaife ZA, He CX, Kalish FS, Schulz-Geske S, Zhao H, Wong RJ, Stevenson DK. Stanford University School of Medicine, Stanford, CA.

Purpose of Study: Neonatal hyperbilirubinemia is a common problem in newborns. Structural heme analogs, metalloporphyrins (Mps), competitively inhibit heme oxygenase (HO), the rate-limiting enzyme in bilirubin production, and are potential compounds for clinical use in the treatment of neonatal hyperbilirubinemia. Although tin mesoporphyrin (SnMP) has been studied in human neonates, its property as a photosensitizer and ability to induce HO-1 may limit its clinical use. We have previously reported on the in vivo efficacy of an alternative Mp, zinc deuteroporphyrin bis glycol (ZnBG) in adult rodents. In this study, we investigated the efficacy of ZnBG towards inhibiting HO activity and its effects on HO-1 expression in 3-day-old mice, establishing a time and range of dosing for phototoxicity testing.

Methods Used: 3–0.5-day-old FVB mice were given vehicle (controls) or ZnBG (0.12, 0.23, 0.47, and 0.94 μmol/kg) IP. After 3h, mice were sacrificed and the liver and brain were harvested and sonicated in phosphate buffer. HO activity was quantified by gas chromatography, calculated as pmol CO produced/hr/mg fresh weight (FW) and expressed as % inhibition of HO activity of control values. HO-1 expression was assessed by measurements of HO-1 protein using Western Blots.

Summary of Results: 3h after IP administration, ZnBG significantly inhibited HO activity in the liver at all doses, ranging from 28% to 65%. When % inhibition of HO activity was plotted against the dose of ZnBG, the IC50 of ZnBG was interpolated to be 0.325 μmol/kg body weight. No significant inhibition of HO activity was observed in the brain. In addition, no significant changes in HO-1 protein were found in either tissue for any dose.

Conclusions: We conclude that ZnBG is effective in inhibiting HO activity rapidly and at relatively low doses in 3-day-old mice, and therefore, is an attractive compound for potential use in the treatment of neonatal hyperbilirubinemia. Further study is required to determine the long-term effects of ZnBG as well as its photoreactivity.

% Inhibition of HO Activity (mean±SD, n ≥4), *p<0.01 vs control

<table>
<thead>
<tr>
<th>ZnBG (μmol/kg)</th>
<th>LIVER</th>
<th>BRAIN</th>
</tr>
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<tbody>
<tr>
<td>0.12</td>
<td>28.3±9.6</td>
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<tr>
<td>0.47</td>
<td>57.8±9.6</td>
<td>10.5±6.8</td>
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<tr>
<td>0.94</td>
<td>65.1±6.9</td>
<td>2.2±9.9</td>
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</table>

296 BACTERIAL NITRATE REDUCTION IN SALIVA OF NEWBORN INFANTS
Kanady JA1, Hopper AO1, Ninnis JR2, Aruni W2, Power GG1, Blood AB3, 1Loma Linda University, Loma Linda, CA; 2Loma Linda University; Loma Linda, CA; 3Loma Linda University; Loma Linda, CA.

Purpose of Study: NEC is an acute, idiopathic disease characterized by infection and necrosis of the bowel wall and occurs in approximately 5–12% of premature infants. Nitric oxide (NO) protects the normal adult GI tract by increasing blood flow, thickening the mucous lining, and killing bacteria. Nitrite is converted to NO under acidic conditions and therefore constitutes a significant source of NO in the adult gastrointestinal tract. Swallowed nitrite is derived by the action of anaerobic bacteria on the dorsal surface of the
tongue that convert ingested nitrates to nitrite. The activity of these bacteria in the oral flora of newborn infants has not been studied. This study tests the hypothesis that premature infants, known to be at increased risk for NEC, have decreased bacterial reduction of nitrate to nitrite in the mouth.

Methods Used: Saliva was collected from preterm infants (n=8) and adults (n=8) by swabbing the back of the tongue with a cotton-tipped applicator. The volume of saliva collected was determined by weight gain of the swab, whose tip was then immediately transferred to 3.0 mL of sterile, anaerobic broth at 37 °C. To assess nitrate-reducing activity, 33 mM nitrate was added to the broth and the increases of nitrite concentration [NO2−] were measured over 30 min by triiodide chemiluminescence.

Summary of Results: Baseline salivary [NO3−] in preterm infants (0.91± 0.12 μM, SEM) was significantly lower than in adults (2.72± 0.35 μM; p<0.001; ANOVA). The nitrate-reducing activity, calculated from the slope of nitrite increase after normalizing to the volume of saliva collected, was minimal in the saliva of preterm infants (2.6±2.7 nmoles/min/mg saliva), and only a fraction of that in adult saliva (200±49 nmoles/min/mg) (p<0.001).

Conclusions: The nitrite concentration in the saliva of preterm infants is ~33% of the salivary [NO3−] in adults. Nitrate reducing activity provided by bacterial flora on the surface of the tongue of preterm infants is only ~1.3% of the activity found in adults. Thus preterm infants have far less nitrite to be converted to NO than adults. This deficiency merits further study as a contributing cause of NEC.

297 PROLONGED NEONATAL INTENSIVE CARE STAY FOR INFANTS OF DIABETIC MOTHERS
Phattraprayoon N1, Al-Ramadhanii R2, Barton L1, Ramanathan R1, 1Keck School of Medicine, University of Southern California, Los Angeles, CA and 2Keck School of Medicine, University of Southern California, Los Angeles, CA.

Purpose of Study: There has been a decrease in morbidity and mortality of infants of diabetic mothers (IDM) over the past decades, but there have been few reports of reasons for IDM babies. We examined only a fraction of the preterm infants had activity results for NICU stay and to see if any improvement occurred over time.

Methods Used: IDM infants greater than A1 classification who came to our NICU for hydropsalycemia screening or other reasons during two time periods (1997–1999 and 2007–2009) were reviewed for stays requiring longer stays in the neonatal intensive care unit (NICU) than just to be monitored for hydropsalycemia. We reviewed our IDM babies from 1997–1999 and compared them to a later cohort from 2007–2009 to look at the length of stay, reasons for NICU stay and to see if any improvement occurred over time.

Summary of Results: Six hundred and thirty five IDM infants (348 born in 1997–1999 and 287 in 2007–2009) were reviewed for this study.

From the 1997–1999 cohort, mean birth weight was 3595 ± 586 g, range 2205–5580 g and gestational age 38 ± 1 weeks, range 37–42 weeks, and length of stay 1–59 days.

From the 2007–2009 cohort, mean birth weight was 3391 ± 476 g, range 2220–5040 g and gestational age 38 ± 1 weeks, range 37–42 weeks, and length of stay 1–64 days.

Incidence of hypoglycemia, large for gestational age (LGA), birth trauma and length of stay were decreased in the later cohort; however, incidence of respiratory distress and feeding intolerance did not improve.

Conclusions: IDM infants still remained at significantly increased risk of requiring NICU care in our study. Major causes of length of stay have not changed from earlier years. Respiratory distress and feeding intolerance are still the main causes of prolonged NICU stay.

Results:

<table>
<thead>
<tr>
<th>Patient Number</th>
<th>Cause of Death</th>
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<tbody>
<tr>
<td>1</td>
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<tr>
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</tr>
<tr>
<td>3</td>
<td>Apoplexy</td>
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<td>Congenital malformation</td>
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<tr>
<td>6</td>
<td>Infection</td>
</tr>
<tr>
<td>7</td>
<td>Prematurity and sepsis</td>
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</table>

299 A META-ANALYSIS OF Dopamine USE in Hypotensive Preterm Infants: Blood Pressure and Cerebral Hemodynamics
Sassano-Higgins S, Friedlich P, Seri 1 Childrens Hospital Los Angeles, Los Angeles, CA.

Purpose of Study: Dopamine administration results in variable effects on blood pressure in hypotensive preterm infants. The clinical benefits of dopamine administration in increasing cerebral blood flow (CBF) and reducing adverse neurological outcomes in hypotensive preterm infants are unclear. The current study was conducted to examine the efficacy of dopamine for treatment of hypotension and to investigate the changes in cerebral hemodynamics and central nervous system injury in hypotensive preterm infants following dopamine administration.

Methods Used: A meta-analytic design was used. All studies published in peer-reviewed journals with sufficient information for statistical analysis were included. Random and fixed effects models were used to calculate effect size estimates and significance levels.

Summary of Results: Random effects meta-analysis found that dopamine increases mean arterial blood pressure (12 studies; N=163; r=0.88, 95%CI=0.76–0.94) and systolic blood pressure (8 studies; r=0.48, 95%CI=0.20–0.32, colloid (2 studies; N=67, r=0.60, 95%CI=0.41–0.74), and hydrocortisone (1 study; N=28; r=0.40, 95%CI=0.034–0.67). CBF increased following dopamine administration.
Dopamine administration increases mean and systolic blood pressure in hypotensive preterm infants, and is more effective than dobutamine, colloid, or hydrocortisone alone. Dopamine administration is associated with increased CBF, with greater increases in CBF in hypotensive than in normotensive preterm infants. Dopamine is not associated with a greater incidence of adverse effects than other therapies used to treat hypotension.

300 PRENATAL METHAMPHETAMINE USE AND NEONATAL AND INFANT NEUROBEHAVIORAL OUTCOME: RESULTS FROM THE INFANT DEVELOPMENT, ENVIRONMENT, AND LIFESTYLE (IDEAL) STUDY

Kiblawi Z1,a, Smith LM2,3, LaCasse L2, Derauf C3, Newman E2, Shah R2, Arrin A2, Huotis M4, Haning W2, Strauss A2, DellaGrotta S1, Donarumo LM2, Lester BM2, Harbor-UCLA Medical Center, Torrance, CA and 1IDEAL Community Research Network, Providence, RI.

Purpose of Study: Methamphetamine (MA) use among pregnant women is an increasing problem in the United States. How MA use during pregnancy affects neonatal and infant neurobehavior is unknown. The purpose of this study is to examine the neurobehavioral effects of prenatal MA exposure in the multicenter, longitudinal IDEAL study.

Methods Used: IDEAL screened 34,833 subjects at 4 clinical centers. 17,961 (67%) were eligible and consented, among which 412 were enrolled. Exposed subjects were identified by self-report and/or GC/MS confirmation of amphetamine and metabolites in infant meconium. Comparison subjects were matched (race, birth weight, maternal education, insurance), denied amphetamine use and had a negative meconium screen. Both groups included prenatal alcohol, tobacco and marijuana use, but excluded use of opiates, LSD, or PCP. The NICU Network Neurobehavioral Scale (NNNS) was administered within the first 4 days of life and again at 1 month old to 380 enrollees (185 exposed and 195 comparison). ANOVA tested exposure effects on NNNS summary scores at birth and one month. GML repeated measures analysis assessed the effect of MA exposure over time on the NNNS summary scores within and without covariates.

Summary of Results: Prenatal MA exposure was associated with decreased arousal (P = 0.001) and excitability (P = 0.043) scores relative to the comparison newborns. By one month of age, both groups showed higher quality of movement (P = 0.037), less lethargy (P = 0.001) and fewer asymmetric reflexes (P = 0.016). Methamphetamine specific changes in NNNS scores over the first month of life were noted with increased arousal (P = 0.031) and decreased total stress scores (P = 0.03).

Conclusions: Prenatal MA exposure in the neonatal period was associated with neurobehavioral patterns of decreased arousal and excitability. Improvements in arousal and total stress were observed in MA exposed newborns by one month of age.

301 EARLY PHYSICAL SIGN OF CONGENITAL RICKETS: HARRISON’S THORACIC GROOVE

Chan GM University of Utah, Salt Lake City, UT.

Case Report: We would like to present two cases of congenital rickets where the presence of Harrison’s groove was noted. Case 1 was a 34 week gestation small for gestation female with a birth weight of 1100 g. This infant was a product of a 33 year old gravidia 7 para 4024. Mother noted decreased fetal movement during the last 3 weeks of the pregnancy. Case 2 was a 30 week gestation appropriately sized female with a birth weight of 920 g. This infant was a product of a 24 year old primigravida mother. Physical exams during the first week of life was unremarkable except for the presence of a linear groove or sulcus across the anterior lower part of the thorax in both infants. Radiographic studies of the chest were reportedly normal for lungs and bones. Laboratory findings included serum calcium levels of 10.9 and 10.6 mg% (normal 6–10 mg%), phosphate of 2.3 and 4.0 mg% (normal 4.2–9 mg%), 25-hydroxyvitamin D of 19 and 23 ng/ml (normal 30–60 ng/ml), 1,25-dihydroxyvitamin D of 107 pg/ml and 89 pg/ml (normal 25–45 pg/ml), and urine calcium/creatinine ratio of 1.7 and 1.4 (normal <0.7). We believe that the physical sign of Harrison’s groove in these infants is indicative of congenital rickets from maternal calcium and/or vitamin D deficiency.

Nephrology and Hypertension

Concurrent Session

1:30 PM Friday, January 28, 2011

302 IL-33-MEDIATED CISPLATIN-INDUCED AKI IS DEPENDENT ON CD4 T CELL-MEDIATED PRODUCTION OF CXCL1

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Purpose of Study: The major complication of cisplatin administration is acute kidney injury (AKI). IL-33 is a novel pro-inflammatory cytokine predominantly present in endothelium. We have previously demonstrated that cisplatin causes endothelial cell apoptosis and necrosis. Thus the effect of cisplatin-induced AKI on IL-33 was determined in mice.

Methods Used: Mice were injected with Cis 25 mg/kg. There is tubule apoptosis (day 1), necrosis (day 2) and elevated BUN and serum creatinine (SCr) (day 3).

Summary of Results: On immunofluorescence (IF) of kidney, IL-33 was predominantly present in the endothelium. On immunoblot, Full-length (active) IL-33 (34 kDa) increased in the kidney in cisplatin-induced AKI. On ELISA, IL-33 increased in the kidney in cisplatin-induced AKI compared to vehicle treated mice. To demonstrate the injurious role of IL-33, it was determined whether soluble ST2 (sST2), a fusion protein that neutralizes IL-33 activity by acting as a decoy receptor, protected against AKI or whether inhibition of recombinant IL-33 (rIL-33) worsened AKI. Infiltration of CD4 T cells into the kidney, serum creatinine (sCr), acute tubular necrosis (ATN) and apoptosis decreased in the kidney in mice with cisplatin-induced AKI treated with sST2. CD4 T cells, sCr, ATN and apoptosis were increased in wild type mice, but not in CD4−/− mice, injected with cisplatin plus rIL-33. Thus, the injurious effect of IL-33 is mediated by CD4 T cells. CXCL1, a proinflammatory chemokine, is produced by CD4 T cells. CXCL1 was increased in kidney in wild type, but not in CD4 T cell −/− mice treated with cisplatin plus rIL-33. CXCR2−/− mice, that are deficient in the receptor for CXCL1, had lower sCr, ATN and apoptosis scores compared to wild type mice with cisplatin-induced AKI, demonstrating the injurious role of CXCL1 in cisplatin-induced AKI.

Conclusions: IL-33-mediated cisplatin-induced AKI is dependent on CD4 T cell-mediated production of CXCL1. Inhibition of IL-33 or CXCL1 has therapeutic potential in cisplatin-induced AKI.

303 VITAMIN D INHIBITS CELL PROLIFERATION WITHOUT INDUCING APOPTOSIS OF HEART-DERIVED CELLS, ENHANCING CARDIAC DIFFERENTIATION

BY MODULATING THE EXPRESSION OF KEY Wnt SIGNALING FAMILY MEMBERS

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Purpose of Study: Cardiovascular disease (CVD) remains the leading cause of death in the US. Low levels of 25D (<15ng/ml) are associated with high risk of myocardial infarction, even after controlling for factors known to be associated with coronary artery disease. A growing body of evidence suggests that the Vitamin D receptor and 1,25D (the active form of vitamin D) plays an important role in several CVD related signaling pathways. The Wnt signaling pathway is pivotal to gene expression and tissue development; it has been shown to control stem cell renewal, lineage selection and more.
importantly heart development. The purpose of this study is to demonstrate that 1,25D induces cardiac differentiation by inhibiting cardiomyocytes cell proliferation without promoting apoptosis through modulation of selected members of the Wnt signaling pathway.

**Methods Used:** H9c2 cardiomyocytes incubated with or without 1,25D were evaluated for cell proliferation, apoptosis, changes in cell area and the expression of genes related to the cell cycle and Wnt family members by RT2PCR, immunocytochemistry and westernblots.

**Summary of Results:** Addition of 1,25D to H9c2 cardiomyocytes: a) promotes nuclear translocation of VDR; b) inhibits cell proliferation without promoting apoptosis; c) decreases the expression of genes related to the regulation of the cell cycle; d) enhances cardiomyotubes formation; e) induces the expression of Casein kinase-I and f) increases the expression of Wnt11.

**Conclusions:** 1,25D promotes cardiac differentiation by negative modulation of the non-canonical Wnt signaling pathway through Casein kinase-I and up-regulating the expression of Wnt11, which has been reported to induce cardiac differentiation. This is the first mechanistic demonstration that supports the hypothesis that VD repletion might attenuate CVD not only by down regulating excess cell proliferation but by promoting cardiac cellular differentiation.

**304 COMPARISON OF METHODS FOR COUNTING PODOCYTES**

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**Purpose of Study:** The podocyte is a specialized epithelial cell that is crucial in maintaining the structure and function of the glomerulus. Some renal diseases report a decrease in podocyte number, but are inconsistent in the podocyte number counted, possibly due to different counting methods. In this study, we report results from three methods comparing podocyte number obtained from two widely used counting methods (Weibel-Gomez and Fractonator/Disector) against a gold standard (Exhaustive Count).

**Methods Used:** Serial 1-μm epon sections (n=250) were cut from a single mouse kidney. Images from all sections through nine glomeruli were used for Exhaustive Count, which allowed all podocyte nuclei to be counted as they were sequentially observed and is the gold standard. For the Weibel-Gomez method, the number of podocyte nuclei profiles were counted, and the volume fraction of podocyte nuclei per glomerulus was determined using a counting grid superimposed over the image. Shape and variance factors were determined and glomerular volume was measured following the calculation of podocyte number. For the Fractonator/Disector method, pairs of consecutive sections were used to determine the number of podocytes. The number of profiles from podocytes that were present in the Sampling Section but absent in the Look-up Section was used to calculate podocyte number.

**Summary of Results:** From the Exhaustive Count method 84.3±7.7 (mean±SD) podocytes were counted compared with 89.3±6.8 podocytes using the Weibel-Gomez method, which was significantly different, p<0.001. From the Fractonator/Disector method, 81.9±13.3 were counted, with no difference compared to the gold standard, p=0.30.

**Conclusions:** These results suggest that the unbiased Fractonator/Disector method is the recommended method for counting podocytes. Study was funded by NIH grant 1U54RR026138.

**305 FACTORS CONTRIBUTING TO THE EFFICACY OF THYMoglobulin AS A TREATMENT FOR ACUTE KIDNEY GRAFT REJECTION**

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**Purpose of Study:** To examine the outcomes of Thymoglobulin treatment for acute rejection and to identify risk factors associated with graft failure using the UCLA Kidney Transplant Database.

**Methods Used:** Between February 2000 and June 2010, there were 517 hospital admissions for possible kidney allograft rejection. Among these, there were 114 unique patients treated with Thymoglobulin for acute rejection, and 98 were included in the analysis, with a median follow up time of 567 days (16 patients did not have sufficient follow-up data). An analytical file was created by retrospective chart-review and baseline characteristics were collected. The Kaplan-Meier product limit method was used to estimate the survival rate. Univariate and multivariate survival analyses were performed using the Cox proportional hazard model.

**Summary of Results:** Graft failure occurred in 36 of the 98 patients (36.7%) with a median time between Thymoglobulin treatment and graft failure of 567 days (25-75%: 152-1265 days). In multivariate analysis, factors associated with increased risk of graft failure included doses of Thymoglobulin received (>9) and number of biopsies performed on the current allograft (>1). Factors associated with decreased risk of graft failure included creatinine level at time of Thymoglobulin treatment (<3.0 mg/dL) and identification of vascular rejection versus non-vascular rejection on the biopsy performed immediately prior to Thymoglobulin treatment.

**Conclusions:** Resolution of acute rejection is vital to allograft survival and Thymoglobulin is a last-line treatment for steroid-resistant acute rejection. Our results indicate that the doses of Thymoglobulin received, the creatinine level at time of Thymoglobulin treatment, the number of prior biopsies, and the presence of non-vascular rejection are associated with increased risk for graft failure. Individual chart-review permitted access to biopsy data not available in database studies, and the analysis suggests that perhaps Thymoglobulin treatment is less efficacious in acute rejection with chronic changes. In patients with risk factors found to be significant for graft failure, nephrologists may choose to lower or omit the use of Thymoglobulin as its benefits may be outweighed by its recognized adverse effects in the long run.

**306 HOSPITALIZATIONS IN ELDERLY HEMODIALYSIS PATIENTS**

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**Purpose of Study:** There is an increasing amount of elderly patients developing end-stage renal disease (HD) for elderly patients has been questioned. Prior studies have not been consistent on whether age is a predictor of hospitalization in chronic HD patients. The purpose of the study was to determine whether hospital admissions and hospital days differed between elderly and younger patients on chronic HD.

**Methods Used:** Hospitalization rate (HR), length of hospitalization (LH) and co-morbidities were compared between patients >70 years (Group A) and <70 years (Group B). HR is compared in number per patient per year (n/pt-yr) and LH in days per patient per year d/pt-yr), both presented as Mean (95% Confidence Interval) between two groups of incident HD patients.

**Summary of Results:** Group A had initiation of HD at 77±5 years (n = 173). Group B had initiation of HD at 57±8 years (n = 213). Diabetes mellitus (DM) was present in 50% of the Group A patients and 65% of the Group B patients. Differences in hospitalizations from all causes were marginal: rate, Group A 2.46 (2.11, 2.81), Group B 1.97 (1.74, 2.20) n/pt-yr; p = 0.061; length, Group A 34.2 (26.3, 42.1), Group B 23.2 (18.1, 28.2) d/pt-yr; p = 0.053. Hospitalizations for individual categories (e.g. cardiac, HD vascular access, infections, etc) did not differ between the two groups. Among patients with DM, group A subjects had a higher admission rate and greater length of hospitalizations from all causes and from infections than Group B subjects. No difference was found for any category of hospitalization between Group A and Group B patients without DM. For hospitalizations from all causes, multiple linear regression identified co-morbidity, quantified by the Charlson index (p < 0.001), poor compliance with the HD schedule (p = 0.069) and only marginally advanced age at HD initiation (p = 0.092) as predictors of a high rate, and high Charlson index (p < 0.001), older age at HD (p = 0.035), and marginally poor compliance (p = 0.069) as predictors of great length.

**Conclusions:** Co-morbidities, which includes DM, are the most potent determinants of frequency and length of hospitalizations in HD patients, while advanced age has only marginal effects.

**307 THE ROLE OF NETRIN-1 RECEPTORS IN MEDIATING ATTENUATION OF ACUTE KIDNEY INJURY**

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Purpose of Study: Ischemia is the most common cause of acute kidney injury (AKI). For example, patients undergoing surgical procedures requiring cross-clamping of the aorta or renal vessels experience AKI in up to 30% of cases. Similarly, AKI after cardiac surgery occurs in approximately 10% of patients and is associated with dramatic increases in morbidity and mortality. Our laboratory has previously shown that netrin-1 attenuates kidney injury and inflammation following renal ischemia. The current study will investigate which of the known netrin-1 receptors (UNC5A, UNC5B, UNC5C, UNC5D, neogenin, DCC, A2BAR) mediate renal tissue protection. These findings could provide new therapeutic options for patients with AKI.

Methods Used: Mice were anesthetized using intraperitoneal pentobarbital. Mice then underwent renal ischemia (30 or 60 minutes) using a previously-described hanging-weight system to selectively occlude the left renal artery. The kidney was allowed to reperfuse (2 or 6 hours), then left nephrectomy was performed and the kidney was flash-frozen. Each experiment was completed on 3 wild-type and 3 mice with partial depletion of netrin-1 expression (Ntn1+/−). Homozygote mice gene-targeted for netrin-1 are not viable and die shortly after birth. Fold-changes in netrin receptor expression were determined via RT-PCR. Confirmatory studies will be completed using Western blot for receptors that show significant fold changes in mRNA expression.

Summary of Results: Preliminary data show an up-regulation of UNC5B and A2BAR in wild type mice and heterozygote mice following renal ischemia and reperfusion compared to controls without ischemia. We have not shown any regulation of the other receptors so far. However, the number of experiments must be increased to confirm this initial data set.

Conclusions: Based on mRNA analysis, the kidneys seem to up-regulate the UNC5B and A2BAR after renal ischemia. Further studies must be performed to confirm these preliminary findings.

RISE IN SERUM SODIUM CONCENTRATION AFTER SALINE INFUSION IN HYPONATREMIA

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Case Report: We investigated factors that may lead to quantitative discrepancies between predicted and measured sodium [Na] in a patient who developed osmotic myelinolysis.

A 55-year-old man with alcoholism was admitted with diarrhea and repeated falls. Blood pressure was 93/49 mm Hg and pulse 90 bpm. Neurologic, cardiac and pulmonary examinations were normal. Edema was not detected. Initial [Na] was 111 mmol/L and serum osmolality 231 mOsm/kg. Six hours later, after infusion of 1.75 L normal saline (NS), [Na] was 120 mmol/L, serum osmolality 254 mOsm/kg, urine osmolality 10 mmol/L. Infusion rate was decreased. After another 12 hours and a total of 2.5 L NS, [Na] was 129 mmol/L. Osmotic myelinolysis manifested two days later.

To compare values observed and those predicted by the Adrogue-Madias formula for an infusion of 1.75 L NS, we classified the potential pitfalls into three categories: (A) Estimates entered in the formula. Volume and sodium concentrations in infusate and serum are measured, but body water is estimated. We varied body water by 10 L (36 and 26 L) with resulting predicted serum [Na] of 113.0 and 113.7 mmol/L. (B) Omission of other factors affecting [Na]. Considering changes in the osmotic coefficients of sodium and potassium and the effects of the Gibbs-Donnan equilibrium, the predicted [Na] for body water of 36 and 26 L is 133.2 and 114 mmol/L. (C) Assuming a closed system. We calculated the effects of external losses of water, sodium and potassium through the lungs, skin, gastrointestinal system and the kidneys. An estimated loss of 0.3 L of water through the first three routes would lead to a [Na] of 114.7 and 116 mmol/L for body water values of 36 and 26 L. The calculated urine volume with sodium and potassium concentrations of 10 mmol/L each, needed to raise the [Na] to 120 mmol/L was 2.0 and 1.1 L, respectively.

External losses of water and solute are the main causes of discrepancies between predicted and final [Na] values in patients treated for hyponatremia. As these losses are unpredictable, monitoring of clinical status and laboratory parameters during saline infusion is imperative.

MAINTENANCE OF PHENOTYPE OVER SERIAL PASSAGES IN FETAL CELL CULTURES

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Purpose of Study: Obtaining fetal samples at specific gestational ages and in timely fashion for investigation can be problematic. The possibility of maintaining the phenotype of a variety of primary human fetal cell cultures through passing and freeze/thawing has not been evaluated. In addition, smaller samples at younger gestational ages limit the total cell number to be studied, while growing cells under stable conditions allows expansion of cell numbers. We sought to determine if cell phenotype could be maintained in retinal and brain cell cultures following freezing and reconstitution.

Methods Used: Human fetal retinal and brain samples were collected between 15–23 weeks of gestation. Single cell suspensions were created and suspended in Dulbecco’s Modified Essential Media, with 10% fetal bovine serum and 1% antimicrobials added. Cells were grown to confluence and passed by incubating in 0.05% trypsin, washing in DMEM, and resuspending at a concentration of 5 × 10⁷ cells per flask. Cells were passed twice before freezing. Cells were grown on coverslips with each passage, and fixed with 3.7% formaldehyde for 5 minutes, then stored in phosphate buffered saline for later immunostaining. Cells were removed from tissue flasks with trypsin, washed and pelleted, and resuspended in 1 mL of 7.5% dimethyl sulfoxide (DMSO) in DMEM. The concentrated cell suspension was slowly frozen in an ethanol freezing chamber. Cells remained frozen at −80°C for 24 hours, then transferred to liquid nitrogen (−196°C Centigrade). Coverslips were stained with antibodies directed against GFAP (astrocytes), Nestin (neuronal stem cells), or MAP-2 (neurons).

Summary of Results: Both neuronal and retinal cell lines were viable after 2 passages and a freeze-thaw step. Cells remained frozen for a minimum of 3 weeks, and a maximum of 5 months. In all cases, cell lines were successfully reconstituted to the next stage without significant change in phenotype. In retinal cell primary cultures, astrocytes comprised 92–94%, neuronal stem cells comprised 3–5%, and neurons comprised 1–2% of total cells. In brain cell primary cultures, cells consisted of 80–85% astrocytes, 5–8% stem cells, and 3–4% neurons.

Conclusions: Both retina and brain cultures were successfully reconstituted. Further studies are required to determine if the phenotype of the cells change with further passages.

DISPARATE CONSERVATION OF GENE EXPRESSION IN LMX1B-DIRECTED LIMP DORSALIZATION

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Purpose of Study: The role of LMX1B in limb development is well established; however, the process by which it regulates the cascade of events required for dorsalization remains unclear. Identification of downstream targets of LMX1B is imperative for understanding limb development and managing dysmorphogenic diseases such as Human Nail Patella Syndrome. Microarray analysis comparing wild-type and Lmx1b knockout embryonic mouse limbs (11.5, 12.5, and 13.5 days post coitum) identified several potential downstream gene targets of Lmx1b. Utilizing whole mount in situ hybridization (WMISH) analysis, four particular genes of interest (Keratocan, Lumican, Decorin, and Lm2) have shown dorsally restricted expression in the mouse model. We hypothesized that Lmx1b-restricted dorsal expression of these target genes would be conserved throughout tetrapod limb development. Demonstration of conserved developmental expression would allow clinical questions to be evaluated in animal models. Characterization of normal expression of these potential target genes in animal models is necessary to characterize their regulation by Lmx1b.

Methods Used: Using WMISH, normal expression patterns for LMX1B, KERATOCAN, LUMICAN, DECORIN, and Lm2 were documented in the chick at Hamburger-Hamilton stages 21, 23, 25, and 27.
Summary of Results: Uniquely individual expression patterns were observed for all four target genes; however, none of these molecules displayed dorsally restricted expression patterns. Although initiation of expression occurred at a stage later than LMX1B, their expression patterns included specifically localized dorsal and ventral regions. Interestingly, each of the genes studied demonstrate an overlap of expression in the presumptive scapular region indicating that LMX1B may utilize these genes to pattern the scapula.

Conclusions: The lack of correlation between chick and mouse expression patterns suggests that the LMX1B-directed gene cascade of limb dorsalization is not tightly conserved across species.

311 INCREASED LEF1 EXPRESSION IMPLICATES THE WNT/β-CATENIN PATHWAY IN THE MEDIATION OF FGFR-INDUCED UP-REGULATION OF SHH

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Purpose of Study: Shh and Fgf play critical roles in limb development and regeneration. Fgf is secreted from the apical ectodermal ridge (AER) of the developing limb to induce palmar Fgf-8 expression. However, the mechanism by which Fgf regulates Shh expression is unknown. We analyzed the expression time course of Shh in response to Fgf. At 3 and 6 hours, WMISH evaluations may help reduce disease burden.

Summary of Results: We confirmed the gene array data at 24 hrs with robust Lef1 up-regulation in response to Fgf. At 3 and 6 hours, WMISH showed no significant Lef1 up-regulation. By 12 hours, there was detectable expression that progressively increased at 18 and 24 hours.

Conclusions: In previous studies, we found other Wnt/β-Catenin pathway genes, for Wnt5a and its receptor Fzd4, to be up-regulated at 12 and 18 hours (respectively) after Fgf bead implantation. In this study, we found that Lef1, the gene for a downstream transcription factor of Fzd4, was up-regulated within the same period as Wnt5a and Fzd4. Collectively, this data further implicates the Wnt/β-Catenin pathway during Fgf induced up-regulation of Shh.

312 DEFINING THE MECHANISM OF LIMP REGENERATION: A POTENTIAL NOVEL ROLE FOR BAMBI IN MEDIATING FGFR-INDUCED SHH UP-REGULATION

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Purpose of Study: Shh is critical to limb patterning during development and regeneration. The apical ectodermal ridge secretes FgfS that induce limb outgrowth and maintain Shh expression in the zone of polarizing activity. Through a reciprocal Fgf-Shh loop, the mechanism by which Fgf regulates Shh expression is unknown. To identify intermediate molecules involved in this mechanism, we performed gene arrays 24 hrs after Fgf2 application to the posterior region of the chick limb bud. The gene array data demonstrated elevated levels of BMP pathway associated genes (BMP7 and BAMBI). We hypothesized that the BMP pathway might participate in the Fgf-induced up-regulation of Shh.

Methods Used: We analyzed the expression time course of BMP7 and BAMBI during FGF-induced SHH up-regulation. FGF2-soaked beads were implanted into the posterior aspect of stage 23 chick wing buds. The chicks were then harvested after 3–24 hr of incubation and an in situ hybridization for BMP7 and BAMBI was performed.

Summary of Results: BMP7 expression was not elevated after 24 hr of FGF2 treatment (in contrast to gene array data), while FGF2-induced BAMBI up-regulation was validated. Furthermore, FGF2 elevated BAMBI expression within 6 hr after application.

Conclusions: These results were unexpected. We anticipated that BMP7 would be up-regulated, followed by its inhibitor, BAMBI. The up-regulation of BAMBI without up-regulation of BMP7 suggested an alternative role for BAMBI. Recent studies have demonstrated that, in addition to its role as a BMP inhibitor, BAMBI can act as a positive modulator of the Wnt/β-catenin pathway, a pathway known to participate in Shh regulation. Our data suggests that BAMBI plays a novel role in Fgf-induced Shh up-regulation by modulating the Wnt/β-catenin pathway.

313 ASSESSING SUSCEPTIBILITY TO AGE-RELATED MACULAR DEGENERATION WITH GENETIC MARKERS AND ENVIRONMENTAL FACTORS

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Purpose of Study: To evaluate the independent and joint effects of genetic factors, environmental factors and their interaction on age-related macular degeneration (AMD) including geographic atrophy (GA) and choroidal neovascularization (CNV) and to develop a predictive model with both genetic and environmental factors included.

Methods Used: Demographic information, including age of onset, smoking status and BMI, was collected in 1844 participants. Genotypes were evaluated for eight variants in five genes related to AMD. Unconditional logistic regression analyses were performed to generate a risk predictive model.

Summary of Results: All genetic variants showed strong association with AMD. Multivariate odds ratios (ORs) were: 3.52 (95% CI: 2.08–5.94) for CFH rs1061170 CC, 4.21 (95% CI: 2.30–7.70) for CFH rs2274700 CC, 0.46 (95% CI: 0.27–0.80) for C2 rs9332739 CC/CG, 0.44 (95% CI: 0.30–0.66) for CFB rs641153 TT/CT, 10.99 (95% CI: 6.04–19.97) for HTRA1/LOC387715 rs10499024 TT and 2.66 (95% CI: 1.43–4.96) for C3 rs2230199 GG. Smoking was independently associated with advanced AMD after controlling for age, gender, body mass index (BMI) and all genetic variants.

Conclusions: CFH confers more risk to the bilaterality of GA whereas LOC387715/HTRA1 contributes more to the bilaterality of CNV. Risk models with combined genetic and environmental factors together has a notable discrimination power; further C3 confers more risk for GA than CNV. Targeting high risk individuals for surveillance and clinical interventions may help reduce disease burden.

Clinical Relevance: Early detection and risk prediction of AMD could help to improve the prognosis of AMD and reduce the outcome of blindness.

314 EXCLUSION OF PATHOGENIC CODING REGION MUTATIONS IN POSITIONAL CANDIDATE GENES FOR POSTERIOR AMORPHOUS CORNEAL DYSTROPHY

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Purpose of Study: Posterior amorphous corneal dystrophy (PACD) is a rare autosomal dominant disorder that is associated with a constellation of disorders of the anterior segment of the eye. Clinical findings include partial or complete posterior lamellar corneal opacification, decreased corneal thickness, scleralization of the peripheral cornea, corneal flattening with an average corneal curvature less than 41 D, iridocorneal adhesions, correctopia, and iris atrophy. Genome-wide linkage analysis has demonstrated linkage to chromosome region 12q21.33. There are 22 genes in the linked interval, and wild type sequences. target of study regions and positional candidate genes for AMD.

Methods Used: PCR amplification of the exonic regions of each positional candidate gene was performed using DNA from two affected individuals, two unaffected individuals, and an unrelated control. Sanger sequencing reactions were carried out, and variants were identified by comparison of the generated and wild type sequences.

Summary of Results: Screening of 16 of the 18 positional candidate genes (ATP2B1, Btg1, C12orf2, CENP2, CLU1, CLU1OS, DUSP6, GALNT4,
Loc100132126, Loc100287355, Loc100287398, Loc100287505, MRPL22P1, MRPS6P4, and WD3D (B) has been completed. Thirty eight sequences were identified in 20 genes, including 35 known variants and 3 novel variants. 10 silent mutations, 8 missense mutations, 1 nonsense mutation, 1 deletion, and 18 non-coding RNA changes were identified. None of the variants segregated with the affected phenotype in the family.

**Conclusions:** The coding regions of 20 of the 22 positional candidate genes for PACD have not revealed the pathogenic mutation in the family in which linkage to chromosome 12q21.33 was demonstrated. The pathogenic mutation may be contained in the two remaining genes in the linkage interval (Loc464153 and RPL21P106), which are currently being screened. Alternatively, the mutation may be located slightly outside the linkage interval, may involve a copy number variant or may be in a non-coding region of one of the genes already screened.

### 315 ENHANCED LEUKOCYTE CHEMOTAXIS INTO SERUM FROM A MOUSE MODEL OF GAUCHER DISEASE

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**Purpose of Study:** Gaucher Disease is a lysosomal storage disorder resulting from mutations in the gene encoding glucocerebrosidase, an enzyme that cleaves glucocerebroside from Glucosylceramide (GC). Defective activity leads to accumulation of GC within macrophages. Affected individuals exhibit hepatosplenomegaly, and CNS deterioration in certain subtypes. Pathologic changes may be attributable to stimulation of macrophages by excess GC, leading to over-secretion of cytokines and subsequent activation of other inflammatory leukocytes. To determine if the cytokine profile and leukocyte activity are abnormal in Gaucher Disease, we analyzed the cytokine contents of serum from a mouse model of the disorder (9V), and measured the chemotactic response of WT and 9V neutrophils and lymphocytes to both WT and 9V serum.

**Methods Used:** Pooled serum was obtained from WT (n=14) and 9V (n=17) mice and analyzed for the presence of leukocyte-specific cytokines using a cytokine array. Neutrophils and Lymphocytes from WT and 9V spleen and lung tissue were then isolated and analyzed by flow cytometry to confirm identity. The serum and cells were placed on opposing sides of a 3-5μm porous membrane in a Boyden Chemotaxis Chamber, and were incubated at 37°C for 30min to allow cell migration. Following incubation, the membrane was removed and stained, and the number of migratory cells was counted.

**Summary of Results:** Cytokine analysis of WT and 9V serum indicated elevated levels of neutrophil (GM-CSF, IL-17), B-Cell (CXCL13, IL-10), and T-Cell (IL-7, CXCL9, SDF-1, IL-27, IL-16, IL-12p70) specific cytokines/chemokines in 9V vs. WT serum. Migration of 9V splenic neutrophils and lymphocytes into 9V serum was characterized by a 4.2 and 1.6-fold increase in cell number as compared to migration of WT splenic neutrophils and lymphocytes into WT serum. Similarly, migration of 9V lung neutrophils and lymphocytes into 9V serum exhibited a 12.6 and 5.7-fold increase, respectively, as compared to their WT counterparts.

**Conclusions:** Enhanced migration of leukocytes into 9V serum as compared to WT serum is likely due to the presence of an elevated cytokine profile in the 9V model. Furthermore, 9V leukocytes may be “activated” and more likely to respond to the presence of cytokines than their WT counterparts.

### 316 FAMILIAL IDIOPATHIC SCOLIOSIS: INVESTIGATION OF SF11 GENE IN ADOLESCENT MALES WITH SEVERE CURVATURES

Smith GA, Dunn JH, Swindle K, Miller NH *University of Colorado School of Medicine, Aurora, CO.

**Purpose of Study:** Idiopathic Scoliosis (IS) is a structural lateral curvature of the spine ≥10 degrees that occurs in 2–3% of individuals of which 3–9% require active treatment. Genetic studies suggest that IS is a complex genetic disorder, with multiple genes and genetic variations contributing to its expression. Genomic screening and subsequent fine mapping has resulted in significant association between a subgroup of families characterized by a male having a ≥20 degree curvature and an area on chromosome 22 inclusive of the SF11, SF3A1 and LARGE genes. The goal of this study is to examine further this association through sequencing analysis of the SF11 gene within this subpopulation.

**Methods Used:** DNA was extracted from blood samples of families with two or more individuals affected by IS (202 families: 1198 individuals). A subgroup of trios comprised of parents, regardless of curvature status, and affected son(s) (18 families, 56 individuals) with curvatures ≥30 degrees were identified for sequencing. Curvature status of parents and son(s) was confirmed by X-ray and noted for analysis. Primers were designed using Primer Express® software to amplify and sequence the 32 coding exons of SF11. Standard Sanger sequencing methods were used with read lengths of ~900 nucleotides.

**Summary of Results:** Results are contingent on ongoing sequencing analysis. Anticipated results will show any significant associations between IS and the SF11 gene.

**Conclusions:** The current work builds on previous data indicating a significant association between the SF11 gene and a phenotypic subset of families with IS. The use of clinical criteria to characterize the scoliosis phenotype may aid in decreasing the heterogeneity of any one large study population, and enhance the successful identification of specific genes responsible for this disorder. SF11 is involved in spindle assembly and is translated in several tissues including bone, nerve, and connective tissues with notable protein interactions relating to bone and connective tissue development. The identification of a genetic locus is of major clinical and therapeutic interest and may allow for a deeper understanding of spinal growth and stability.

### 317 ASSESSING INTEREST AND ATTITUDES TOWARD GENETIC PREDICTIVE TESTING FOR COLON CANCER: METHODOLOGY AND INITIAL PREDICTORS

Tuong W\(^1\), Leventhal K\(^2\), Graves K\(^3\) *UC Davis, Sacramento, CA and \(^2\)Georgetown University, Washington, DC.

**Purpose of Study:** With continued expansion of personalized medicine through both commercial availability of predictive SNP testing and emerging clinical translation of certain genomic applications, we sought to assess interest in, attitudes toward, and initial predictors of SNP testing in a primary care population.

**Methods Used:** Based on prior data, we developed hypothetical scenarios to assess interest in SNP testing. We assessed demographics, interest in learning about genetic testing, genetics knowledge, and positive/negative attitudes about SNP testing. We conducted cognitive testing of the survey with 42 primary care patients.

**Summary of Results:** Participants (n=19 men, 23 women) reported a mean age of 50.6 (SD=16.2 years). Most participants (74%) reported they would probably or definitely have a colon cancer SNP test. Greater interest in SNP testing was related to more interest in learning about genetic testing (X^2=10.14, p=0.001), and having both higher positive (t=-2.258, df=33, p=0.031) and negative attitudes (t=-2.314, df=33, p=0.027) toward SNP testing. Further, participants with higher genetics knowledge had more positive attitudes toward testing (t=-2.260, df=33, p=0.024). In this initial exploration, demographics, genetics knowledge, and personal and family history of cancer were unrelated to interest in SNP testing.

**Conclusions:** Primary care patients indicated strong interest in SNP testing and in learning more about genetic testing. Interestingly, both positive and negative attitudes toward SNP testing were related to a greater desire to have SNP testing. Future development of educational tools may help patients clarify the pros and cons of SNP testing in order to promote more informed decisions about personalized genomic medicine.

### 318 FACTOR VIII (FVIII:C) LEVELS WITH AGE IN A COHORT OF INDIVIDUALS WITH MILD HEMOPHILIA A

Lee L\(^1\), Ludlow A\(^2\), Deb G\(^2\), Vickars L\(^3\), Wu J\(^3\), Jackson S\(^4\) *UBC, Vancouver, BC, Canada; \(^1\)St. Paul's Hospital, Vancouver, BC, Canada and \(^3\)BC Children's Hospital, Vancouver, BC, Canada.

**Purpose of Study:** Hemophilia A is a disorder resulting in absent or reduced FVIII: C levels. Baseline FVIII:C levels are important in determining management in mild hemophilia (FVIII:C 0.06-0.40U/mL). In healthy individuals, FVIII:C increases with age. This trend has not been well characterized.
in mild hemophilia. This is important to consider because overtreatment with factor concentrate may paradoxically increase the thrombosis risk.

The study objectives are to describe the changes in baseline FVIII:C levels with age in a cohort of subjects with mild hemophilia and to identify any factors (blood type, co-infection status, FVIII mutation type) which may affect FVIII:C levels.

Methods Used: Medical records for subjects with FVIII levels 0.06-0.40IU/mL registered with the BC Provincial Bleeding Disorder Program were reviewed. Subjects with a minimum of 2 FVIII:C levels at least 5 years apart were included in the linear mixed effects model. Retrospective data was extracted from medical records including age, sex, blood type, FVIII muta- tion, historical FVIII:C levels, DDAVP response, and co-infection (Hep.C, B and HIV) status. Hypothesis testing was applied to estimate the effects of co-variates on the variability and baseline FVIII:C.

Summary of Results: 137 subjects with mild hemophilia were reviewed for eligibility. 83 subjects were excluded due to charts not available (n=27) or inadequate FVIII:C data (n=56). Median age was 49 yrs (IQR 33, 59). All subjects were male. Median duration of observation was 15.8 yrs (IQR 9.4, 23.9). Linear mixed effects modeling using 3 different models showed mean trend of zero. There were fewer data points for subjects over the age of 50 as compared to other age groups, limiting the ability to assess FVIII:C trends in older subjects. There was no observable effect of the co-variates assessed on baseline or variability of FVIII:C.

Conclusions: Our study showed no trend in changing baseline FVIII:C levels with age in individuals with mild hemophilia. Co-variate effect on baseline FVIII:C levels and variability was not observed. Further analysis using similar methods with data from additional hemophilia centers, partic- ularly in subjects >50 yrs, is necessary to confirm these observations.

319 SURVIVAL OUTCOMES OF ADULTS WITH ACUTE MYELOID LEUKEMIA - A STUDY OF NATIVE AMERICAN, HISPANIC, AND NON-HISPANIC WHITES TREATED IN NEW MEXICO (1986-2006)
Rivera N, Armijo B, Quintana D, Abdul-Jaleel M, Lee S, Wiggins C, Libby E. UNM School of Medicine, Albuquerque, NM; University of New Mexico Cancer Center, Albuquerque, NM and University of New Mexico, Albuquerque, NM.

Purpose of Study: New Mexico is composed of three main ethnic pop-ulations Caucasians, Native Americans, and Hispanics. As the largest tertiary care center in the state, we offer treatment to significant numbers of all three groups. Also, many residents of the state live in rural areas. The goal of this study is to identify potential differences in outcome be- tween ethnic groups from urban or rural areas in patients with newly diag-nosed acute myelogenous leukemia (AML) treated at this center from 1986-2006.

Methods Used: A retrospective chart review was performed on adult patients with a new diagnosis of AML treated from 1986 through 2006. 144 charts were available for review. Data was collected for race/ethnicity, sex, zip code, cytogenetics, treatments received, total number of chemotherapy cycles, achievement of complete remission, and overall survival from time of diagnosis and from achievement of remission after induction chemotherapy. Date of death was determined from New Mexico tumor registry data. Sta- tistical analysis was performed to examine the effect of the variables men-tioned above on overall survival.

Summary of Results: The study included 144 patients (mean age 49.98, range 18-85). Patients who received standard treatment of 7-8 cycles followed by high dose cytarabine (1–4 cycles) or similar regimen; there were no statistical differences between survival rates when comparing the three ethnic groups, Caucasians 78 (52%), Hispanics 43 (28%), and Native Americans 23 (15%) (P=0.2496). In agreement with previous reports, statistically significant dif- ferences were seen for treatment versus palliative care, cytogenetics and age. The study included 61 urban patients with a mean survival of 426 days and 83 rural patients with a mean survival of 278 days. Analysis revealed a statistically significant difference in survival for urban versus rural patients who achieved complete remission after induction chemotherapy (P=0.0443).

Conclusions: Survival was improved for urban patients who achieved a complete remission after induction chemotherapy vs rural patients (P=0.0443). Further study should be undertaken to identify the causes of this disparity.

320 NOVEL COMBINATIONS OF CYTOSINE DEAMINASE AND URACIL PHOSPHORIBOSYLMUTASE FOR SUICIDE GENE THERAPY
Runyan R University of Washington School of Medicine, Seattle, WA.

Purpose of Study: Suicide gene therapy (SGT) is a process of selectively introducing genes into tumor cells causing them to express enzymatic ac- tivities, making them susceptible to specific antimetabolites. Administration of a prodrug results in the formation of the toxic antimetabolite in tumor cells. Cytosine deaminase (CD), a promising promod drug gene therapy enzyme, converts cytosine to uracil. CD also converts 5-fluorocytosine (5FC) to 5-fluorouracil, which is a potent antimetabolite that inhibits DNA synthesis and RNA function. Uracil Phosphoribosyltransferase (UPRT) is also a key enzyme in the conversion of 5FC to the toxic metabolite. Both the yeast and E. coli CD and UPRT genes have been utilized in SGT. A major problems associated with this suicide gene/prodrug combination is a low affinity dis- played by the gene product for the prodrug. One way to overcome the limited production of 5FdUMP is by the fusion of key enzymes in the prodrug activation pathway. Fusing the two enzymes responsible for the initial and secondary limiting steps in prodrug activation has shown substantial im- provement in tumor reduction.

Methods Used: Using the gEHTH vector as a backbone, two novel combina- tions of CD and UPRT were constructed: bCD/bUPRT and yCD/yUPRT. These new constructs were then transformed into the E.coli strain GIA39 (DE3) and plated, after which plasmid DNA was isolated from single colo- nies. Restriction digestion and genetic sequencing were performed to confirm successful cloning. Genetic complementation studies tested protein synthesis and function using E.coli stains that are deficient in UPRT and CD.

Summary of Results: Sequencing confirmed successful genetic cloning of bCD/bUPRT and yCD/yUPRT. Through genetic complementation studies we show that both constructs exhibit increased CD and UPRT enzymatic activity, when compared to the control bCD/bUPRT and yCD/yUPRT con- structs. These novel gene constructs have also demonstrated increased sta- bility and productivity.

Conclusions: These experiments will ascertain the best possible combina- tion of yeast and bacterial CD and UPRT fusions. A suicide gene therapy with greater sensitivity to the prodrug will lead to a faster and more potent way to suppress tumor growth with reduced side effects.

Western Student Medical Research Forum
Student Session V – Surgery
1:30 PM
Friday, January 28, 2011

321 IMPROVED METHOD FOR CHRONIC CANNULATION OF THE RAT THORACIC DUCT FOR MULTIPLE POOL KINETIC STUDIES
Douglas MJ, Flake N, Witte M University of Arizona College of Medicine, Tucson, AZ.

Purpose of Study: Few studies of pharmaco/metabolokinetics incorporate lymph sampling for more accurate and insightful multiple pool analysis and translational implications. This deficiency is in part due to relative inacces- sibility of the lymphatic compared to blood vasculature and the difficulties in initial cannulation and longer term lymph collection/drainage experiments particularly in small animals.

Methods Used: To surmount these challenges, we refined techniques and performed pilot multiple pool sampling experiments in 6 male Wistar-fuzzy/ Sprague Dawley rats weighing ~350 gm. Following a corn oil meal to vi- sualize milky intestinal lymphatics and ketamine-xylazine anesthesia, the abdominal cavity was entered, and under a Wick dissecting microscope, the cisterna chyli dissected up to the thoracic duct. The thoracic duct was then ligated and a 0.12id x 2.400d silicone tube passed into the cisterna chyli, secured with tissue glue, and serial timed samples of lymph collected. Si- multaneous samples of central venous blood/serum were obtained by can- nulation of the external jugular vein. Lymph drainage was maintained for a period of up to 6 hours at which time collections were terminated.
Summary of Results: Cisterna chyli cannulation and drainage was performed successfully and the lymph/blood sampling protocol carried out effectively. Milky lymph flow at equilibrium averaged 454 ± 246 microliters/hr (mean ± SD), but declined gradually over the 6-hour collection period.

Conclusions: This rat preparation should provide the basis for further dynamic experiments examining multiple pool kinetics of absorption, distribution and fate of a wide variety of drugs, toxins, microorganisms, and trafficking cells. Such experiments would contribute to improved fundamental understanding of the action and effectiveness of these agents, fresh insights into related processes under normal and pathologic conditions, and potential treatment approaches.

322 THE INCIDENCE OF USE OF PLATELET RICH PLASMA IN ORTHOPEDIC SURGERY IN IDAHO AND WASHINGTON
Butkofe S1, 2, Seegmiller J1 1University of Washington, Seattle, WA and 2University of Idaho, Moscow, ID.

Purpose of Study: To measure the frequency of platelet rich plasma (PRP) use in orthopedic surgery in Idaho and Washington, and to report the factors that influence orthopedic surgeons to use or not use PRP.

Methods Used: A questionnaire was developed to gather data in three areas: physician knowledge of PRP applications, PRP use in orthopedic practices, and factors influencing PRP use. 45 orthopedic physicians in Idaho and Washington responded to the questionnaire. Analysis of variance was used to determine if any significant differences existed in PRP use when influential factors, such as high or low PRP knowledge, were compared. In addition, correlation tests were run to identify any relationships between use and potential influencing factors.

Summary of Results: Respondents described their own familiarity with PRP in orthopedic surgery as more ‘somewhat familiar’ on a four point scale (mean=3.27 ± 0.72). Our sample used PRP less than ‘rarely’ (mean=1.89 ± 1.02) and a Pearson’s correlation test found that physician knowledge of PRP and PRP use was weakly correlated (r=0.235). Physician knowledge of PRP, experience using PRP, and improved patient outcomes were rated on a four point scale as the most influential factors for physicians who use PRP with means of 3.48 ± 0.59, 3.16 ± 0.87, and 3.22 ± 0.95, respectively. For physicians who choose not to use PRP, the most highly rated factor that influenced this decision was a lack of randomized controlled trials involving platelet rich plasma use in orthopedic surgeries with a mean of 3.54 ± 0.74.

Conclusions: Orthopedists are generally knowledgeable about PRP in orthopedic applications. Despite high levels of knowledge and familiarity with PRP, 46% of our respondents do not use PRP in their practices. Knowledge, training, and experience have little effect on whether physicians choose to use PRP. Notwithstanding, knowledge, experience, and positive patient outcomes are influential to physicians who do choose to use PRP. Whether the physician used PRP or not, all seemed to recognize a lack of literature evidence for some applications and considered this an important influencing factor.

323 GENDER DIFFERENCES IN NONUNION AND MALUNION FOLLOWING TIBIAL SHAFT FRACTURES: A REVIEW OF 5 TREATMENT MODALITIES
Chapman LW, Hame S, Wang JC David Geffen School of Medicine at UCLA, Los Angeles, CA.

Purpose of Study: Tibial shaft fractures are the most common type of tibia fracture and predominately occur following car accidents, sports injuries, and falls. The purpose of this study was to compare 5 treatment modalities for tibial shaft fractures and to evaluate the potential benefit of undergoing a specific treatment given gender. Potential benefit in this study involves minimizing the incidence of nonunion and malunion following treatment.

Methods Used: Patients undergoing treatment for tibial shaft fractures from years 2004–2009 were retrospectively reviewed using a commercially available online database of private insurance billing records. Fracture treatment was identified by CPT code. The 5 treatment modalities were external fixation, intramedullary nailing, open treatment with internal fixation, closed treatment without manipulation/reduction, and closed treatment with manipulation/reduction. Records were cross referenced for nonunion and malunion.

Summary of Results: 23,418 patients were identified. 1,643 (7.02%) patients developed nonunion and 356 (1.52%) patients developed malunion following treatment. Open treatment with internal fixation yielded the highest incidence of nonunion (16.8%). Incidence of nonunion following external fixation was 13.6%, and incidence of nonunion following intramedullary (IM) nailing was 14.2%. 22 patients (4.2%) of the external fixation group and 158 patients (2.6%) of the IM nailing group developed malunion. Of all males undergoing external fixation, 39 (10.83%) suffered nonunion, whereas 33 (19.4%) of all females undergoing external fixation suffered nonunion (p=0.0079). 122 (1.8%) of males and 62 (1.3%) of females in the closed treatment without manipulation group developed nonunion (p=0.0412).

Conclusions: Our study demonstrated a significant difference in gender with respect to nonunion incidence in two of the five treatment modalities: external fixation group and closed treatment without manipulation. Our study also generated a significant difference in gender with respect to malunion incidence in two of the five treatment modalities: open treatment with internal fixation and closed treatment without manipulation. Further studies should examine this gender disparity.

324 POOR CORRELATION BETWEEN PRE-TRANSPLANT VISUALIZED ESTIMATED OF DECEASED DONOR LIVER STEATOSIS AND REPERFUSION BIOPSY ASSESSMENT
Fuller SD, Mangus S, Fridell J, Vianna R, Milgrom M, Tector J Indiana University School of Medicine, Indianapolis, IN.

Purpose of Study: Pre-transplant deceased donor liver steatosis is estimated by the procuring surgeon by gross appearance of the organ, by a frozen section biopsy, or a combination of the two. This estimate is often the determining factor for the use or non-use of the organ. Permanent section biopsy of the reperfused liver may give a more accurate and reliable assessment of steatosis. This paper assesses the correlation between the surgeons’ estimate of hepatic steatosis and the steatosis measured on permanent section biopsy from the reperfused liver.

Methods Used: This study is a retrospective review of all deceased donor liver transplants at a single center over 5 years’ time. The surgeons’ estimates for all procured livers were recorded from the original on-site records from the organ procurement organization. There was no standardization to the reporting such that each recorded value reflected the estimate recorded by the on-site coordinator as stated by the procuring surgeon. All reperfusion biopsies were read by experienced liver transplant pathologists at a single center. A scatter plot with best fit line was utilized to determine correlation.

Summary of Results: Data were available for 485 liver transplants. Correlation between the surgeon estimated steatosis and assessment by permanent biopsy was r²=0.26. The surgeon underestimated steatosis in 25 cases (5%), overestimated in 110 cases (23%), and was in agreement (+/- 5%) in 350 (72%).

Conclusions: These results suggest a reasonable ability of the procuring surgeon to determine the presence of liver graft steatosis on site at the time of organ procurement, but the estimate of the extent of steatosis correlates poorly with that seen on permanent reperfusion biopsy.
measured by determining adherence to a set of 32 evidence-based quality indicators that were created using the RAND/UCLA Modified-Delphi expert panel methodology.

**Summary of Results:** The results given represent a small initial sample of thirteen patients (of a total of two-hundred and seven). The overall rate of adherence for this sample was 56% (95% CI 50-62%). There was significant variation between individual quality indicators. For example, pre-operative risk assessment was conducted in all cases in this sample (100%), while a complete physical examination was rarely documented (8%).

**Conclusions:** The initial results indicate an overall poor rate of adherence to quality measures in this small sample. Specifically, adherence rates were lower for preoperative and postoperative indicators than for intraoperative indicators. We are in the process of collecting and analyzing data for the remainder of patients from SM-UCLA. Concurrently, collaborators at the UCLA Medical center, a high-volume academic center, and Harbor-UCLA Medical Center, a lower volume public hospital, are undertaking this process. Once completed, we will conduct statistical analyses to determine whether there is a significant difference in adherence to these measures amongst these three sites. The finding of variations in care may be key in identifying correctable causes for variable outcomes of total joint replacement.

326 USE OF NEGATIVE PRESSURE THERAPY ON CLOSED SURGICAL INCISIONS

Huberty SA,1, Gabriel A2, Gupta S2,1 University of Washington, Vancouver, WA and 2Loma Linda University Medical Center, Loma Linda, CA.

**Purpose of Study:** Management of complicated wounds pose a continual challenge to health care providers, particularly in patients with associated risk factors and co-morbidities. Negative pressure wound therapy (NPWT) is becoming the standard of care in open wounds. This study evaluated the role of NPWT in closed incisions in patients with high risk factors for wound breakdown.

**Methods Used:** 15 consecutive patients were evaluated in this pilot study. VAC therapy (KCI) was utilized as our NPWT of choice and was applied in the operating room to clean, closed surgical wounds. GranuFoam Silver dressing (KCI) was applied to all closed wounds. The therapy was initiated at 125mmHg below ambient pressure in a continuous mode for 3–5 days.

**Summary of Results:** All patients completed their treatment successfully. Surgical incisions ranged from abdominal, chest and lower extremity incisions. There were no returns to the operating rooms and no wound breakdowns. No other complications were seen in our pilot study.

**Conclusions:** These results are suggestive of NPWT role over incisional use in high-risk patients. Clearly randomized trials need to address this in the near future; however, we recommend its use on the high-risk patients who are undergoing elective or emergent procedures to minimize wound breakdown and improve healing.

327 INCIDENCE AND CORRELATES OF FAILURE FOR VENTRICULOPLACEMENTAL AND LUMBOPERITONEAL SHUNTS IN PATIENTS WITH IDIOPATHIC INTRACRANIAL HYPERTENSION

Martin RL,1,2, Lambert W2, Tanne E2, Choi D2, Lob B2, Loboy S2,1University of Washington School of Medicine, Seattle, WA and 2Oregon Health and Science University, Portland, OR.

**Purpose of Study:** Idiopathic Intracranial Hypertension (IIH) is a condition of raised intracranial pressure with unknown cause. Symptoms include headache, pulsatile tinnitus, stiff neck and radicular pain, transient visual obscurations due to papilledema, and in severe cases, visual loss and blindness. Although IIH can occur in people of every demographic group, studies have found that gender, age, and obesity are strong risk factors. For example, men have an estimated incidence of 1.5 cases of IIH per 100,000, while overweight women of childbearing age (15–44) have a significantly higher incidence at 20 cases per 100,000. With the number of overweight and obese women on the rise, the health burden of IIH is expected to increase. One of the primary surgical options for IIH patients includes cerebral spinal fluid (CSF) shunt procedures. Two major types of shunts are used to treat IIH: lumboventricular (LPS) and ventriculoperitoneal (VPS). While successful at providing acute symptom relief, LPS and VPS frequently require one or more revisions and have a number of complications. Current information concerning the comparative incidence of failure in LPS and VPS is inconclusive.

**Methods Used:** Using the largest IIH patient database in the world, our study will be able to assess the comparative incidence of failure between LPS and VPS in a large cohort of geographically diverse patients. Using life tables analysis and Chi-square test for difference of two proportions, we will determine whether life table estimated failure rates differ between LPS and VPS at 3, 6, 12, and 24 months. Additionally, we will determine whether BMI, lumbar opening pressures, and time from initial diagnosis to first surgery, are associated with failure.

**Summary of Results:** Pending: will be available upon presentation.

**Conclusions:** This information will provide patients and providers with critically important evidence needed to make informed treatment decisions.
rate of I&D (0.084%) than was the non-microscope group (0.048%), however, this association also did not reach significance.

Conclusions: While microscope use was not associated with a higher rate of I&D by itself, when combined with allograft use it was associated with a non-significant increase in the rate of I&D. Since even these small variations can be useful in clinical practice, it is important to note that 7 of the 11 (64%) reported I&D cases were from allograft patients. This may seem small, but it should not be ignored because bringing the patient back into the operating room is time consuming, costly, and increases the patient’s recovery time.

330 SURGICAL MANAGEMENT IN THE TREATMENT OF FIBROUS DYSPLASIA
Richards SM, Adetayo OA, Salcedo SE, Ray AO, Gupta SC Loma Linda University Medical Center, Loma Linda, CA.
Case Report: The patient is a 34 year old otherwise healthy male who was diagnosed with fibrous dysplasia at age 16. He presented with complaints of new onset frequent headaches and blurry vision in the left eye. The patient underwent a left fronto-temporo-orbitozygomatic craniotomy, cranioplasty and microsurgical decompression of the optic nerve. This was followed by allograft reconstruction of the forehead and supraorbital rim as well as orbital wall reconstruction. This was achieved using a Synthes PEEK implant which was prefabricated based on the patient’s preoperative CT images. The implant was made in two pieces to allow flexible contouring of the forehead and orbital rim.

Preoperative CT illustrating fibrous dysplasia of the orbital region.

331 AN ATYPICAL PRESENTATION OF A PANCOLONIC VENOUS MALFORMATION
Wong F, Blair G University of British Columbia, Vancouver, BC, Canada.
Purpose of Study: To report a unique case of gastrointestinal venous vascular malformation.
Methods Used: The patient is a 13 month-old girl. Methods used to elucidate her medical situation included CBC & Peripheral Smear, Coagulation Investigations, Ultrasound, Abdominal X-ray, CT angiogram, Laparotomy, and Pathology and Histology of the surgical specimen.
Summary of Results: Venous vascular malformations (VM) are common in infants in the head, neck, extremities, trunk and especially cutaneous regions. The occurrence of a VM in deeper structures such as the colon, however, is uncommon. The location of reported gastrointestinal venous vascular malformation (GIVM) cases is usually in the sigmoid colon and rectum. Almost all cases in literature presented with hematochezia. Herein we describe a unique case of a pancolonic VM with atypical location—between the transverse colon and mid-sigmoid colon—and atypical presenting symptom—an upper GI bleed. Lab results suggested a consumptive coagulopathy and imaging revealed the section of colon involved. The hypothesized pathogenesis of the presenting upper GI bleed is a Kasabach-Merritt like syndrome caused by the VM. The venous malformation resulted in stasis of blood that activated the coagulation cascade. This led to the formation of blood clots and phleboliths and depleted clotting factors such as fibrinogen and platelets. The thrombosed vessels caused injury to passing erythrocytes resulting in anemia. The coagulopathy complicated the upper GI bleed. In order to correct the source of the coagulopathy, the VM was resected. Pathology of the surgical specimen confirmed thrombi and phleboliths in venous channels as well as recanalized thrombosed vessels. The findings confirmed the suspicion of coagulopathy within the malformation that may have led to the consumptive coagulopathy.
Conclusions: In conclusion, we note that gastrointestinal venous vascular malformations can present in an atypical way. The presenting symptom may not be hematochezia. In this case, an upper GI bleed was the only presenting symptom that hinted at the underlying coagulopathy and the rare gastrointestinal venous malformation.

General Internal Medicine and Aging Concurrent Session
8:30 AM
Saturday, January 29, 2011

332 PERIORAL WRINKLES ARE ASSOCIATED WITH GENDER, AGING, AND SMOKING: DEVELOPMENT AND ANALYSIS OF A GENDER-SPECIFIC PHOTONUMERIC SCALE
Cheng N, Chien A, Kang S Johns Hopkins University, Baltimore, MD.
Purpose of Study: Perioral rhytides, or wrinkles around the mouth, are a highly prevalent phenomenon and common chief complaint among older adults. Proposed etiologies include sun exposure, phototype, cigarette smoking, and hormone replacement. Clinical impression suggests perioral wrinkling affects females to a greater extent than males. Yet, no existing grading scale accounts for this gender difference. To this end, we developed an objective, gender-specific photonumeric scale for the assessment of perioral rhytides and investigated possible associated factors.
Methods Used: A total of 143 individuals were enrolled (72 F, 71 M, aged 21 to 91). Standardized photographs were taken of each participant’s entire face and perioral area. Five photographs were selected as standards and were
assigned grades of 0, 2, 4, 6, and 8, in ascending severity of perioral wrinkling. Subjects were evaluated by three blinded graders and also completed a questionnaire to document associated demographic and lifestyle factors. **Summary of Results:** Perioral wrinkling was statistically significantly correlated with females over age 45 (mean score 4.8 F, 2.9 M, p < 0.001) and duration of smoking (mean score 3.4 smokers, 2.1 nonsmokers, p = 0.0005). Other correlations included number of children birthed (p = 0.004), years of recreational sun exposure (p = 0.001), years of lip product use (p < 0.0001), years of hormone replacement therapy (0.0001), and years of vitamin use or other health supplementation (p < 0.0001). Analysis of the three graders’ maximum range of disagreement (MRD) showed good inter-rater reliability, and repeat grading and MRD analysis two weeks later showed good intra-rater reliability. A modified multiple linear regression model selected gender, age, and smoking as the best predictors of severity of perioral wrinkling.

**Conclusion:** These results indicate that the effects on bone toughness are duration-dependent. Interestingly, in the animals treated for 9 months with zoledronate plus dexamethasone, the dexamethasone-treatment attenuated age-related changes in the mechanical properties of bone, including decreased toughness, the amount of energy that can be absorbed by bone before fracturing. We hypothesized that cancer dosing regimens of zoledronate, an intravenous BP, reduce bone toughness, and that this effect would be dose- and duration-dependent.

**Methods Used:** The model for the experiment was rib bones from skeletally mature beagle dogs, which were part of three separate experiments. The first experiment tested monthly dosing of zoledronate for 3 and 6 months. In the 9-month study, we also found reduced remodeling and increased mineralization of the bone but did not alter mechanical properties at 3 or 6 months. In the 9-month study, we also found reduced remodeling and increased mineralization, but in addition, the bones showed significantly lower biomechanical properties in the zoledronate-treated samples compared to the controls, especially in energy to failure (938 mJ to 497 mJ, p = 0.02). We develop (41.64 M J/m2 to 17.06 M J/m2, p ≤ 0.05).

**Conclusions:** These results indicate that the effects on bone toughness are duration-dependent. Interestingly, in the animals treated for 9 months with zoledronate plus dexamethasone, the dexamethasone-treatment attenuated the effects of zoledronate and kept bone properties at control levels. Additional studies will be needed to understand how dexamethasone is altering the effects of zoledronate on bone toughness.

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**333 THE EFFECTS OF CANCER DOSING OF BISPHOSPHONATES ON THE MECHANICAL PROPERTIES OF BONE DEPEND ON DURATION OF TREATMENT**

Luo T, Allen M Indiana University School of Medicine, Indianapolis, IN.

**Purpose of Study:** Bisphosphonates (BPs) are a class of drugs used to treat osteoporosis as well as bone metastases related to various cancers (breast, prostate, multiple myeloma). They reduce bone loss via inhibition of osteoclasts. Our goal was to study side effects associated with long-term and high dose bisphosphonate use, such as in cancer regimens. Previous animal studies have shown that BP dosing for osteoporosis produce significant changes in the mechanical properties of bone, including decreased toughness, the amount of energy that can be absorbed by bone before fracturing. We hypothesized that cancer dosing regimens of zoledronate, an intravenous BP, reduce bone toughness, and that this effect would be dose- and duration-dependent.

**Methods Used:** The model for the experiment was rib bones from skeletally mature beagle dogs, which were part of three separate experiments. The first experiment tested monthly dosing of zoledronate for 3 and 6 months. The second experiment assessed biweekly dosing for 3 months. The third experiment examined biweekly dosing for 9 months with secondary treatment of dexamethasone. Each experiment had a set of untreated controls. Histological analysis, peripheral quantitative computed tomography (pQCT), and biomechanical testing were performed on all bones.

**Summary of Results:** Dosing of zoledronate either monthly or biweekly reduced remodeling and increased mineralization of the bone but did not alter mechanical properties at 3 or 6 months. In the 9-month study, we also found reduced remodeling and increased mineralization, but in addition, the bones showed significantly lower biomechanical properties in the zoledronate-treated samples compared to the controls, especially in energy to failure (938 mJ to 497 mJ, p = 0.02). We develop (41.64 M J/m2 to 17.06 M J/m2, p ≤ 0.05).

**Conclusions:** These results indicate that the effects on bone toughness are duration-dependent. Interestingly, in the animals treated for 9 months with zoledronate plus dexamethasone, the dexamethasone-treatment attenuated the effects of zoledronate and kept bone properties at control levels. Additional studies will be needed to understand how dexamethasone is altering the effects of zoledronate on bone toughness.

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**334 CHARACTERIZATION OF ALPHA-CRYSTALLIN UPTAKE INTO EYE LENS CELLS AS A NOVEL TREATMENT FOR CATARACT AND RETINAL DEGENERATION**

Christopher K, Mueller N, Petrush JM University of Colorado School of Medicine, Aurora, CO.

**Purpose of Study:** Protein aggregation disorders are among the most common diseases afflicting humans and tend to have the fewest treatments available. In the eye, cataracts can be formed by aggregation of γ-crystallin protein subunits in the lens. Similarly, a mutation in the rhodopsin gene leads to aggregation and blindness in patients with retinitis pigmentosa. A family of small heat shock proteins called the α-crystallins show chaperone-like activity (CLA) in the eye as well as elsewhere in the body. During cataract formation, these proteins tend to accumulate at the membrane of lens cells and lose their CLA function. Replacing the pool of α-crystallin proteins is a novel therapeutic strategy to restore CLA in the eye and prevent protein aggregation.

**Methods Used:** Using human lens epithelial (HLEB3) cells, we characterized the cellular uptake of one of the α-crystallin proteins, αB-crystallin, and monitored its activity once inside the cell. Recombinant αB-crystallin was purified and labeled with a fluorescent probe to enable us to monitor its real-time uptake and subcellular distribution.

**Summary of Results:** Uptake of wild-type αB-crystallin after packaging into a cationic polymer transfection reagent proved most effective, however the reagent caused reduced cell viability. Additional mechanisms to enhance protein delivery are being studied such as our modified αB-crystallin fused to a protein transduction domain. Stability of αB-crystallin once inside the HLEB3 cells was also investigated. Using gel electrophoresis methods, we observed that the protein was not being rapidly degraded by the cell. Additionally, a series of point mutants of αA-crystallin have been developed, expressed, and purified. These mutants have the potential for increased CLA and may be a more effective therapeutic option. Furthermore, a cell line with inducible aggregation-prone rhodopsin expression was developed and is being used to test the efficacy of treatment with our therapeutic α-crystallins to prevent or decrease the amount of protein aggregation in the cell.

**Conclusions:** The α-crystallin family of small heat shock proteins remains a promising approach to treatment of protein aggregation disorders in the eye and elsewhere in the body.

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**335 PLASMA TESTOSTERONE IS ASSOCIATED WITH FRAMINGHAM RISK SCORE**

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**Purpose of Study:** The Framingham risk score predicts a patient’s 10 year risk of developing cardiovascular disease. Many risk factors included in the calculation of this score influence (or are influenced by) circulating testosterone. To further clarify the possible association between testosterone and cardiovascular risk, as defined by the Framingham score, we analyzed data from a VA database.

**Methods Used:** A retrospective chart review was performed. Inclusion criteria were male sex and age ≥ 20. Exclusion criteria included pre-existing cardiovascular disease, stroke, and diabetes. Testosterone supplements, antiandrogen therapy, antihypertensives, or lipid-lowering medications were not exclusion criteria, but were factored in data analysis. Laboratory and clinical data were collected on veterans who had total plasma testosterone calculated in the year 2008; Framingham scores were calculated using an online tool from the National Heart Lung and Blood Institute. Using SAS v9.2, robust linear regression assessed the association between total testosterone and Framingham score. The Spearman rank correlation coefficient, Wilcoxon rank-sum test, and Kruskal-Wallis test evaluated associations between total testosterone and the other patient data. A p-value ≤ 0.05 was considered significant.

**Summary of Results:** 1,479 patients were included in the study. Mean age was 61 years. There was a significant negative association between total testosterone and Framingham score (estimate of regression parameter for total testosterone −0.0042, p < 0.0001). Total testosterone was also positively associated with HDL and negatively associated with total cholesterol and triglycerides.

**Conclusions:** Lower plasma testosterone may suggest the presence of other more traditional cardiovascular risk factors and potentially increased risk for heart disease.
Purpose of Study: Skeletal muscle wasting is a serious public health problem associated with aging, chronic disease, kidney dialysis and AIDS. Vitamin D (Vitamin D3) is widely recognized for its regulation of calcium and phosphate homeostasis in relation to bone development and maintenance and for its synergistic effects on target organs such as intestines, kidneys and PTH glands. It has been shown to improve muscle performance and reduce falls in VD deficient older adults. Little is known of the underlying mechanism or role it plays in association with myogenic differentiation. In this study, we examined the effect of 1,25-D3 the active form of VD3 at the molecular level on myoblast cell proliferation, progression and differentiation into myotubes.

Methods Used: C2C12 myoblasts were treated with and without 1,25D in a time course manner. VDR expression was analyzed by: Immunofluorescence (IF), Real Time PCR (qRT-PCR) and Western Blots. Expression of muscle lineage and proliferation markers was assessed by immunocytochemistry (ICC) and western blots. Expression of pro-myogenic markers and angiogenic growth factors was analyzed by qRT-PCR arrays and confirmed by qRT-PCR.

Summary of Results: Addition of 1,25D to C2C12 myoblast induces: a) increase expression and nuclear localization of the VDR; b) decrease PCNA (a cell proliferation marker) cell expression; c) increase expression of MyoD and myogenin (early and intermediate myogenic markers respectively); d) increase expression of IGF-2, FST (follistatin—a myostatin inhibitor), FGF1 (fibroblast growth factor 1, involved in muscle development and regeneration) and VEGF which has been demonstrated to promote myobute hyper trophy and increased myogenic differentiation; e) no changes in GDF11 expression; and f) decrease expression of IGF-1 and Mstn- the only known negative regulator of muscle mass.

Conclusions: The study provides a mechanistic justification for VD replenishment in muscle waste conditions such as AIDS, cancer, congestive heart failure, COPD, renal failure, and also in VD deficient older adults who are known to have age-related loss of muscle mass and strength and an increased rate of falls.

337 ALCOHOL USE DISORDER IDENTIFIERS
TEST - CONSUMPTION QUESTIONNAIRE (AUDIT-C): HOW OFTEN DO SCREENING RESULTS CONTRADICT REPORTED DRINKING LEVELS?
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1-3 Units of Washington, Seattle, WA and 1-4 Paget Sound, Seattle, WA.

Purpose of Study: The AUDIT-C is a 3-item screening tool that asks about quantity and frequency of alcohol consumption. Screening thresholds of 24 (men) or 13 (women) points have been found to balance sensitivity and specificity for identifying alcohol use disorders and/or exceeding recommended limits (≤14 drinks/week and ≤4 drinks/day for men; ≤7 d/wk and ≤3 d/day for women). However, some patients who report drinking within these limits on the 3 questions can screen positive according to AUDIT-C scores and some who report exceeding limits can screen negative on the AUDIT-C. Thus, AUDIT-C screening results (positive or negative) and reported drinking on the AUDIT-C questions (above or below recommended limits) can be discordant, though how often this occurs is unknown. The purpose of this study was to evaluate the prevalence of AUDIT-C screening results that are discordant with reported drinking among male and female drinkers in two national samples: US general population and VA outpatients.

Methods Used: All combinations of responses to the 3 AUDIT-C questions ("response patterns") were evaluated to identify screening results that were discordant with reported level of drinking. Proportions of men and women with discordant response patterns were estimated among drinkers in the US (N=26,610) and VA (N=468,245) samples.

Summary of Results: For men, 14 AUDIT-C response patterns were discordant. Eleven responses patterns yielded negative screens but indicated exceeding recommended limits and 3 response patterns yielded positive screens but indicated drinking within limits. In the US and VA samples, 13.8% and 21.1% of male drinkers, respectively, had discordant response patterns. For women, there were 7 discordant response patterns: 4 yielded negative screens but indicated exceeding limits and 3 yielded positive screens but indicated drinking within limits. In the US and VA samples, 10.6% and 12.1% of female drinkers, respectively, had discordant response patterns.

Conclusions: Many individuals who drink will have AUDIT-C screening results that appear to contradict their reported alcohol consumption. Response patterns that yield these discordant results need to be considered when brief alcohol interventions are provided.

338 AGE-RELATED CHANGES IN THE RETINAL NERVE FIBER LAYER OF AFRICAN AMERICAN AND CAUCASIAN HEALTHY SUBJECTS USING SPECTRAL DOMAIN OPTICAL COHERENCE TOMOGRAPHY
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Purpose of Study: To evaluate the effect of aging and scan quality on spectral domain optical coherence tomography (SDOCT) retinal nerve fiber layer (RNFL) thickness in healthy participants of African descent (AD) and European descent (ED).

Methods Used: AD (34 eyes) and ED (161 eyes) of 98 healthy participants from the UCSD Diagnostic Innovations in Glaucoma Study (DIGS) and the African Descent and Glaucoma Evaluation Study (ADAGES) underwent SDOCT imaging with Cirrus SDOCT (Carl Zeiss Meditec, Inc, Dublin, CA). AD (30 eyes) and ED (120 eyes) of 75 healthy participants who underwent SDOCT imaging with Cirrus SDOCT also underwent RNFL imaging with Spectralis SDOCT (Heidelberg Engineering, Inc, Heidelberg, Germany). Univariate and multivariate models were used to predict RNFL (Cirrus SDOCT quality score, race, disc size and axial length on RNFL thickness was completed.

Summary of Results: There was significant (p<0.05) thinning with increasing age for the inferior Cirrus RNFL sector and the global, inferotemporal, and inferonasal Spectralis RNFL sectors. There was also significant RNFL thinning with a poorer quality score for all of the Cirrus RNFL sectors and for the temporal and superotemporal Spectralis RNFL sectors. The effect of age and quality score was similar in AD and ED eyes and explained approximately 20% and 7%, respectively of the variation in the univariable models of the inferior RNFL quadrant.

Conclusions: AD and ED eyes demonstrated age-related thinning of the RNFL in the inferior quadrant, which is a location that is often affected early in glaucoma disease progression. These results suggest that SDOCT shows promise in providing quantitative data about the extent of age-related changes in the RNFL of healthy eyes. Due to the observation that the SDOCT quality score affects the RNFL measurement, future studies will need to account for this relationship in order to ensure that measured RNFL changes in healthy eyes are due to changes in age alone rather than changes in age-related SDOCT quality scores.

339 DO DIABETES AND HYPERTENSION HAVE A SYNERGISTIC EFFECT ON COGNITIVE FUNCTION IN MIDDLE AGE?
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Purpose of Study: In the first half of this century, the number of individuals over 60 years of age is expected to more than triple, representing the most rapidly growing segment of the world population. Intact cognitive function is essential for the well-being of older adults—it determines engagement in the workforce and social life. Hypertension (HTN) and diabetes (DM) in midlife have each been associated with poorer cognitive functioning in later life. Few studies have explored the effect of both diseases on cognitive function in middle age, concurrently, and understanding these relationships may shed light on how these common diseases alter cognitive function.

We used cross-sectional data from the second wave of a large population-based study (the Midlife in the United States Study (MIDUS Study)) to assess the impact of HTN and DM on the risk of cognitive function impairment in middle-age (n=919), to test for synergistic effects between disease states, and to examine changes in disease effect with age.

Methods Used: Multivariable linear regression (using SAS 9.2) was used to evaluate the relationships between predictors (systolic blood pressure, diastolic blood pressure, log(hemoglobinA1c), HTN, DM) and outcomes (episodic memory, executive function), controlling for age, gender, education, and race.

Summary of Results: Adjusted for age, gender, education, and race, higher systolic blood pressure was associated with slightly worse episodic memory (p<0.05) and executive function (p<0.01). Diabetic disease state was associated with worse executive function (p<0.01), whereas hypertension was not. No significant interactions were identified between continuous or categorical variables.
categorical variables representing diabetic and hypertensive status. There was a significant age interaction: Log(hemoglobinA1c) was associated negatively with cognitive function only in older ages (p=0.05).

**Conclusions:** Higher blood pressure and diabetic status are associated with worse cognitive function, even in a middle-aged cohort. Poor glycemic control (as measured by higher hemoglobinA1c) has a more pronounced effect on cognitive function in older age, when vascular compensatory mechanisms may not be as effective. No synergy was documented between HTN and DM in their associations with cognitive function.

**Hematology and Oncology II**

**Concurrent Session**

8:30 AM

Saturday, January 29, 2011

340 DEVELOPMENT OF AN INTEGRATED INTERDISCIPLINARY ONCOLOGY ELECTIVE

Lai L, Ingledew P UBC Faculty of Medicine. Vancouver, BC, Canada.

**Purpose of Study:** A deficit in oncology education for medical students exists throughout North America. Many medical programs expose students to oncology sporadically, often in a discipline-specific manner which limits the experience of the unique interdisciplinary nature of oncology. The goal of this project is to develop an integrated interdisciplinary oncology clerkship, supplemented by online modules and virtual patients (VP).

**Methods Used:** The Kern approach to curriculum development was employed. A web-based survey was sent to 224 UBC medical students finishing their first year of clerkship (3rd year of medicine), regarding adequacy of oncology training. The survey polled interest levels for an integrated oncology clerkship, and student preference for mode of delivery for educational content. Following survey analysis, development of an online module covering common types of cancer was initiated. In addition, a branching-logic Virtual Patient (VP) case was developed using OpenLabyrinth (OL), a software tool for authoring VP cases. Several software tools were evaluated for ability to extract and analyze data from the OL database for purposes of education research.

**Summary of Results:** The survey indicated a high interest in an integrated oncology clerkship with an online module. 37% (82 of 224) of the target audience responded. 50% (41 of 82) of students had not interacted with cancer patients. 70% (57 of 82) felt that an integrated oncology elective would be useful. Nearly 90% (64 of 82) felt that online modules would enhance learning. A large proportion of students (62%, 51 of 82) rated their ability to discuss oncology issues with patients as poor to fair (the two lowest scores out of a 5-point rating scale). To date, the learning modules for lung, prostate and colorectal cancer have been written. A branching VP case for colorectal cancer was authored. Reports that analyze and display performance data derived from students working through VP cases were created using Jasper iReports. Remaining work includes development of a breast cancer module, and authoring of more VP cases.

**Conclusions:** The gaps in oncology education can be met by the needs-based development of a new integrated oncology clerkship, especially if supplemented by an online module. VP cases offer a unique learning environment, where students have the opportunity to practice clinical reasoning.

341 ACUTE MYELOID LEUKEMIA OUTCOMES FOLLOWING BREAST CANCER TREATMENT

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**Purpose of Study:** Patients with breast cancer treated with chemotherapy (alkylating agents/topoisomerase II inhibitors) and/or radiation are predisposed to therapy-related myelodysplastic syndrome or acute myeloid leukemia (tMDS/AML). Outcomes for tMDS/AML are poor compared to de novo AML. Proposed causes are high frequency of unfavourable karyotypes, prolonged cytopenias and injury to organs from therapy. Investigation is needed to determine factors that contribute to tMDS/AML development.

**Methods Used:** Retrospective chart review was done on 63 patients who presented to the BMJ clinic with tMDS/AML following previous breast cancer treatment from 1985 to 2009. The median age at tMDS/AML diagnosis was 59 yrs (R=39–84). The median latency between breast cancer and tMDS/AML was 47 months (R=1–447). Surgery only patients were excluded.

**Summary of Results:** After tMDS/AML diagnosis 21 patients had palliative/supportive/no treatment and were excluded from further analysis. 45 patients received chemotherapy and 27 patients achieved remission. Of these 9 patients received allologeneic bone marrow transplant and 4 had autologous transplant. 12 patients are alive without evidence of tMDS/AML or breast cancer at time of study.

Patients were divided into 3 groups based on previous breast cancer treatment: chemotherapy and radiation (C+R)(n=32), rad only (R)(n=23) and chemo only (C)(n=8). The median time between breast cancer and tMDS/AML was longest for R (med=129 mos) vs. C+R and C (med=39; 31.5 mos). The median overall survival time between tMDS/AML and death was 187 days (R=2–2424).

Bone marrow karyotype was characterized as good (8 pts), intermediate (29 pts) or poor (17 pts). The median overall survival for good, intermediate and poor karyotype was 547, 212, and 167 days respectively.

Causes of death for patients were relapse of breast cancer (5 pts), cardiac complications (3 pts), pulmonary failure (2 pts), sepsis (3 pts) and graft-versus-host disease (3 pts (prophylactically transplant). The remainder of patients died from a progression of tMDS/AML.

**Conclusions:** Our descriptive study provides support that cytogenetics and treatments for cancer affects outcomes and overall survival of tMDS/AML.

**References:**


342 TARGETING FOXM1 TRANSCRIPTION FACTOR IN RHABDOMYOSARCOMA

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**Purpose of Study:** Forkhead Box (Fox) M1 is a transcription factor involved with regulation of the G1/S, G2/M cell cycle checkpoints as well as the mitotic spindle assembly checkpoint. FoxM1 has been reported in the literature to be upregulated in different types of cancer. In a recent shRNA screen, our lab showed that FoxM1 is necessary for growth and proliferation in rhabdomyosarcoma (RMS) cell lines. Recent studies have also demonstrated that FoxM1 expression can be blocked using the thiione antibiotic Siomycin A. The purpose of this study was to determine the effects of Siomycin A on FoxM1 in two human RMS cell lines, RD and Rh30.

**Methods Used:** To investigate our hypothesis, we used Western blot analysis and MTT proliferation assays. Western blots were prepared from cell lysates of human RMS cell lines RD and Rh30 cultured in DMEM supplemented with FBS. Additional lysates from healthy donors and a variety of other pediatric cancers were also used. Cells were cultured both in the presence and absence of drug. Experiments were directed at reported targets of FoxM1 involved with cell cycle progression as well as member of the AKT/mTOR pathway.

**Summary of Results:** In this study, we show that FoxM1 is highly expressed in multiple pediatric cancer cell lines, including RMS, when compared to normal fibroblasts. Our results suggest that Siomycin A inhibits FoxM1 and leads to decreased proliferation and increased cell death in a dose dependent fashion. Interestingly, we found that in addition to blocking FoxM1, Siomycin A inhibits the serine/threonine kinase Bub-1b. Bub-1b is a mitotic spindle assembly checkpoint protein also found to be essential for growth and survival of RMS cells in vitro and in vivo.

**Conclusions:** These data suggest FoxM1 and Bub-1b as potential therapeutic targets in rhabdomyosarcoma. Current studies are continuing to characterize the relationship between these targets.

343 MELANOMA PATIENT INFORMATION: ASSESSMENT OF THE QUALITY OF INTERNET-BASED INFORMATION RESOURCES

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Purpose of Study: Melanoma is the most serious form of skin cancer. Many patients seek web-based information to guide their decision-making; however, the internet is largely unregulated with few safeguards to regulate its content. The purposes of this study were to apply a structured rating tool to melanoma websites and to evaluate the quality of internet-based melanoma information.

Methods Used: The study consisted of the creation of a standardized rating tool and a structured review of the top 100 melanoma-related websites. A rating tool was created based on a combination of existing methodologies. It assessed websites based on affiliation, authorship, attribution, currency, interactivity, readability, accuracy and coverage. The keyword “melanoma” was input into three search engines. Exclusion criteria were applied and the results were sorted to produce a list of the top 100 sites. The validity of the tool was supported by assessing inter-rater reliability for a random subset of sites.

Summary of Results: Websites were largely commercial in nature (48%, n=48) with a smaller minority of non-profit sites (29%, n=29). Only half of the sites clearly indicated the author (n=50) and the author’s credentials (n=51) and 58% (n=58) indicated the author’s institutional affiliation. Less than one-third identified the sources used to compile the information (n=31). While 80% of sites (n=80) identified the date of creation of the entire site, only 45% (n=45) had been updated within the past two years. The majority of websites (87%, n=87) had interactive components; however, only 24% (n=24) featured a discussion board and 9% (n=9) provided educational support. The majority of websites (86%, n=86) provided information that was accurate and 13% (n=13) were mostly accurate. 36% (n=36) published information via treatment options by stage and only 15% (n=15) published information on prognosis by stage.

Conclusions: Web-based melanoma information could be significantly improved. This study revealed deficits in identifying authorship and attribution and less than half of the sites had been recently updated. While most sites contained basic facts about melanoma, few contained accurate information based on disease stage. Information from this study can help to improve the quality of web-based information for melanoma patients.

344 LARGE SCALE CHEMOENZYMATIC SYNTHESIS OF THE NON-HUMAN SIALIC ACID, N-GLYCOLYNEURAMINIC ACID
Haidar Y. Pearce O, Varki A University of California, San Diego School of Medicine, La Jolla, CA.

Purpose of Study: Classic and recent epidemiological studies continue to reveal a link between human consumption of red meats/dairy products and the incidence of carcinomas. While saturated fats or mutagens in grilled meats are often blamed, these observations can also be explained by the consumption of a non-human sialic acid, Neu5Gc, which is enriched in red meats and milk. In addition, all humans have circulating anti-Neu5Gc antibodies. The interaction of consumed Neu5Gc with these circulating anti-Neu5Gc antibodies produces a chronic inflammatory state that could have an important role in the progression of carcinomas. Major rate limiting factors to further cellular and animal studies of the influence of Neu5Gc are the relative inaccessibility of Neu5Gc in large quantities.

Methods Used: A novel method for the chemoenzymatic synthesis of Neu5Gc was attempted (Figure 1). The synthetic scheme utilizes the bacterial enzyme, N-glycolyneuraminic acid lyase, which is purified by lysing cells and running a manual Ni-sepharose high performance resin column. The enzyme was covalently attached to an Amberzyme Oxirane resin which simplifies purification and allows reutilization of the enzyme. Enzyme activity was checked via a thiobarbituric acid assay to determine optimal reaction conditions.

The cheap precursor, glucosamine, is converted to the synthetic intermediate, GlcNGc, by a simple chemical reaction. ManNGc, which is in constant equilibrium with GlcNGc, is converted to the desired product, Neu5Gc, via addition of the enzyme. After filtration of the resin, column chromatography was run to purify the Neu5Gc.

Summary of Results: Large scale synthesis of Neu5Gc is achieved in two simple reaction steps from the cheap precursor glucosamine and subsequent purification by filtration and column chromatography.

Conclusions: The large-scale chemoenzymatic synthesis of Neu5Gc will allow further studies of the link between human consumption of red meat/dairy products containing Neu5Gc and the incidence of carcinomas.

345 MITOCOンドRIONAL DNA AND MINIMAL RESIDUAL DISEASE ASSESSMENT IN PEDIATRIC ACUTE LYMPHOBlastic LEUKEMIA
Villanueva N, Asgalda M, Chang L, Ernst T, Saito A, Pritchett L, Shiramizu B University of Hawaii John A. Burns School of Medicine, Honolulu, HI.

Purpose of Study: Children treated for acute lymphoblastic leukemia (ALL) have a risk for developing neurocognitive problems, which may be associated with relapse. Relapse is contributed by residual disease or persistent disease (MRD/PD) present in CSF or blood. MRD/PD-positive specimens may have high mitochondrial DNA (mtDNA) copy numbers/cell, a possible compensatory survival mechanism for a unique lymphoblastic cellular phenotype in the brain. This study analyses CSF for MRD/PD and mtDNA copy numbers/cell in the subjects who are also undergoing special MRI scans and neurocognitive tests.

Methods Used: CSF DNA from two consented newly diagnosed children with ALL were assessed for MRD/PD status and mtDNA levels. Neurocognitive testing and special MRI scans (magnetic resonance spectroscopy, diffusion tensor imaging, and high resolution MRI) without sedation were also completed on these children and in age-matched non-ALL controls. MRD/PD status was assessed by semi-nested real-time PCR using pooled primer sets from the FR1/variable region of the IgH and two consensus 3’ primers. mtDNA copy numbers/cell were assessed using real-time PCR.

Summary of Results: Two children (001 and 002) were enrolled and had CSF specimens available for MRD/PD and mtDNA copy number analyses. Results summarized in Table 1. Subject 001 completed MRI scans and neurocognitive tests while Subject 002 tests are pending. Baseline neurocognitive test for subject 001 were normal while MRI scans indicated presence of mild to moderate atrophy throughout the brain. Four control children have also completed MRI scans and neurocognitive tests with normal results.

Conclusions: Although the results are too early to provide any conclusions or statistical comparisons, MRD and mtDNA analyses of the CSF specimens demonstrate the feasibility to perform the assays on CSF cells. Additional children with ALL are continuing to be recruited.

TABLE 1. Summary of MRD and mtDNA Analyses of CSF

<table>
<thead>
<tr>
<th>Entry</th>
<th>Visit 1</th>
<th>Visit 2</th>
<th>Visit 3</th>
<th>Visit 4</th>
<th>Visit 5</th>
<th>Visit 6</th>
<th>Visit 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject 001</td>
<td>Neg</td>
<td>Equiv (VH1) 156</td>
<td>Neg</td>
<td>Equiv</td>
<td>Neg</td>
<td>Pos (VH3) 201</td>
<td>Equiv (VH3) 174</td>
</tr>
<tr>
<td>Subject 002</td>
<td>Equiv (VH1) 285</td>
<td>Neg 241</td>
<td>Pos (VH3) 360</td>
<td>Neg 156</td>
<td>Equiv (VH3) 198</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Equiv: Equivocal for MRD; Neg: Negative for MRD; Pos: Positive for MRD; VH1: VH1 family; VH3: VH3 family; *mitochondrial DNA copy number per cell; N/A: no specimen available.

346 DYNAMIC CONTRAST-ENHANCED MRI FOR PREDICTION OF PATHOLOGIC RESPONSE TO NEOADJUVANT CHEMOTHERAPY IN BREAST CANCER PATIENTS
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Purpose of Study: To determine if changes in dynamic contrast-enhanced (DCE) MRI pharmacokinetic parameters can be used to predict which patients achieve pathologic complete response (pCR).

FIGURE 1: Synthesis of Neu5Gc.
Methods Used: 57 breast cancer patients (mean age, 49 ± 10 years) received neoadjuvant chemotherapy (NAC) and underwent DCE-MRI. 26 patients were HER-2 positive and 31 patients were HER-2 negative. All patients received doxorubicin and cyclophosphamide (AC) followed by a taxane regimen. HER-2 positive patients received trastuzumab with taxane. DCE-MRI were obtained before NAC and at first follow-up, which was after 2 cycles of AC. For each MRI study, the whole tumor region of interest (ROI) was drawn manually, slice-by-slice. A computer algorithm selected and labeled the hotspot that showed the greatest percentage enhancement within each ROI. Enhancement kinetics from the whole tumor and hotspot were fitted with the Tofts model to obtain the transfer constant (Ktrans) and rate constant (kep). Pathology samples were categorized as pCR or non-pCR. The ability of pharmacokinetic parameters to predict pCR was analyzed using paired, two-tailed t-tests. An artificial neural network was also used to select a classifier based on Ktrans and kep parameters that could predict pCR. Receiving operating characteristic (ROC) analysis was performed and the area under the ROC curve (AUROC) was examined.

Summary of Results: 30 patients (30/57, 53%) achieved pCR, including 19 HER-2 positive (19/26, 73%) and 11 HER-2 negative (11/31, 35%). In the pCR group, the hotspot Ktrans value at the first follow-up was lower compared to the pre-treatment value (p<0.05). There was no significant difference in Ktrans or kep between the pCR and non-pCR group. The AUROC to differentiate between pCR and non-pCR was 0.76.

Conclusions: With a 53% pCR rate the NAC regimen used was effective, especially in HER-2 positive patients (73%) who received trastuzumab with taxane. While DCE-MRI pharmacokinetic parameters may not strongly predict pCR after patients received only 2 cycles of AC, further analysis after the administration of trastuzumab with the taxane regimen may demonstrate a higher accuracy in prediction because pCR is most likely achieved using targeted therapy with trastuzumab.

### 347 WHOLE ORGAN SEGMENTATION VERSE CYLINDRICAL REGION OF INTEREST IN MEASURING CT RADIATION DOSE OF ABDOMINAL ORGANS

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**Purpose of Study:** To determine whether accurate measurements of CT radiation dose of abdominal organs can be estimated using cylindrical regions of interests (ROIs) instead of whole organ dosages. Evaluations would allow large dataset dose measurements and the development of organ dose maps based on patient specific parameters. The end goal being, to track lifetime patient radiation dose from CT.

**Methods Used:** Using a program developed by the lab, the livers, spleens and kidneys of 19 female and 15 male contrast enhanced CT scans were manually segmented. Cylindrical ROIs (2 cm diameter/1.5 cm height for spleen/liver; 1 cm diameter/1.5 cm height for kidneys) were also taken at the approximate volumetric centroid. A Monte-Carlo N-Particle (MCNPX) simulation, using both fixed tube current (FTC) and tube current modulation (TCM), calculated organ dose.

**Summary of Results:** Table 1 and 2 shows dosage RMSE and average percent difference as well as the relation between patient abdominal circumference to organ dose for FTC and TCM respectively. As shown, full organ and ROI organ dose measurements are comparable, organ dose is dependent on patient size and organ dose does not differ across abdominal organs.

**Conclusions:** Manual organ segmentation and its dosage calculations are time consuming (~5 hr / case), costly, challenging and require anatomical expertise. The development of pre-calculated organ dose measurements using accurate organ dose models would minimize the complications seen in manual measurements. Thus, lifetime tracking of patient radiation dose would be feasible, allowing investigators and clinicians to assess risk from medical procedures.

| Table 1: Fixed Tube Current (FTC) |
|-----------------|--------|--------|----------------|--------|
| Organ          | RMSE (mGy) | Full/ROI Percent Difference | Patient Size vs. Organ Dose (R) |
| Liver          | 1.9    | 7.9%   | 0.81           |
| Spleen         | 1.9    | 5.9%   | 0.75           |
| R.Kidney       | 2.3    | 7.1%   | 0.89           |
| L.Kidney       | 2.5    | 7.9%   | 0.89           |

| Table 2: Tube Current Modulation (TCM) |
|-----------------|--------|--------|----------------|--------|
| Organ          | RMSE (mGy) | Full/ROI Percent Difference | Patient Size vs. Organ Dose (R) |
| Liver          | 2.4    | 9.2%   | 0.45           |
| Spleen         | 2.0    | 5.3%   | 0.50           |
| R.Kidney       | 3.1    | 9.8%   | 0.33           |
| L.Kidney       | 4.0    | 10.0%  | 0.50           |

### 348 ORTHOVOLTAGE ROTATIONAL EXTERNAL BEAM RADIATION THERAPY ON A BREAST CT PLATFORM: A SIMULATION STUDY

Prionas ND, McKenney SE, Boone JM. University of California Davis, Sacramento, CA.

**Purpose of Study:** To evaluate the feasibility of a dedicated breast computed tomography (bCT) platform to deliver orthovoltage rotational external beam radiation therapy through clinically relevant dose distributions, specifically partial breast irradiation, whole breast irradiation, and dose painting.

**Methods Used:** Monte Carlo simulations, using MCNPX 2.6.0, were based on the geometry of a prototype bCT platform. A 178 keV monoenergetic photon source was used to simulate a 320 kVp spectrum filtered by 4 mm of copper, as validated by their depth-dose characteristics in polyethylene. The source was rotated around a 14 cm voxelized polyethylene disk (0.1 cm tall) or cylinder (9 cm tall) to simulate primary and primary + scattered photon interactions, respectively. Beam collimation was varied in the x-y plane of rotation (1–14 cm) and in the z-direction (0.1–10 cm). Dose deposition was measured in the voxelized phantoms as mGy per incident photon.

**Summary of Results:** Varying collimation in x-y generated a family of 2D dose profiles; dose distributions showed a cupped profile with high edge dose using an uncollimated (14 cm) beam and increasingly peaked central dose with low edge dose as collimation narrowed. The ratio of central dose to cylinder edge dose was 14.8 for 1 cm and decreased to 0.6 for 14 cm collimation. Dose painting for multiple foci, a fine distribution, and a ring distribution was demonstrated using multiple rotations with varying collimation. A homogeneous dose distribution (~5% fluctuation) with skin sparing was demonstrated using a weighted sum of four dose profiles. The mean scatter to primary ratio across the central axial section of the cylinder ranged from 0.007 (0.1 cm z-collimation) to 0.619 (10 cm z-collimation). Using 2 cm z-collimation, scatter tails decreased exponentially towards the cylinder top/bottom to 3% of maximum dose deposition.

**Conclusions:** A BCT system is a feasible platform to deliver orthovoltage rotational external beam radiation therapy for the treatment of breast cancer. A variety of dose distributions can be generated that allow for partial breast irradiation to a single focus, dose painting, and whole breast irradiation with skin sparing. Dedicated BCT may serve as a platform for both diagnostic breast imaging and radiation therapy.

### 349 PERSISTENT FEVER, SPLENOMEGBALY, AND ANEMIA IN A 2 YEAR OLD: A CASE OF SLOWLY PROGRESSING HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS

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**Case Report:** HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS (HLH) is a rare disorder of cytokine dysfunction which may be familial, or associated with infection, malignancy, or autoimmune disorders. Diagnosis is made by molecular analysis or by clinically meeting 4 major criteria (fever, splenomegaly, cytopenia, hypertriglyceridemia and/or hypofibrinogenemia, and hemophagocytosis). Additional criteria include low or absent natural killer cell activity, ferritin>500 mcg/L, or soluble CD25 activity. We report a 2 year old with fever of unknown origin, massive splenomegaly, and persistent anemia, who early-on did not meet HLH criteria. Given a high suspicion for HLH, the child received an extensive workup, which revealed a diagnosis of HLH.

A 2 year old presented to the ER with 1 week history of fever, splenomegaly, and anemia (hemoglobin 5.5). Inpatient workup revealed evidence of past EBV infection. Patient received 2 blood transfusions and was discharged home. Patient returned to ER 2 weeks later with persistent fever and hemoglobin 8.8. Patient was readmitted for a workup, with HLH test results summarized as follows:
**Methods Used**: 337 cases seen at the Children's Hospital of Central California. Demographic data, initial presentation, initial and follow-up lab results, type of treatment, response to treatment, cardiac outcome, and non-cardiac complication were reviewed, there were no significant differences between the two groups in terms of age of onset, gender, race, course of fever, season of onset, lab test values, response to treatment, cardiac outcome, and non-cardiac complication incidence. As for the initial clinical presentation, except for the course of fever, typical KD presents with cervical lymphadenopathy, rash, peripheral extremity change, oral mucosa change and conjunctival injection more than the incomplete KD (all p<0.000).

**Conclusions**: Typical KD and incomplete KD most likely share the same pathophysiological pathway, have no significant differences in lab values, response to treatment or outcome. This reconfirms that the diagnosis of incomplete KD should not change the clinical management strategy.

**355 MEASUREMENT OF THERAPY INDUCED NEUTRALIZING ANTIBODIES TO INTERFERON (IFN)-β IN MULTIPLE SCLEROSIS PATIENTS UTILIZING A NEW FUNCTIONAL CELL BASED IFN-β REPORTER GENE ASSAY**

Martins TB1, Rose JW2, Jaskowski T1, Kasukawa N1, Gardiner GL1, Husebye D1, Seraj HS1, Hill HR1,2. ARUP Institute for Clinical and Experimental Pathology, SLC, UT. 2University of Utah School of Medicine, SLC, UT and 3University of Utah School of Medicine, SLC, UT.

**Purpose of Study**: Low birth weight (LBW) infants exhibit an increased risk of infection, a condition that persists into adulthood. Specifically, LBW adults demonstrate an attenuated immune response to T-cell independent antigens. B-1 lymphocytes are the effector cells responsible for T cell independent antigen responses as well as early immune responses against pathogens. B-1 lymphocytes are comprised of B-1a and B-1b cells. The co-receptor CD5 is expressed on B-1a cells, but not on B-1b cells. The expression of CD5 on the cell surface depends on the translation of two alternative exons termed E1a and E1b. Transcripts initiated from the E1a exon robustly express CD5 on the cell surface, while transcripts made from E1b are retained in the cytoplasm. The alternative promoter for E1b resides in a human endogenous retrovirus, which contains CpGs that can be methylated. We hypothesize that LBW infants possess decreased numbers of B-1b cells, and this will be associated with altered DNA CpG methylation of the CD5 retroviral insert.

**Methods Used**: B cells were isolated from cord blood of LBW and normal birth weight (NBW) infants. Cell populations were analyzed using flow cytometry and the ratio of B-1b to B-1a cells was determined by counting sorted cells. DNA from FACS-sorted B cells was isolated and subject to bisulfite sequencing to determine the methylation patterns of the CD5 retroviral insert.

**Summary of Results**: Analysis has been completed on 40 patients. 3 were LBW (birth weight of 10th percentile or lower). LBW patients have a decreased ratio of B-1b to B-1a cells compared to NBW patients (0.149 ± 0.68 vs. 0.771 ± 0.2745). B-1b cells from LBW infants also exhibit 30% and 33% methylation of the CD5 retrovirus insert, compared to 45% and 50% methylation in NBW patients at two CpG sites within the CD5 retroviral insert.

**Conclusions**: LBW infants contain fewer B-1b cells and exhibit less methylation within the CD5 retroviral insert. These are among the first human findings that associate an epigenetic change within a gene directly related to the targeted cell phenotype. We speculate that DNA CpG changes of the CD5 retroviral insert will function as a marker for postnatal decreased B-1b cell numbers.
Purpose of Study: Interferon (IFN)-α is well established as first line therapy in relapsing/remitting multiple sclerosis (MS). Patients with therapy induced neutralizing antibodies (Nabs) to IFN-α have shown reduced responses to treatment with IFN-α, having higher relapse rates, increased MRI activity and a higher risk of disease progression.

Methods Used: We employed a new functional assay which measures IFN-α neutralizing antibody utilizing a division arrested, cryopreserved human cell line which have been transfected with a luciferase reporter gene. Summary of Results: This new IFN-α reporter gene assay demonstrated 100% sensitivity compared with the traditional cytopathic effect assay (CPE), correctly identifying 25 of 25 samples as positive for the presence of IFN-α neutralizing antibody. Specificity was also 100% with 58 of 58 normal donors testing negative. Additionally, 165 multiple sclerosis patients undergoing IFN-α therapy were tested for IFN-α neutralizing antibody by the IFN-α reporter gene assay. The percent of patients testing positive for neutralizing antibodies to IFN-α were, according to the IFN-α therapy utilized: Avonex (1α) 34.3%, Rebif (1α) 44.7%, and Betaseron (1β) 38.1%.

The IFN-α cell surface receptor did show slight cross-reactivity in samples spiked with high concentrations (1000 pg/ml) of recombinant IFN-γ, but showed no cross-reactivity to interleukin (IL)-1β, 2, 4, 5, 6, 8, 10, 12, 18, and TNF-α.

Conclusions: The IFN-α reporter gene assay showed excellent correlation with the well established CPE assay and also offered clear advantages. Results can be reported within 20 hours compared to 4-6 days for the CPE assay, the cell line has been well characterized and is cryopreserved assuring better run to run reproducibility, and there is no need to maintain a viral culture system. The use of this new functional assay should be a valuable tool for the monitoring of IFN-α treated MS patients for the presence of neutralizing antibodies, hopefully leading to more effective therapy.

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INVESTIGATION OF STAT3-DEPENDENT NEUTROPHIL MIGRATION IN JOB SYNDROME
Kumanovic A,1*, Augustine NH,1, Hill HR,1,2,3 *Univ of Utah, SLC, UT; 1Univ of Utah, SLC, UT and 3ARUP Institute for Clinical and Experimental Pathology, SLC, UT.

Purpose of Study: Hyper-immunoglobulin E (IgE) syndrome (HIES), or Job syndrome is a primary immunodeficiency, characterized by high serum concentration of IgE, eczema, eosinophilia, indolent cold abscesses, candidiasis, and pneumonias complicated by pneumocele formation and bronchiectasis. In addition to the immune system defects, HIES is also characterized by connective tissue, skeletal and dental development defects. Mutations in STAT3 gene have been identified in classical autosomal dominant HIES patients. STAT3 participates in the signal transduction of many cytokine pathways and thus provides a possible explanation for the diverse dysfunctions. However, direct connections between the various clinical manifestations of HIES and STAT3 mutations have not yet been clearly established. Chemotactic defects in the peripheral blood neutrophils from HIES patients was first described by us and verified by other investigators. Many noted that the chemotactic defect is a variable feature of the disease, but these early studies used a variety of methods and chemotactic agents to study neutrophil migration. The discovery of the genetic etiology of autosomal dominant HIES makes it possible to re-examine neutrophil chemotaxis in molecularly-defined HIES patients using chemokines affecting STAT3-dependent and independent signaling pathways.

Methods Used: Chemotaxis of peripheral blood neutrophils from HIES patients and healthy donors were examined in response to various stimuli in multwell chemotaxis chamber with 5-μm pore-size filters. Actin polymerization was detected by measuring FITC-phallolidin fluorescence intensity by flow cytometry.

Summary of Results: Neutrophils from HIES patients have a chemotaxis and actin polymerization defect in response to CXCL1 and CXCL2, but normally respond to the complement co-factor to the response to formylated peptide fMLP is variable. CXCL1 and CXCL2 signals are mediated by the CXCR2 chemokine receptor. CXCR2, like the receptors for fMLP and C5a, belongs to the G protein-coupled receptor family, but it is unique in that it can reportedly activate the STAT3 signaling system.

Conclusions: Our results demonstrate that neutrophils from HIES patients have an intrinsic chemotaxis defect in response to chemotactic signals mediated by CXCR2.

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SEVERE URTICARIA AND ABDOMINAL PAIN DUE TO ANTIBODIES TO IGE RECEPTOR TREATED WITH CYCLOSPORINE
Wang KY, Roberts RL. UCLA, Los Angeles, CA.

Purpose of Study: Urticaria and abdominal pain due to antibodies to the IgE Fc Receptor was successfully treated in children with oral cyclosporine.

Methods Used: We described 2 children with urticaria due to antibodies to IgE Fc receptor went into complete remission with oral cyclosporine. Their abdominal pain also resolved with cyclosporine, suggesting that the pain could have also due to the antibodies to IgE receptor which may have caused local abdominal pain by histamine release in the gut.
357
A RANDOMIZED CONTROLLED TRIAL OF COST-EFFECTIVENESS OF ULTRASOUND-GUIDED INTRAARTICULAR INJECTION OF INFLAMMATORY ARTHRITIS

Norton H, Sibbitt WL, Bankhurst A, Delea S, Chavez-Chiang N, Band P. University of New Mexico, Albuquerque, NM and New York University School of Medicine, New York, NY.

Purpose of Study: The present randomized controlled study addressed whether sonographic needle guidance affected the outcomes of intraarticular injection for inflammatory arthritis.

Methods Used: 244 symptomatic joints (3 small, 51% intermediate, and 46% large joints) with inflammatory arthritis (76% rheumatoid arthritis) were randomized to injection by conventional palpation-guided anatomic injection (120 joints) or sonographic image-guided injection enhanced with a one-handed RPD (the reciprocating procedure device) mechanical syringe (124 joints). A one needle, two-syringe technique was used. After intraarticular placement and synovial space dilatation were confirmed by sonography, a syringe exchange was performed, and triamcinolone acetonide was injected with the second syringe through the indwelling intraarticular needle. Baseline pain, procedural pain, pain at outcome (2 weeks and 6 months), responders, therapeutic duration, reinjection rates, total cost, and cost per responder were determined.

Summary of Results: Relative to conventional palpation-guided methods, sonographic guidance for injection of inflammatory arthritis resulted in 81% reduction in injection pain (p < 0.001), a 35% reduction in pain scores at outcome (p < 0.02), 38% increase in the responder rate (p < 0.003), 34% reduction in the non-responder rate (p = 0.003), a 32% increase in therapeutic duration (p < 0.01), a 8% reduction ($7) in cost/patient/year, and a 33% ($64) reduction in cost/responder/year for a hospital outpatient (p < 0.001).

Conclusions: Sonographic needle guidance improves the performance, clinical outcomes, and cost-effectiveness of intraarticular injections of inflammatory arthritis.

358
TECHNIQUE-BASED IMPROVEMENTS IN THE COST-EFFECTIVENESS OF INTRAARTICULAR INJECTIONS FOR OSTEOARTHRITIS OF THE KNEE: A RANDOMIZED CONTROLLED STUDY


Purpose of Study: This randomized controlled study compared the cost-effectiveness of two different low-cost, palpation-directed intraarticular injection techniques for osteoarthritis (OA) of the knee.

Methods Used: 128 symptomatic osteoarthritic knees were randomized to palpation-guided intraarticular injection with either 1) a conventional syringe (CS), or 2) a single-handed aspiration syringe (AS), the RPD (the reciprocating procedure device). 3 ml of 1% lidocaine were used for anesthesia, followed by arthrocentesis, and then injection of 80 mg of triamcinolone acetonide. Baseline pain, procedural pain, aspirated fluid volume, pain at outcome (2 weeks and 6 months), responder rates, time to re-injection, cost/patient/year and cost/responder/year were determined.

Summary of Results: Baseline pain scores and demographics were comparable between the groups (p > 0.2, for all). Both techniques significantly reduced pain scores at outcome from baseline (p < 0.001). Relative to the CS technique, the AS technique resulted in a 110% greater volume of aspirated fluid, a 37% reduction in procedural pain (p < 0.01), a 43% greater reduction in pain scores at outcome (p < 0.01), a significant increase in responder rate (p < 0.01), a significant reduction in non-responder rate (p < 0.01), 29.7% increase in the time to next injection (p < 0.01), and a 23% reduction ($28 US) in cost/patient/year (p < 0.001).

Conclusions: Utilization of a low-cost, single-handed aspiration syringe improved outcomes and reduced overall costs or intraarticular injections of osteoarthritides of the knee. Larger multicenter studies are needed to confirm these results in diverse practice settings.

359
NEW DEVICE TECHNOLOGIES FOR SUBCUTANEOUS FAT BIOPSY

Kettwich LG, Sibbitt WL, Chavez-Chiang N, Delea S, Michael A, Bankhurst A. University of New Mexico Health Sciences Center, Albuquerque, NM and Texas Tech Health Sciences Center, El Paso, TX.

Purpose of Study: Subcutaneous fat biopsy by needle aspiration is useful for the evaluation of amyloidosis, environmental contaminants, lipid metabolism, genetic studies, and diabetes research; however, aspiration with conventional syringes is awkward and can result in needle trauma to patient tissues. The present study examined new technologies for needle aspiration biopsy.

Methods Used: Subcutaneous fat biopsy in 10 high-risk individuals with arthralgias and neuropathic symptoms was randomized to 1) a 10 ml RPD (reciprocating procedure device) mechanical syringe, or 2) a 60 ml vacuum syringe. In each case an 18 gauge 2 inch (5 cm) Chiba biopsy needle was utilized. Outcome measures included patient pain by the 10 cm Visual Analogue Pain Scale (VAS), adequacy of biopsied tissue, complications, and diagnosis. The operator’s ability to control the syringe was quantitatively measured by the linear displacement method.

Summary of Results: Both the vacuum and mechanical syringes permitted facile aspiration of subcutaneous fat; in all cases, there was adequate sample obtained for analysis without complications. The mechanical and the vacuum syringes enhanced control of the needle compared to conventional syringes, reducing unintended forward penetration by 75% (3.6 ±0.5 mm) and 87% (12.0 ±1.4 mm), respectively (p < 0.0001). Although adipose cells were obtained in abundance, small blood vessels and connective tissue septa were also obtained intact permitting precise microhistological examination. One case of primary AL amyloidosis (kappa light chain disease) was diagnosed in each group.

Conclusions: Subcutaneous fat biopsy by needle aspiration can be facilely achieved with new aspiration syringe technologies with improved needle control and enhanced patient safety.

360
MIDDLE CEREBRAL ARTERY RESISTIVITY AND PULSATILITY INDICES IN SYSTEMIC LUPUS ERYTHEMATOSUS

Boyer NM, Roldan C, Yonen KA, Sharrar JM, Sibbitt WL, Greene ER. University of New Mexico, Albuquerque, NM and New Mexico Highlands University, Las Vegas, NM.
Purpose of Study: Systemic lupus erythematosus (SLE) is associated with significant cerebrovascular and neuropsychiatric disease for which multiple pathogenesis have been proposed. Although global and focal cerebral hyperfusion have been proposed, there are limited data about intracerebral arterial hemodynamics. Transcranial Doppler (TCD) allows portable, high temporal (6 ms) and spatial (0.3 mm) resolution, and noninvasive blood velocity measurements in the middle cerebral arteries and calculations of standard resistivity (RI) and pulsatility (PI) indices. Determined from the blood velocity waveforms, RI and PI correlate linearly with cerebral hemispheric arterial tone, blood flow resistances, and impedances. Accordingly, we hypothesized that there would be a significant differences (p<0.05) in RI and PI between SLE patients and healthy, age and gender matched controls. With similar blood pressures, decreased RI and PI would be consistent with a compensatory mechanism to maintain relative hyper or normal cerebral perfusion.

Methods Used: Thirty four stable SLE patients (35±11 years) and 15 control subjects (34±10 years) underwent TCD to measure RI and PI bilaterally. Patients and controls had simultaneously measured similar, normal blood pressures and were examined in the supine position during normal, resting respiration (minimize PCO2 changes). RI and PI were determined by a single observer blinded to all subjects’ clinical data.

Summary of Results: SLE patients had lower (p<0.05) average RI values as compared to controls: 0.45±0.10 versus 0.52±0.05; respectively. Similarly, SLE patients had lower (p<0.05) average PI than controls: 0.65±0.19 versus 0.77±0.12; respectively. There were no significant differences in RI and PI bilaterally within each cohort.

Conclusions: Measured and robust RI and PI values in the middle cerebral artery are significantly lower in SLE as compared to controls. These indirect indices suggest that middle cerebral arterial resistances and impedances are decreased in SLE, and under normotensive conditions, are consistent with increased arteriolar dilatation, increased medium size arterial cerebral blood flow, and a compensatory mechanism for hyper or normal cerebral perfusion in SLE.

Metabolism II
Concurrent Session
8:30 AM
Saturday, January 29, 2011

361 ISLET MATRIX METALLOPROTEINASE-9 IS DOWNREGULATED IN A MOUSE MODEL OF TYPE 2 DIABETES
Svy D2,1, Aston-Mourney K2,1, Kahn S2,1 1University of Washington, Seattle, WA and 2VAPSHCS, Seattle, WA.

Purpose of Study: A pathological hallmark of the pancreatic islet in type 2 diabetes is aggregation of islet amyloid polypeptide (IAPP) extracellular amyloid deposits, the process of which is toxic to β cells. We have previously shown that (a) glucose dose-dependently increases amyloid deposition in human IAPP (hIAPP) transgenic (T) mouse islets, (b) matrix metalloproteinase (MMP)-9 degrades hIAPP, and (c) inhibition of MMP-9 activity increases amyloid deposition in hIAPP T islets. Whether high glucose regulates MMP-9 or tissue inhibitor of metalloproteinase (TIMP)-1, a natural inhibitor of MMP-9, is unknown. Thus, we determined if downregulation of MMP-9 occurs in islets in vitro when cultured in high glucose and/or in vivo in a diabetic mouse model.

Methods Used: Amyloid-forming hIAPP T and non-amyloid forming non-transgenic (NT) mouse islets were cultured for 7 days in 11.1, 16.7, and 33.3 mM glucose (n=4–6). Islets were also isolated from diabetic db/db and control db/+ mice (n=8). To confirm the presence of amyloid in T islets after culture, islet sections were stained with thioflavin S. MMP-9 and TIMP-1 mRNA levels were determined by real-time PCR using 18S as the endogenous control. MMP-9 activity was measured in islet conditioned medium by gelatin zymography (n=3).

Summary of Results: In cultured T and NT islets, neither MMP-9 nor TIMP-1 mRNA changed with increasing glucose. In contrast, MMP-9 activity tended to increase at 33.3 mM glucose (NT at 11.1, 16.7, and 33.3 mM glucose: 1.09±0.21, 3.61±0.95; T at 11.1, 16.7, and 33.3 mM glucose: 1.22±0.06, 1.03±0.18, 3.92±1.60; p=0.06), but was not affected by amyloid formation which was observed at 16.7 and 33.3 mM glucose. In db/db mouse islets, MMP-9 mRNA decreased by 41% when compared to db/+ islets (p<0.01). The mechanism by which glucose induces amyloid formation in this model. However, in a whole animal model, MMP-9 mRNA was significantly decreased in diabetes. This suggests that downregulation of MMP-9 by another component of the diabetic milieu, such as elevated free fatty acids, may contribute to the increased amyloid deposition associated with type 2 diabetes.

362 INCREASED EXPRESSION OF THE VASOCONSTRICCTOR ENDOTHELIN-1 MAY CONTRIBUTE TO ISLET ENDOTHELIAL DYSFUNCTION IN DIABETES
Otley A1, Kahn S2,1, Hull R2,1 1University of Washington, Seattle, WA and 2VAPSHCS, Seattle, WA.

Purpose of Study: Increased production of the vasoconstrictor endothelin-1 (ET-1) and decreased activity of the vasodilation-promoting enzyme prostacyclin synthase (PGIS) contribute to diabetic macrovascular endothelial dysfunction. We have demonstrated that the pancreatic islet is also affected by diabetic endothelial dysfunction. We thus determined if ET-1 and PGIS are expressed in islets and whether islet ET-1 expression increases and PGIS expression decreases with diabetes.

Methods Used: ET-1 and PGIS mRNA levels were measured by real-time PCR in immortalized murine endothelial cells (MS-1) and β (β-TC3), and whole islets. Islets were isolated from diabetic C57BL/6J/db/db mice or non-diabetic db/+ mice at 8 and 16 weeks of age. E-selectin was used as a marker of endothelial dysfunction, PECAM as a marker of endothelial cell number, and insulin as a marker of β-cell number.

Summary of Results: ET-1 mRNA was detectable in MS-1 cells. PGIS mRNA was detectable in both MS-1 and β-TC3 cells. As expected, E-selectin and PECAM were strongly expressed in MS-1 cells and insulin was strongly expressed in β-TC3 cells. At the time of islet isolation db/db mice were hyperglycemic compared to db/+ controls (Table). ET-1 mRNA levels tended to increase in db/db islets at 16 weeks (Table; p<0.09). Similarly, PGIS mRNA levels did not significantly differ from control at 8 or 16 weeks. As expected, E-selectin mRNA levels were increased in db/db islets at 16 weeks, while PECAM mRNA levels were decreased at both 8 and 16 weeks.

Conclusions: ET-1 is selectively expressed in islet endothelial cells while PGIS is expressed in both islet endothelial and β cells. If PGIS activity decreases in diabetes it is not via decreased mRNA levels. Increased expression of the vasoconstrctor ET-1 occurs with prolonged diabetes and may, therefore, contribute to islet endothelial dysfunction. This, in turn, may contribute to islet dysfunction in type 2 diabetes.

<table>
<thead>
<tr>
<th></th>
<th>8 week db/+ (n=8)</th>
<th>8 week db/db (n=8)</th>
<th>16 week db/+ (n=8)</th>
<th>16 week db/db (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fed Plasma Glucose (mg/dL)</td>
<td>209±14</td>
<td>582±22**</td>
<td>204±12</td>
<td>395±55**</td>
</tr>
<tr>
<td>ET-1 mRNA</td>
<td>1.00±0.09</td>
<td>1.06±0.12</td>
<td>1.00±0.13</td>
<td>1.46±0.91</td>
</tr>
<tr>
<td>PGIS mRNA</td>
<td>1.00±0.22</td>
<td>0.69±0.15</td>
<td>1.00±0.32</td>
<td>1.14±0.27</td>
</tr>
<tr>
<td>E-selectin mRNA</td>
<td>1.00±0.27</td>
<td>1.38±0.25</td>
<td>1.00±0.37</td>
<td>2.33±0.58*</td>
</tr>
<tr>
<td>PECAM mRNA</td>
<td>1.00±0.05</td>
<td>0.76±0.05**</td>
<td>1.00±0.04</td>
<td>0.77±0.06**</td>
</tr>
</tbody>
</table>

Data are mean±SEM. *p<0.05, **p<0.01 vs db/+ age-matched control.

363 OXIDATIVE STRESS PROMOTES ISLET AMYLOID DEPOSITION
Morcos M2,1, Zraika S2,1, Kahn S2,1 1University of Washington, Seattle, WA and 2VAPSHCS, Seattle, WA.

Purpose of Study: Type 2 diabetes and chronic hyperglycemia are associated with oxidative stress in pancreatic β cells. Islet amyloid, a pathological feature of the pancreas in type 2 diabetes, contributes to the loss of β-cell mass but its relationship to oxidative stress is not fully understood. Previous studies have shown that amyloid deposition increases oxidative stress in...
islets; however, extended exposure to amyloid-induced oxidative stress may also increase amyloid deposition in a positive feedback manner. Therefore, oxidative stress may promote amyloid deposition and the loss of β-cell mass.

Methods Used: We used an in vitro model of islet amyloid formation where human islet amyloid polypeptide (hIAPP) transgenic mouse islets produce amyloid in a glucose-dependent manner and non-transgenic islets are incapable of producing amyloid. Islets were incubated in 11.1 or 16.7 mmol/L glucose for 7 days with or without 0.5 mM glyceraldehyde, a known oxidative stressor. Oxidative stress, amyloid and β-cell area were visualized using nitrotyrosine, thiolavin S and insulin staining respectively. Amyloid severity was calculated as the proportion of islet area occupied by amyloid and β-cell area was calculated as the proportion of islet area occupied by insulin.

Summary of Results: Glyceraldehyde treatment increased oxidative stress in both 11.1 and 16.7 mmol/L glucose-cultured islets. In the 16.7 mmol/L glucose-cultured hIAPP transgenic islets, treatment with glyceraldehyde did not significantly increase amyloid deposition (2.91 ± 0.84% vs. 2.65 ± 0.45%; p=0.66, n=5), nor did it change β-cell area (66.1 ± 2.0% vs. 67.2 ± 2.4%; p=0.21, n=5). These findings suggest that in islets with amyloid-induced oxidative stress, further induction of oxidative stress with glyceraldehyde does not promote additional amyloid formation or β-cell loss. Conversely, in the 11.1 mmol/L glucose-cultured hIAPP transgenic islets, where amyloid deposition is normally minimal, glyceraldehyde treatment increased amyloid deposition (0.29 ± 0.10% vs. 1.22 ± 0.33%; p=0.02, n=5) and reduced β-cell area (71.1 ± 0.8% vs. 68.6 ± 0.7%; p=0.01, n=5). As expected, the non-transgenic islets did not form amyloid.

Conclusions: The induction of oxidative stress under conditions of minimal islet amyloid deposition promotes amyloid formation and may contribute to the loss of β-cell mass in type 2 diabetes.

365 NICOTINE WORSENS HIGH FAT DIET-INDUCED HEPATIC AND MUSCLE STEATOSIS IN MICE

Nzenwa I1,2, Vides R1,3, Mangubat M4, Garcia L1,5, Shen R1, Sinha-Hikim AP1,2, Friedman TC1,2, Sinha-Hikim I1,2 1Charles Drew University, Los Angeles, CA; 2David Geffen School of Medicine-UCLA, Los Angeles, CA and 3Stanford University, Stanford, CA.

Purpose of Study: Smoking is a major risk factor for many diseases. There is evidence that smoking may contribute to non-alcoholic fatty liver disease (NAFLD) and skeletal muscle abnormalities. The health risk associated with smoking is exaggerated by obesity and is the leading causes of morbidity and mortality worldwide. We hypothesize that nicotine can have additive effects on HFD-induced hepatic steatosis and skeletal muscle abnormalities in obese mice.

Methods Used: Adult C57BL6 male mice were fed with normal diet (ND) or HFD with 60% of calories derived from fat (Research Diets, New Brunswick, NJ) with or without twice daily injections of 0.75 mg/kg BW of nicotine for 10 weeks.

Summary of Results: Compared with mice on ND, mice fed with HFD exhibited significant weight gain and increased abdominal as well as epididymal fat mass. Nicotine-treated mice on a HFD showed significantly less weight gain than mice fed a HFD alone. Abdominal fat and overall fat content was substantially reduced as determined by DEXA scan. Food intake was unchanged. Nicotine-treated mice on a HFD also had greater oxidative stress, as indicated by low GSH/GSSG ratio in comparison to HFD alone. Light and electron microscopy revealed increased lipid accumulation of varying sizes in hepatocytes from mice on a HFD. Addition of nicotine to HFD resulted in a further increase in lipid accumulation and in the size of lipid droplets. Intriguingly, compared with mice fed with HFD alone, where no or minimal lipid droplets were detected, combined treatments of nicotine and HFD resulted in intramitochondrial fat (IMF) accumulation in myofibers. We further observed mitochondrial swelling and vacuolization after combined treatment with nicotine and HFD than that of the mice on HFD alone.

Conclusions: These results indicate that nicotine further accelerates hepatic steatosis and skeletal muscle abnormalities induced by HFD and provide a basis for further studies aimed to elucidate the mechanisms of additive effects of nicotine and HFD on NAFLD and IMF accumulation in skeletal muscle. We surmise that nicotine plus HFD is likely to be a very toxic combination in patients.

366 MONounsaturated fatty acids incompletely rescue 3T3-L1 cells from effects of SCd1 inhibition

Yee JK, Wahjudi P, Patterson ME, Lim S, Mao C, Lee W Los Angeles BioMedical Research Institute at Harbor-UCLA, Torrance, CA.

Purpose of Study: Enhanced adipogenesis during adipocyte differentiation contributes to obesity development. Stearoyl-CoA desaturase enzyme 1 (SCd1) converts the saturated fatty acids palmitate and stearate to the monounsaturated fatty acids (MUFA)s palmitoleate and oleate, respectively. SCd1 is induced during adipocyte differentiation, is upregulated in obesity, and is a potential target for obesity prevention. Oleate can induce lipid accumulation before differentiation gene induction. The objectives are: 1) to determine the effects of SCd1 inhibition during adipocyte differentiation on desaturation and cell proliferation using stable isotypes; 2) to determine if the effects of SCd1 inhibition are altered by addition of MUFA.

Methods Used: Confluent 3T3-L1 primary adipocytes were treated with or without differentiation induction cocktail in the medium for four days. Differentiating cells were further treated in 4 groups during induction: 1) no additional treatment 2) SCd1 inhibitor CXG0290 3) CXG0290 and palmitoleate or 4) CXG0290 and oleate. In the first two days of induction, all groups received medium with 25% [13C]glucose. After induction was completed, BEB-content medium with [13C]stearate. Cells were harvested and counted on day 8, fatty acid and DNA isomers were analyzed by GC/MS. The 13C-enriched oleate/stearate ratio was calculated as the desaturation index (DI), a measure of SCd1 activity.

Summary of Results: CXG0290 decreased the DI based on isomers of newly formed fatty acids. Addition of palmitoleate to CXG0290 partially restored the DI, but addition of oleate decreased the DI further. After induction was completed, the DI as measured by desaturation of [13C]stearate was...
increased in the CGX0290 group. Cell counts were decreased in the CGX0290 group, although the 13C DNA enrichment was increased.

Conclusions: CGX0290 is an effective SCD1 inhibitor. CGX0290 does not prevent differentiation of 3T3-L1 cells, but decreases cell numbers likely through increased apoptosis. Addition of MUFA does not reverse effects of SCD1 inhibition, and oleate appears to feedback inhibit SCD1 further. Effects of SCD1 inhibition may be sensitive to modulation by nutrition. Potential use of CGX0290 in obesity prevention or treatment definitely requires further investigation.

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IS 1,5 ANHYDROGLUCITOL (GLYCOMARK®) A CLINICALLY USEFUL TEST IN TYPE 1 DIABETES?
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Purpose of Study: Glycomark® is an FDA approved test that measures 1,5 anhydroglucitol concentration in plasma. It is promoted as being a good assessment of postprandial hyperglycemia in diabetes. However, its clinical utility in type 1 diabetes has not been established. We utilized established glucose parameters, both in the blood and the interstitial fluid, to assess its ability to predict glucose control in type 1 diabetes.

Methods Used: Eighty-four studies were performed in type 1 diabetic volunteers. A fasting blood sample was drawn for both A1C and the 1,5 anhydroglucitol assay. We then performed five days of blinded-Continuous Glucose Monitoring (CGM) with simultaneous capillary blood glucose measurements. The data supplied from the CGM recording device was correlated with the 1,5 anhydroglucitol value. The following parameters were examined: blood A1C, mean capillary blood glucose, mean interstitial glucose, percent time spent above 180mg/dl, mean postprandial integrated interstitial glucose, percent time spent above 180mg/dl, and mean interstitial glucose.

Summary of Results: The first four of the parameters are shown below, correlated with the 1,5 anhydroglucitol value. None of the correlations was strong enough to be clinically useful.

Conclusions: 1,5 anhydroglucitol (Glycomark®) did not demonstrate any significant correlation to commonly accepted glucose control parameters. 1,5 anhydroglucitol should not be used to draw conclusions about other systemic glucose parameters until its reliability and clinical utility have been established in patients with diabetes.

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A NOVEL MECHANISM TO PREVENT CARDIAC CONSEQUENCES OF IN UTERO COCAINE EXPOSURE
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1Charles Drew University, Los Angeles, CA; 2David Geffen School Of Medicine-UCLA, Los Angeles, CA and Stanford, Stanford, CA.

Purpose of Study: Abuse of cocaine during pregnancy exposes ∼100,000 infants per year to cocaine in the United States. Cocaine readily crosses the placenta into the fetal circulation affecting the placenta and fetus with numerous adverse outcomes, including fetal cardiac myocyte death. This study was designed to test the hypothesis that minocycline, a second generation tetracycline, prevents fetal cardiac myocyte death induced by prenatal cocaine exposure through inhibition of c-Jun NH2-terminal kinase (JNK) and p38 mitogen-activated protein kinase (MAPK)-mediated mitochondria intrinsic pathway signaling.

Methods Used: Timed mated pregnant SD rats received one of the following treatments twice daily from gestational day 15 to 21: (i) IP injections of saline (control); (ii) IP injections of cocaine (15 mg/kg BW); and IP injections of cocaine + oral administration of 25 mg/kg BW of minocycline. Neonatal pups were killed on day 15 after birth. Additional pregnant dams received twice daily IP injections of cocaine (from days 15 to 21 of gestation) + oral administration of 37.5 mg/kg BW of minocycline. Minocycline treatment continued from day 15 of gestation until the pups were killed on day 15 after birth. Pups were kept with their mothers and allowed to be breast-fed ad libitum. Moms were not given cocaine after delivery.

Summary of Results: In utero cocaine exposure resulted in an increase in oxidative stress and fetal cardiac myocyte apoptosis through activation JNK and p38 MAPK-mediated mitochondria-dependent apoptotic pathway. Continued minocycline treatment from day 15 of gestation until the pups were killed significantly prevented oxidative stress, kinase activation, perturbation of BAX/BCL-2 ratio, caspase activation, and significantly attenuated fetal cardiac myocyte apoptosis after prenatal cocaine exposure.

Conclusions: Our results show in vivo cardioprotective effects of minocycline in preventing fetal cardiac myocyte death after prenatal cocaine exposure. Minocycline with its proven clinical safety and its ability to cross placental barrier and enter into the fetal circulation may become an effective therapy for preventing cardiac consequences of in utero cocaine exposure.
IUGR ALTERS HEPATIC mRNA LEVELS OF HMGA1, RBPF, AND INSR IN A GENDER SPECIFIC MANNER IN D120 RATS

Burshears B, Hale MA, Fu Q, Yu X, Callaway C, McKnight R, Joss-Moore L, Lane RH University of Utah, Salt Lake City, UT.

**Purpose of Study:** IUGR individuals are at higher risk for adult-onset metabolic disturbances, such as insulin resistance (IR). Furthermore, IUGR induced by uteroplacental insufficiency in rats has been previously characterized by increased adiposity and IR, with males being more severely affected than females. High mobility group A1 (HMGA1), an architectural transcription factor, contributes to or regulates the development of IR. HMGA1 alters transcription of the insulin receptor gene (INSR) and retinoic binding protein 4 (RBPF), genes involved with IR. Hepatic expression of HMGA1 and INSR is local, while liver secretes RBPF. It is unknown what affect IUGR has on expression of HMGA1, INSR, and RBPF in rat liver. We therefore hypothesized that IUGR would decrease the expression of HMGA1 and INSR in the rat liver. Additionally, we hypothesized that RBPF would be increased in IUGR.

**Methods Used:** IUGR was generated by bilateral uterine artery ligation at term (e21.5) or Day 0 (D0), D21, and D120. mRNA was isolated. cDNA was synthesized. RT-PCR was used to analyze HMGA1, INSR, and RBPF.

**Summary of Results:** IUGR did not alter mRNA levels of HMGA1, INSR, and RBPF at D7 when compared to control. However, At D21, IUGR significantly decreased levels of RBPF mRNA in males (p<0.05) but not in females. IUGR increased RBPF mRNA levels in D120 liver in males, but not females. Moreover, female D120 mRNA levels of HMGA1 and INSR in liver were increased when compared to control (p<0.05).

**Conclusions:** IUGR increased mRNA levels of HMGA1, INSR, and RBPF at D120 in a gender specific manner. Recent studies have shown that increased adiposity correlates positively with RBPF levels in males, possibly through increased hepatic production. Therefore, we speculate that decreased RBPF levels in D21 males and increased RBPF levels in D120 males may represent the change in adiposity seen in our IUGR model over time.

A BIOASSAY OF INSULIN BIOACTIVITY USING HEPG2 CELLS IN CULTURE

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**Purpose of Study:** Insulin is known to increase glucose phosphorylation and glycogen synthesis in hepatocytes. The purpose of this experiment was to develop a bioassay to examine the dose dependent effects of insulin on the metabolism of glucose using HepG2 cells.

**Methods Used:** HepG2 cells were grown to a density of 25 × 10^6 cells/mL, and were incubated in medium containing 100 mg/dL glucose, 10 mM 50%-enriched U-13C3 lactate, and 1 mM sodium pyruvate for 4 hours. Three sets of conditions were tested: (A) no insulin, (B) low dose insulin, and (C) high dose insulin. Medium glucose and cell glycogen quantitation were carried out by GC/MS analysis with U-13C6 glucose as the internal standard for the calculation of glucose uptake and glycogen synthesis. Contribution from glycogen synthesis was assessed by determining 13C isotopomer in glycogen and medium glucose.

**Summary of Results:** Insulin stimulated glucose uptake in a dosedependent manner. By contrast, glucose release as determined by the increase in 13C label in medium glucose was decreased with increasing insulin concentrations. Glycogen production was increased with higher insulin concentrations. However, the fraction of labeled glucose in glycogen did not change suggesting the relative contribution from glucose uptake and glycogen synthesis to glycogen synthesis was not affected by insulin. Increasing insulin concentrations also resulted in increased synthesis of fatty acids.

**Conclusions:** Consistent with the known metabolic effects of insulin on glucose metabolism, we have shown that increasing concentrations of insulin are associated with increased glucose uptake, increased glycogen production, and decreased glucose release in HepG2 cells. The new assay system gives additional insight into the role of insulin in the regulation of glucose release and glycogen synthesis.

MEASUREMENT OF MOLECULAR DETERMINANTS OF BODY WEIGHT REGULATION IN THE HUMAN BRAIN USING MAGNETIC RESONANCE SPECTROSCOPY

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**Purpose of Study:** Body weight is regulated such that alterations in bodyfat stores trigger compensatory changes in appetite and energy expenditure that resist weight change. Increasing evidence from animal models demonstrates that hormonal and nutrient-related signals interact in key brain areas, such as the hypothalamus, to regulate energy balance. For example, under the influence of insulin and leptin, increases in intracerebral long-chain fatty acyl-CoA (FACoA) lipids in hypothalamic neurons are hypothesized to convey a state of energy abundance, thereby decreasing food intake. The relevance of such pathways in the human brain is unknown because these molecules have not been measured. The goal of our project is to measure FACoA in the human brain using proton magnetic resonance spectroscopy (MRS).

**Methods Used:** 13 lean and obese healthy men and women will be studied at baseline and following an intravenous injection of glucose and insulin to stimulate FACoA. Serial hypothalamic MRS acquisitions will be obtained over 1 hour to capture the FACoA peak. Fasting blood samples for glucose, free fatty acids, insulin, leptin, and adiponectin will be obtained, as well as insulin, glucose, and free fatty acids during the 1-h post-infusion period to model the acute insulin response to glucose. We will determine whether changes in peripheral biomarkers correlate with FACoA concentrations, and whether these differ between lean and obese.

**Summary of Results:** Preliminary studies were completed to optimize spectral acquisition parameters in human subjects. Using water suppression (11x13x9 mm3 voxel) with 4.8 min spectral acquisition binning, we determined that decreased RBP4 levels in D21 males and increased RBP4 levels in D120 males may represent the change in adiposity seen in our IUGR model over time.

**Conclusions:** Preliminary analyses indicate no competing lipid peak exists in the region of interest for FACoA, and the voxel size and location does not limit our ability to measure metabolites of comparable concentration for stimulated FACoA.

**Metabolite** | **Chemical Shift (ppm)** | **Signal/Noise** | **Concentration (mM)**
--- | --- | --- | ---
N-Acetyl Aspartate | 2.018 | 8.1 | 0.024
Phosphocreatine/Creatine | 3.023 | 6.5 | 0.016
Choline | 3.216 | 8.4 | 0.016
Myo-Inositol | 3.565 | 6.4 | 0.019

Neonatology – Developmental Biology
Concurrent Session
8:00 AM Saturday, January 29, 2011

MESENCHYMAL-SPECIFIC DELETION OF APC DISRUPTS PARABRONCHIAL AND PERIVASCULAR SMOOTH MUSCLE CELLS DIFFERENTIATION

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**Purpose of Study:** During embryonic development, Mesenchymal cells that are programmed to become smooth muscle cell (SMC) elagante and express alpha-smooth muscle actin (alpha-SMA). Disregulation of SMC proliferation and differentiation leads to debilitating pulmonary diseases including pulmonary hypertension and asthma. The Wnt signaling pathway plays a key role in cell proliferation and cell fate determination during development. In the absence of a Wnt signal, Adenomatous Polyosis Coli (APC) forms a destruction complex and inhibits beta-catenin activity. APC also regulates cytoskeleton formation and controls cell migration. The role of APC on SMC development is unknown. The current study was designed to address this knowledge gap by examining the consequences of inactivating the Apc gene and stabilizing beta-catenin via a Cre-loxP approach, specifically in mesenchymal cells.
We used the Dermo1-cre driver line to induce recombination in Apc flox/flox mice. Double-transgenic Dermo1-cre; Apc flox/flox embryonic lungs were isolated to determine different stages of development. The lungs were analyzed by real-time PCR, western blot and immunohistochemistry.

**Summary of Results:** Apc is expressed in normal developing lungs. Apc is co-localized with markers for smooth muscle and ciliated cells. Apc deletion in mesenchymal cells leads to embryonic lethality at E12.5. In mutant mesenchymal cells, immunofluorescent staining and western blot confirmed a significant decrease in Apc and a commensurate accumulation of beta-catenin. The Apc mutant lungs showed decreased parabronchial SMC differentiation. Gaps in the layer of alpha-SMA expressing cells surrounding blood vessels were also found in the mutant embryos. Conditional stabilization of beta-catenin also generates a similar phenotype indicating that the effect of Apc deletion on SMC differentiation is mediated via the Wnt pathway.

**Conclusions:** APC plays a vital role in mesenchymal cell lineages during late embryogenesis. Mesenchymal-specific deletion of Apc blocks parabronchial and perivascular SMC differentiation. The effect of Apc on the SMC development is mediated via the beta-catenin-dependent Wnt signaling.

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**374 EFFECTS OF ERYTHROPOIETIN AND DARBEPOETIN ON GROWTH AND DIFFERENTIATION OF CULTURED HUMAN FETAL CARDIAC MYOCYTES**

Stewart EM, Maconaghy S, Ohls R University of New Mexico, Albuquerque, NM.

**Purpose of Study:** Erythropoietin (Epo) is a regulator of differentiation of erythroid progenitor cells. In myocardioc tissue, Epo, through its interaction with its receptor, reduces tissue ischemia by inhibiting apoptosis, dampening of inflammatory response, and stimulation of angiogenesis. We evaluated the effects of Epo and darbepoetin (Darbe), a long acting erythropoiesis stimulating agent (ESA), on cell growth, gene expression, and histological characterization of fetal cardiac myocytes between 10 and 18 weeks gestation. We hypothesized that Epo receptor (EpoR) expression would increase with gestational age, cell counts would increase with increasing ESA concentrations, and phenotype would not be altered by ESA exposure.

**Methods Used:** Primary myocyte cultures were grown following hand suspension of 10-18 week gestational age (GA) human fetal ventricular cardiac tissue. Cells were cultured at 106 cells/ml for 5 to 7 days in culture medium containing 0, 10, or 100 units/ml Epo or in 1, 10, or 1000 ng/ml Darbe. Cells were identified histologically and cell counts performed. Myocyte phenotype was confirmed by positive troponin staining using fluorescent microscopy, and myocyte progenitors were determined using CD133, a glycoprotein expressed in cardiac progenitor cells. Total RNA was isolated and quantified. RNA was reverse transcribed and quantitative PCR performed using primers and probes to identify EpoR gene expression. GAPDH was used as an internal control in duplex reactions.

**Summary of Results:** All cells stained positive for troponin, demonstrating a myocyte phenotype. An inverse relationship was observed between the number of cells with CD133 staining and increasing gestational age. There was no significant difference in cell growth between cells grown in maximum concentrations of Epo or Darbe compared to controls, and there was no change in phenotype with ESA exposure. Epo receptor expression increased with increasing ESA concentrations.

**Conclusions:** The percentage of progenitor cells in primary cultures of human fetal myocytes decreased significantly with increased GA. Cells in culture increased Epo receptor expression with increasing concentrations of Epo or Darbe. Unlike other fetal tissue types, ESAs do not appear to be mitogenic to fetal myocytes.

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**375 IUGR ALTERS MRNA LEVELS OF CHOLINERGIC AND GABAERGIC RECEPTORS IN A DEVELOMENTALLY-SPECIFIC MANNER**

Kunkel MD1, Wang Y2, Callaway C3, Yu X4, McKnight R2, Joss-Moore L2, Lane RH2 University of Utah, Salt Lake City, UT and 2University of Utah, Salt Lake City, UT.

**Purpose of Study:** Intratracheal growth restriction (IUGR) causes abnormal lung development leading to increased risk of impaired lung function in adulthood. This is true in both humans and rats. In rat lungs, IUGR increases mesenchymal thickness, which is associated with decreased apoptosis. Apoptosis, morphology, and cell proliferation are affected by cholinergic and GABAergic autocrine and paracrine signaling systems. Nicotinic acetylcholine receptors (nAChR) and GABA receptors (GABAR) are expressed in the lung. Specifically, the nAChR α7 and α4 subunits and the GABAR subunits β2 and β3 are important in the lung. Despite the importance of acetylcholine and GABA signaling in lung morphology and apoptosis, little is known about the effect of IUGR on these cell signaling systems. We hypothesize that IUGR will decrease mRNA levels of nicotinic acetylcholine (α4 and α7) and GABA (β3) receptors in the rat lung at birth (pre-alveolarization), and that this decrease will persist through day 21 (post-alveolarization).

**Methods Used:** IUGR (induced by bilateral uterine artery ligation) and control rat lungs were harvested at birth, day 7, and day 21. Real-time RT-PCR was used to measure the mRNA levels of nAChR subunits α4 and α7 and GABAR subunits β2 and β3.

**Summary of Results:** Results are IUGR as a % of control. At birth, IUGR significantly decreased mRNA levels of nAChR α4 (73%*) and α7 (59%*) relative to control; mRNA levels of GABAR β3 trended lower (79%, p=0.094) in IUGR rats. At day 21, IUGR significantly increased the expression of nAChR α4 (146%); the expression of nAChR α7 (128%, p=0.099) and GABAR β3 (126%, p=0.078) trended higher in IUGR rats. p<0.001.

**Conclusions:** We conclude that IUGR affects cholinergic receptors differentially before and after alveolarization. Specifically, IUGR decreases mRNA levels of two types of nAChR at birth, prior to the onset of alveolarization. We speculate that the protein levels of both nicotinic acetylcholine receptors will be similarly affected. Interestingly, mice with a knock-out α7 nAChR gene exhibit increased cellular hyperplasia. Therefore, we further speculate that the decreased expression of the cholinergic receptors play an important role in the changes in morphology and apoptosis seen in IUGR.

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**376 MATERNAL TOBACCO SMOKE EXPOSURE ALTERS RAT LUNG PPARγ AND SETD8 ABUNDANCE IN A GENDER-SPECIFIC MANNER**


**Purpose of Study:** Erythropoietin (Epo) is a regulator of differentiation of erythroid progenitor cells. In myocardial tissue, Epo, through its interaction with its receptor, reduces tissue ischemia by inhibiting apoptosis, dampening of inflammatory response, and stimulation of angiogenesis. We evaluated the effects of Epo and darbepoetin (Darbe), a long acting erythropoiesis stimulating agent (ESA), on cell growth, gene expression, and histological characterization of fetal cardiac myocytes between 10 and 18 weeks gestation. We hypothesized that Epo receptor (EpoR) expression would increase with gestational age, cell counts would increase with increasing ESA concentrations, and phenotype would not be altered by ESA exposure.

**Methods Used:** Primary myocyte cultures were grown following hand suspension of 10-18 week gestational age (GA) human fetal ventricular cardiac tissue. Cells were cultured at 106 cells/ml for 5 to 7 days in culture medium containing 0, 10, or 100 units/ml Epo or in 1, 10, or 1000 ng/ml Darbe. Cells were identified histologically and cell counts performed. Myocyte phenotype was confirmed by positive troponin staining using fluorescent microscopy, and myocyte progenitors were determined using CD133, a glycoprotein expressed in cardiac progenitor cells. Total RNA was isolated and quantified. RNA was reverse transcribed and quantitative PCR performed using primers and probes to identify EpoR gene expression. GAPDH was used as an internal control in duplex reactions.

**Summary of Results:** All cells stained positive for troponin, demonstrating a myocyte phenotype. An inverse relationship was observed between the number of cells with CD133 staining and increasing gestational age. There was no significant difference in cell growth between cells grown in maximum concentrations of Epo or Darbe compared to controls, and there was no change in phenotype with ESA exposure. Epo receptor expression increased with increasing ESA concentrations.

**Conclusions:** The percentage of progenitor cells in primary cultures of human fetal myocytes decreased significantly with increased GA. Cells in culture increased Epo receptor expression with increasing concentrations of Epo or Darbe. Unlike other fetal tissue types, ESAs do not appear to be mitogenic to fetal myocytes.

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**377 IUGR ALTERS MRNA LEVELS OF CHOLINERGIC AND GABAERGIC RECEPTORS IN A DEVELOPMENTALLY-SPECIFIC MANNER**

Kunkel MD1, Wang Y2, Callaway C3, Yu X4, McKnight R2, Joss-Moore L2, Lane RH2 University of Utah, Salt Lake City, UT and 2University of Utah, Salt Lake City, UT.

**Purpose of Study:** Intratracheal growth restriction (IUGR) causes abnormal lung development leading to increased risk of impaired lung function in adulthood. This is true in both humans and rats. In rat lungs, IUGR increases mesenchymal thickness, which is associated with decreased apoptosis. Apoptosis, morphology, and cell proliferation are affected by cholinergic and GABAergic autocrine and paracrine signaling systems. Nicotinic acetylcholine receptors (nAChR) and GABA receptors (GABAR) are expressed in the lung. Specifically, the nAChR α7 and α4 subunits and the GABAR subunits β2 and β3 are important in the lung. Despite the importance of acetylcholine and GABA signaling in lung morphology and apoptosis, little is known about the effect of IUGR on these cell signaling systems. We hypothesize that IUGR will decrease mRNA levels of nicotinic acetylcholine (α4 and α7) and GABA (β3) receptors in the rat lung at birth (pre-alveolarization), and that this decrease will persist through day 21 (post-alveolarization).

**Methods Used:** IUGR (induced by bilateral uterine artery ligation) and control rat lungs were harvested at birth, day 7, and day 21. Real-time RT-PCR was used to measure the mRNA levels of nAChR subunits α4 and α7 and GABAR subunits β2 and β3.

**Summary of Results:** Results are IUGR as a % of control. At birth, IUGR significantly decreased mRNA levels of nAChR α4 (73%*) and α7 (59%*) relative to control; mRNA levels of GABAR β3 trended lower (79%, p=0.094) in IUGR rats. At day 21, IUGR significantly increased the expression of nAChR α4 (146%); the expression of nAChR α7 (128%, p=0.099) and GABAR β3 (126%, p=0.078) trended higher in IUGR rats. p<0.001.

**Conclusions:** We conclude that IUGR affects cholinergic receptors differentially before and after alveolarization. Specifically, IUGR decreases mRNA levels of two types of nAChR at birth, prior to the onset of alveolarization. We speculate that the protein levels of both nicotinic acetylcholine receptors will be similarly affected. Interestingly, mice with a knock-out α7 nAChR gene exhibit increased cellular hyperplasia. Therefore, we further speculate that the decreased expression of the cholinergic receptors play an important role in the changes in morphology and apoptosis seen in IUGR.
MECHANICAL TACTILE STIMULATION IN A NEONATAL STRESS MODEL ALTERS DEPOT-SPECIFIC ADIPOSE TISSUE EXPRESSION OF TNF-ALPHA AND IL-6 OF ADULT RATS

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Purpose of Study: Preterm hospitalized infants experience numerous to stressful events. Early life stress is associated with greater fat mass and preferential visceral adipose tissue (VAT) deposition. VAT produced inflammatory cytokines, TNF-alpha and IL-6, are linked to obesity and insulin resistance. Infant massage has been shown to decrease stress. Using mechanical-tactile stimulation (MTS, a surrogate for infant massage), we tested the hypothesis that MTS administered during neonatal stress would reduce VAT depot mRNA expression of TNF-alpha and IL-6.

Methods Used: Timed pregnant dams were delivered at term (E21), litters culled to 10 pups (5 M, 5 F), and randomized to: control (CTL; 60 min maternal separation), neonatal stress (NS; maternal separation + injection + hypoxia/hyperoxia) and NS + MTS (10 min of stroking and limb movement). Treatments were given from D5-9 with tissue harvested on D21 and D120. Subcutaneous (SAT) VAT depots were detected by MRI. Serum TNF-alpha and IL-6 levels were measured with ELISA with VAT/SAT depot TNF-alpha and IL-6 mRNA levels by real-time PCR.

Summary of Results: At weaning (D21) body weight and SAT/VAT depots were similar. In adults (D120), MTS decreased VAT depot versus NS or CTL (p<0.04); body weight and SAT depot were not different. At weaning VAT depot IL-6 and TNF-alpha expression was higher in males than females (p<0.05). In adults, VAT depot TNF-alpha mRNA levels were higher in NS than CTL (p<0.02) with higher IL-6 mRNA expression in both NS and MTS compared to CTL (p<0.05). SAT depot TNF-alpha and IL-6 mRNA expression and serum TNF-alpha and IL-6 levels were similar at D21 and D120.

Conclusions: MTS administered during neonatal stress decreased adult VAT deposition and VAT TNF-alpha mRNA expression. Neonatal stress elevated VAT depot expression for both IL-6 and TNF-alpha mRNA in adult rats. VAT depot TNF-alpha expression is associated with obesity and reduced insulin sensitivity. We speculate that MTS attenuates early life stress and promotes age specific changes to signaling pathways affecting adipose tissue deposition and inflammatory cytokine expression.

INTRAUTERINE GROWTH RESTRICTION ALTERS EXPRESSION OF PPARγ CO-REPRESSORS: Ncor1, SMRT, AND SIRT1 IN JUVENILE MALE RAT ADIPOSE


Purpose of Study: Intrauterine growth restriction (IUGR) predisposes individuals to adult onset obesity. In a well characterized rat model of IUGR, we demonstrated altered adipogenesis in juvenile males with increased visceral adiposity and increased expression of peroxisome proliferator activated receptor gamma (PPARγ). PPARγ is a key regulator in adipogenesis. PPARγ activity is inhibited by the binding of a co-repressor complex composed of nuclear receptor co-repressor 1 (Ncor1), silencing mediator of retinoic acid and thyroid receptor hormone (SMRT), and NAD+ dependent deacetylase SIRT1. Increased expression of PPARγ causes adipogenesis and weight gain; however, the expression of Ncor1, SMRT, and SIRT1 in response to altered adipogenesis in the setting of IUGR remains unknown. We hypothesize that IUGR will alter mRNA and protein levels of the co-repressor complex components, Ncor1, SMRT, and SIRT1, in the visceral adipose tissue of juvenile male rats.

Methods Used: To test this hypothesis we used a well characterized UPI induced IUGR rat model. Expression of Ncor1, SMRT, and SIRT1 protein and mRNA were measured in subcutaneous (SAT) and visceral adipose tissue (VAT) at day 7 and day 21.

Summary of Results: In VAT, IUGR increases mRNA levels of SMRT in day 7 and day 21 male rats (224±72* and 234±14*) and Ncor1 in day 21 male rats (227±32*) when compared to controls. IUGR increases SIRT1 protein levels of day 21 male rats (321±69*) with no change in SAT IUGR mRNA levels. In SAT, IUGR increases mRNA levels of Ncor1 and SMRT in day 7 male rats (164±20* and 180±12*) and SIRT1 in day 7 female rats (114±10*) when compared to controls. IUGR decreases SIRT1 protein levels of day 21 female rats (47±10*). (*p<0.05).

Conclusions: IUGR increases mRNA levels of NcoR1, SMRT and alters protein levels of SIRT1, in an age, gender, and adipose depot specific manner. In metabolic models, gender variation is a persistent observation and males are often more affected than females. We speculate that this increase in co-repressor complex expression in IUGR male rats is an appropriate response to their increased PPARγ expression; however, the complex is ineffective in inhibiting PPARγ activity.

TISSUE SPECIFIC ROLE OF DNA METHYLTRANSFERASE 4 DURING ZEBRAFISH DEVELOPMENT

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Purpose of Study: DNA methylation is a heritable epigenetic marker. The mechanism by which DNA methylation and gene expression are regulated during embryonic development is an area of active research. The zebrafish presents a unique model which is well suited to investigate mechanisms of normal embryonic development because of its easily observed rapid embryonic development and availability of techniques to manipulate gene expression. Recent research has started to establish tissue specific roles for the various DNA methyltransferases (DNMTs) during development. These studies have also shed some light on the basic mechanisms by which DNMTs regulate expression of targeted genes. DNMT3 is one of the three groups of DNMTs found in humans and it has six known zebrafish orthologs: Dnmt3a/4/5/6/7/8. The focus of this project was to investigate the role of zebrafish dnmt4 in normal development. Whole mount in situ hybridization assays revealed that dnmt4 is robustly expressed in hematopoietic compartments as well as the site of hematopoietic stem cell generation. We hypothesized that dnmt4 controls zebrafish hematopoiesis by regulating the transcription of hematopoietic genes.

Methods Used: We took a candidate approach and investigated whether the knockdown of dnmt4 caused transcriptional up regulation of aid, mbd4 and gadd45a. These targets were chosen based on recent findings both in published studies and our lab’s unpublished work that indicates they play a role in hematopoiesis. Antisense morpholino knockdown technology was used to create zebrafish embryos with substantially decreased dnmt4 expression. RNA was isolated from whole fish samples and then CDNA was created. RT-PCR was conducted to test the relative expression normalized to 28S of the target genes.

Summary of Results: Expression of our target genes was found to be two to three times higher in dnmt4 morphants than in controls.

Conclusions: Taken together these data provide evidence that dnmt4 plays a role in hematopoietic differentiation and establishes a possible tissue specific role for dnmt4 during embryonic development. Future studies will be aimed at further characterizing the hematopoietic phenotype of dnmt4 morphants and verifying that changes in gene expression correlate with tissue specific changes in DNA methylation of target genes.

MECHANICAL-TACTILE STIMULATION DURING NEONATAL STRESS DECREASES VISCERAL ADIPOSE TISSUE AND PREVENTS HYPERINSULINEMIA IN ADULT RATS


Purpose of Study: Preterm infants are continually challenged by physiologic and environmental stressors in early life. Early life stress is associated with greater abdominal visceral adipose tissue (VAT). Increased VAT is linked to adult metabolic consequences such as glucose intolerance and insulin resistance independent of obesity. Infant massage decreases stress biomarkers. Using mechanical-tactile stimulation (MTS) as a surrogate for infant massage, we tested the hypothesis that MTS administered to rat pups during neonatal stress would decrease VAT depot and improve circulating levels of glucose, insulin, and the adipokines leptin and adiponectin.

Methods Used: Timed pregnant dams were delivered at term (E21). Litters were culled to 10 pups (5 M, 5 F) and divided into 3 groups: naïve control (CTL), neonatal stress (NS; maternal separation + injection + hypoxia/hyperoxia) and NS + MTS (10 min of tactile stimulation and limb movement). Treatments...
381 INTRAUTERINE GROWTH RESTRICTION BY UTEROPLACENTAL INSUFFICIENCY AND MATERNAL TOBACCO SMOKING EXPOSURE CAUSES GENDER-SPECIFIC CHANGES IN MRNA EXPRESSION OF HIPPOCAMPAL ESTROGEN RECEPTOR


Purpose of Study: 2 major causes for intrauterine growth restriction (IUGR) in the United States are uteroplacental insufficiency (UPI) and maternal tobacco smoking exposure (MTSE). Both UPI and MTSE impacts neurodevelopmental outcomes in offspring born with IUGR, particularly in males. A common characteristic of IUGR animal models is increased hippocampal apoptosis. Neuronal apoptosis is modulated by estrogen signaling - estrogen is anti-apoptotic in the hippocampus. Estrogen mediates its effects on hippocampal apoptosis through estrogen receptors (ER), which are encoded by the genes esr1 and esr2. Currently, little is known about the effects of IUGR by either UPI or MTSE on hippocampal expression of ERs.

Methods Used: IUGR was induced by 2 methods in 2 separate experimental groups: (1) uterine ligation in pregnant rats at day 19 of gestation to mimic UPI, and (2) exposure of pregnant rats to tobacco smoke from day 11 of gestation to term to mimic MTSE. Both groups underwent caesarean section at term. The pups were killed, hippocampi were dissected and RNA extracted. Reverse transcriptase real-time PCR was performed to assay expression of esr1 and esr2.

Summary of Results: UPI decreased esr1 (51% reduction, p=0.01) and esr2 (55% reduction, p=0.02) expression in male pups compared to controls. UPI did not alter esr1 or esr2 expression in female pups. In contrast, MTSE increased esr2 expression (123% increase, p=0.05) in female pups compared to controls, but no changes were seen in males. MTSE did not alter esr1 expression in either gender.

Conclusions: We conclude that the consequence of UPI and MTSE on hippocampal ER mRNA expression is different, despite both UPI and MTSE causing IUGR. The mechanisms behind MTSE induced IUGR is unknown, but the impact is likely through MTSE induced fetal exposure to UPI, nicotine, and/or some other compounds. Given the different observed effects of MTSE and UPI on hippocampal ER gene expression, we speculate that mechanisms through which MTSE affects ER expression occur through exposure to nicotine and/or some other compounds.

382 MATERNAL SMOKE EXPOSURE DURING PREGNANCY DECREASES RAT HEPATIC IGF-1 mRNA VARIANT LEVELS IN A GENDER SPECIFIC MANNER


Purpose of Study: Maternal cigarette smoking during pregnancy causes intrauterine growth restriction (IUGR). IUGR is the result of fetal adaptation to a suboptimal in-utero environment. Insulin like growth factor 1 (IGF-1) plays a key role in fetal growth and serum levels are directly correlated to birthweight. Serum IGF-1 levels are controlled by hepatic IGF-1 gene expression. IGF-1 gene expression is characterized by multiple mRNA variants, which affect IGF-1 translation efficiency via multiple promoters and alternative splicing. The IGF-1 gene and mRNA variants are highly conserved among mammals. IUGR affects IGF-1 in the setting of perinatal malnutrition with males more affected than females, however, the effects of IUGR from maternal cigarette smoke exposure on hepatic IGF-1 mRNA levels remain unknown. We hypothesize that maternal cigarette smoke exposure (MS) during pregnancy will decrease hepatic IGF-1 mRNA variant level (IGF-1A, IGF-1B, P1, and P2) in the newborn offspring, with males more affected than females.

Methods Used: Cigarette smoking was initiated on day 11 of gestation and continued to day 21 when rat pups were delivered via c-section. Liver harvested at day 0 and mRNA quantified via real time RT-PCR.

Summary of Results: MSE during pregnancy decreased IGF-1 mRNA variants P1 and IGF-1A in day 0 females (62.5%* and 73%***) when compared to controls. MSE during pregnancy decreased IGF-1 mRNA variant P1 in day 0 males (70%*** when compared to controls. (*p<0.05).

Conclusions: We conclude that MSE during pregnancy decreases IGF-1 mRNA variant levels similarly in both genders. Gender variation is a persistent observation in metabolic models, and males are often more affected than females. Uteroplacental insufficiency (UPI) IUGR showed an ongoing decrease in males IGF-1 expression into adolescence. Thus far, MSE IGF-1 mRNA variant levels are very similar to findings in the UPI IUGR model. Thus, we speculate the primary fetal effects of MSE are due to placental insufficiency as opposed to tissue exposure.

383 INTRAUTERINE GROWTH RESTRICTION ALTERS CARDIAC GENE mRNA LEVELS

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Purpose of Study: Intrauterine growth restriction (IUGR) predisposes individuals to cardiovascular disease in adulthood that results in cardiac hypertrophy. IUGR also causes cardiac hypertrophy in adult rats. Several genes are specifically expressed during heart development that are abnormally upregulated in cardiac hypertrophy including ANP, BNP, HCN4, NR2B, and α-skeletal actin. These genes are all partially regulated by a common master regulatory complex. However, the effect of IUGR on expression of these genes is unknown. We hypothesize that IUGR will increase mRNA levels of the genes that code for ANP, BNP, HCN4, NR2B and α-skeletal actin in rat hearts.

Methods Used: Bilateral uterine artery ligation surgery was performed on rat dams at E19. Rat pup hearts were harvested at term (D0) and D21. mRNA was isolated and cDNA was produced. Real-time RT-PCR was performed to assess mRNA levels of Nppa gene (that encodes ANP), Nppb (BNP), HCN4, Grin2b, (NR2B) and Acta1 (α-skeletal actin).

Summary of Results: Results are presented as IUGR as % of Control ± SEM. IUGR increased mRNA levels of Nppa(306±20%***), HCN4 (167±14%*), and Grin2b (187±19%*) in D21 males. IUGR also increased mRNA levels of HCN4 (150±12%**) in D21 females. IUGR decreased mRNA levels of Acta1 (75%**) in D21 males. No differences were seen in Nppb mRNA levels or in any D0 animals. *p<0.05, **p<0.01, ***p<0.001.

Conclusions: We conclude that IUGR increases mRNA levels of Nppa, HCN4 and Grin2b in male rats, but only HCN4 in female rats. Contrary to our hypothesis, IUGR decreased Acta1 mRNA levels in male rats. Gene specific binding with the master transcriptional repressor complex, REST (repressor element 1-silencing transcriptional factor), may account for this variability. REST binding involves variable co-repressors that alternately cause transcriptional expression or repression in response to different stimuli. We speculate that IUGR will increase protein levels of ANP, HCN4, and Grin2b in the rat heart. We also speculate that IUGR will alter REST expression and binding.

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HEMODYNAMIC RESPONSES TO MODERATE HYPEROXIA ABUNDANCE IN NEONATAL RAT LUNG EXPOSED TO MODERATE HYPEROXIA


Purpose of Study: Maternal tobacco smoke exposure (MTSE) is detrimental to fetal brain development. In humans and animal models, MTSE predisposes to postnatal learning disabilities with effects persistent throughout the life span. Changes in gene expression due to changes in epigenetic markers are believed to be responsible for the persistence of a phenotype long after an initial insult. Despite the known detrimental effect of MTSE on the developing brain, it is unknown how MTSE affects expression of genes involved in hippocampal development and learning processes. We hypothesize that in the hippocampi of d0 rats, MTSE alters the mRNA expression of genes involved in hippocampal development, such as insulin like growth factor 1 (IGF1), NMDA receptors (NMDA-NR1) and glucocorticoid receptor (GR) pathways and variants.

Methods Used: To test our hypothesis we used hippocampal tissue from Sprague-Dawley rats exposed to cigarette smoke from day 11 of gestation until term and control rats (n = 12/group). We quantified hippocampal mRNA expression of genes and gene variants of IGF1, NMDA receptors and GR pathways.

Summary of Results: Our most important findings are: In d0 hippocampal mRNA expression, total IGF1 variant levels in females and increased IGF1 mRNA to (to 200-350% of control levels) in males. 2) increased mRNA expression of NMDA-NR1 and variants (to 250-700% of control levels) in females and decreased mRNA of total NMDA-NR1 in males (to 22% of control levels). 3) decreased mRNA of GR in males (to 40% of control levels) while no change was seen in females.

Conclusions: Two conclusions arise from this data. First, the fetal hippocampal mRNA levels are exceptionally sensitive to MTSE and the effects are gene specific. Most of the genes studied present with changes in mRNA expression following MTSE. Second, the fetal hippocampal response to MTSE is gender specific. We speculate that upon further testing, we will continue to identify gene and gender specific differences in hippocampal protein expression and epigenetic markers, associated with gender specific behavioral changes later in life.

Neonatal – Pulmonary II
Concurrent Session
8:30 AM
Saturday, January 29, 2011

DECREASED PULMONARY VESSEL DENSITY AND PULMONARY ARTERY ENDOTHELIAL CELL FUNCTION IN FETAL SHEEP WITH INTRAUTERINE GROWTH RESTRICTION


Purpose of Study: Intrauterine growth restriction (IUGR) increases the risk for neonatal respiratory disease including bronchopulmonary dysplasia and associated pulmonary vascular dysfunction. Abnormal lung growth and structure have been noted in animal models of IUGR but whether IUGR adversely impacts fetal pulmonary vascular development and pulmonary artery endothelial cell (PAEC) function is unknown. Therefore, we hypothesized that fetal pulmonary alveolarization and vascular growth are decreased in experimental IUGR and that PAECs from IUGR animals have abnormalities of growth and tube formation in vitro.

Methods Used: Studies were performed in an established model of placental insufficiency and IUGR induced by exposing pregnant ewes to elevated temperatures (day 37–120; term 148). Late gestation alveolarization was quantified by radial alveolar counts (RAC) and pulmonary vessel density by staining for von Willebrand Factor. Proximal PAECs were isolated by enzymatic digestion and cultured by standard methods. Cell culture was tested between passages 4–6 by measuring growth and tube formation assays in the presence or absence of insulin (50–200 nmol/L) or rhVEGFA (50ng/ml).

Summary of Results: RACs were decreased 20% (p<0.001) and pulmonary vessel density was decreased 44% in IUGR (5.3±0.6 vs. 9.5±0.9 vessels/mm², P<0.01). In normal PAECs, tube formation as assessed by branch point counting was increased to a maximum of 39% in a dose response fashion with insulin treatment (p<0.0001) and by 48% with VEGFA treatment (p<0.0001). In comparison with normal PAECs, tube formation by IUGR PAECs at baseline was reduced by 34% and was not responsive to treatment with insulin or VEGFA (p<0.0001). In normal PAECs, insulin increased cell growth to a maximum of 68% in a dose response fashion (p<0.01). Cell growth was reduced in IUGR PAECs by 29% at baseline (p<0.05) and the response to insulin was also attenuated (p<0.01). Conclusions: Alveolarization, lung vasculature, and in vitro PAEC function are impaired in IUGR fetuses. We speculate that the pulmonary circulation of IUGR fetuses is characterized by endothelial dysfunction, which may contribute to abnormal growth, development, and the increased risk for chronic lung disease and bronchopulmonary dysplasia in IUGR.
888 A NOVEL MEANS FOR DELIVERING NASAL INTERMITTENT POSITIVE PRESSURE VENTILATION (NIPPV) IN INFANTS VIA THE NASAL CANNULA (NC): MEASUREMENTS OF DELIVERED PARAMETERS

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Purpose of Study: Nasal ventilation using NIPPV is on the rise in preterm infants to decrease extubation failures, bronchopulmonary dysplasia, and for the treatment of apnea of prematurity. NIPPV is typically applied using continuous positive airway pressure (CPAP) bi-nasal short prongs which can result in nasal trauma. Nasal Cannula Intermittent Mandatory Ventilation (NC-IMV) is a novel means of delivering pressure controlled NIPPV breaths noninvasively to neonates requiring respiratory support. We previously reported that NC-IMV is feasible and well tolerated in a large number of neonates. However, pressures/volume delivered to the patient is not known. Our goal is to determine the magnitude of pressure, volume, and positive end-expiratory pressure (PEEP) delivered to an infant nasal airway/lung model using different sized NC at different peak inspiratory pressure (PIP) settings during time-cycled, pressure-limited mode.

Methods Used: We configured a neonatal test lung to simulate an apneic premature infant with compliance of 0.8 mL/cmH2O, resistance 75 cmH2O/L/sec. An infant nasal airway model was attached to the test lung. The ventilator set in IMV mode, rate 40 breaths/min, inspiratory time 0.5 s, Flow 7–9 L/min, and PEEP 5 cmH2O. The nasal airway was ventilated at PIP of 10, 15, 20, 25, and 30 cmH2O using infant and intermediate high-flow NC (Fisher Paykel, Auckland, NZ) and a new prototype NC (NeoTech Nasal Cannula®). Pressure, volume, and PEEP were measured in the test lung.

Summary of Results: Under all testing conditions, there was detectable pressure, volume and PEEP during NC-IMV. There was a linear relationship between the PIP applied by the ventilator and volume up to 30 cmH2O. The NeoTech Nasal Cannula® provided greater pressure, volume, and PEEP than the other infant NC.

Conclusions: NC-IMV can provide a significant amount of a neonate’s volume/pressure requirements with less nasal trauma than CPAP prongs. Further studies are underway to evaluate the pressure/volume deliveries and reduction of nasal injury in spontaneously breathing neonates on NC-IMV support.

389 PROTEIN AND CATION CONCENTRATIONS IN THE EXHALED BREATH FLUID OF PREMATURE NEONATES

Morales EM Harbor UCLA, Torrance, CA.

Purpose of Study: Ventilator associated pneumonia (VAP) and pulmonary edema are common events in premature neonates. Airway fluid can be collected from these babies for diagnostic and investigative studies by lavaging the lungs with saline, but this involves some risk in terms of gas exchange and infection. Alternatively, respiratory fluid and solutes can be collected from filters in the expiratory tubing of the ventilator. This noninvasive approach can be repeated without endangering the child. We hypothesize that data obtained from expiratory collections can be used to estimate airway solute concentrations and eventually, early detection and quantification of pathogens in airway fluid.

Methods Used: Exhaled breath fluid (EBF) samples were collected from the expiratory filters of conventional ventilators from 4-6 hours of ventilation. The filters were washed with 3 mL of water and measurements were made of protein, Na+,NH4+, K+, Ca2+ and Mg2+ concentrations in these samples. (6 samples with disproportionately high Na+ levels due to saline lavage were excluded). Protein concentration was assayed with a modified colorimetric (BCA) assay. Cation concentrations were assayed by ion chromatography.

Summary of Results: Protein concentrations (mg/L) averaged 6.0: 6.2 (SD, n=10) EBF samples. Ion concentrations (micromol/L) averaged: Na+: 112.0±11.0, K+: 5.9±7.0, Ca2+: 11.8±11.4, Mg2+: 2.7±1.29, and NH4+: 2±1.94. It is assumed that the sum of Na+ and K+ in airway lining fluid (ALF) equals that in the plasma (assumed to be 150 mM/L), then dilution (D) of the EBF by water vapor, (released as a gas from airway surfaces and delivered by humidifier) averaged 2.35±2.140 (n=10). Protein concentrations in the ALF averaged 14±2.14 (estimated estimated from the product of average values of D and EBF filter concentrations). Protein concentrations measured after washing the airways with 3 mL of saline in 6 neonates averaged 1.8 g/L.

Conclusions: These observations suggest that the ALF is diluted by a factor of nearly 10 during conventional airway lavage. These studies suggest that measurements of solutes in EBF may provide a simple and safe approach for following abnormalities in the airway fluid.

[Supported partially by grants from the NIH (HL-55268, HL-075405, HD058948, and HD-051857) and the TRDRP (14RT-0073, 15IT-0250, 17RT-0170)].
M1-polarized AM did not correlate with the risk for BPD or death. Interestingly in a small number of Group 2 neonates M2-polarized AM increased as a function of postnatal age.

Conclusions: To our knowledge, this is the first study to analyze polarization of AM in the lungs of neonates at risk for BPD. The relationship between AM polarization and BPD appears complex. In the neonates examined, M1 polarization did not correlate with BPD risk. Additional studies are underway to elucidate the role of AM polarization in BPD.

391 CAFFEINE WORSENS INFLAMMATION AND ALVEOLAR HYPOPLASIA IN HYPEROXIA-EXPOSED NEWBORN MICE

Lopez B1, Maisonet TM1, Dayanur S1, Grewal S1, Aghai ZH1, Londhe VA1,2 UCLALos Angeles, CA and 3UMDNJ, Camden, NJ.

Purpose of Study: To determine the impact of daily caffeine administration on alveolar development in normoxia and hyperoxia. Caffeine therapy is a current mainstay of NICU management to treat apnea of prematurity. Although caffeine has also been shown to reduce the incidence of BPD, the precise mechanism of action remains poorly understood.

Methods Used: Newborn mice from timed-pregnant dams were divided into 4 experimental groups: 1) saline normoxia (FiO2 21%); 2) caffeine normoxia; 3) saline hyperoxia (FiO2 80%); and 4) caffeine hyperoxia. Starting at P1, pups were treated with daily i.p. injection of saline or caffeine (20 mg/kg load, 10 mg/kg daily caffeine citrate) under constant normoxia or hyperoxia conditions. Animals were sacrificed at P3 or P15 to collect BAL and lung tissue for mRNA, protein, and histological analysis.

Summary of Results: Caffeine-treated pups showed poorer weight gain compared to saline controls. At P3, caffeine-treated lungs showed increased neutrophil infiltration in normoxia (p = 0.013) and hyperoxia (p = 0.002). Lung histology at P3 also exhibited larger saccular airspaces in caffeine groups when compared to saline controls. At P15, morphometric analysis showed fewer alveoli in caffeine-treated pups exposed to hyperoxia (p = 0.006).

Conclusions: Daily caffeine exposure increases lung inflammation and inhibits alveolar development under normoxia and hyperoxia in newborn mice. Caffeine is known to inhibit adenosine receptor activation and thereby blocks down-regulation of inflammation in response to injury. Caffeine may also induce epigenetic changes that alter transcription of critical lung morpho-regulatory genes during alveolar development. Our findings suggest that caffeine may adversely impact alveolar development under hyperoxic conditions.

392 EXPRESSION OF CARCINOEMBRIONIC ANTIGEN CELL ADHESION MOLECULE 6 (CEACAM6) IN HEALTHY AND INJURED CEABAC TRANSGENIC MICE

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Purpose of Study: CEACAM6 is a glycosylated, GPI-anchored protein expressed in epithelial cells of many human tissues. It is overexpressed in Crohn’s disease, facilitating bacterial colonization, and in tumors where it is expressed in epithelial cells of many human tissues. It is overexpressed in Crohn’s disease, facilitating bacterial colonization, and in tumors where it is important for bacterial adherence, immune surveillance, and tumor metastasis.

Methods Used: Caffeine exposure increases lung inflammation and inhibits alveolar development under normoxia and hyperoxia in newborn mice. Caffeine is known to inhibit adenosine receptor activation and thereby blocks down-regulation of inflammation in response to injury. Caffeine may also induce epigenetic changes that alter transcription of critical lung morpho-regulatory genes during alveolar development. Our findings suggest that caffeine may adversely impact alveolar development under hyperoxic conditions.

393 CURCUMIN AUGMENTS NEONATAL LUNG INJURY/REPAIR BY INHIBITING TGF-ß SIGNALING

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Purpose of Study: There is no effective intervention to prevent or treat BPD. Curcumin has potent antioxidant and anti-inflammatory properties, and it modulates PPARγ signaling, a key molecule in the pathobiology of BPD. However, its role in the prevention of BPD is not known. The purpose of this study is to determine if curcumin i) enhances neonatal lung maturation; ii) protects against hyperoxia-induced neonatal lung injury; iii) protection is mediated by blocking TGF-ß.

Methods Used: Embryonic day (e) 19 fetal rat lung fibroblasts (FRLF) were exposed to 21% or 95% O2 for 24h following 1h pretreatment with curcumin. Cell proliferation (thyidine incorporation), differentiation (Western for para-thyroid hormone-related protein receptor, PPARY and adipoocyte differentiation-related protein, (ADRP); triolein uptake) and TGFß signaling (Western blot analysis and confocal immunofluorescent staining for Smad3) were determined. In a separate set of experiments, neonatal Sprague Dawley rat pups were categorized in the following groups: normoxia (21% O2), normoxia + curcumin (5 mg/kg BW), hyperoxia (7d exposure to 95% O2), and hyperoxia + curcumin (5 mg/kg BW). Curcumin was administered i.p. once daily. At the end of the experimental period, the pups were sacrificed and the lungs analyzed for markers of lung injury/repair (Western analysis and lung morphology).

Summary of Results: In vitro, curcumin dose-dependently accelerated e19 fibroblast differentiation (increased PThrP receptor, PPARY, and ADRP expression and triolein uptake) and proliferation (increased thymidine incorporation). Furthermore, pretreatment with curcumin blocked the hyperoxia-induced decrease (PPARY and ADRP) and increase (n-SMA and fibronectin) in markers of lung injury/repair. Similarly, in vivo hyperoxia-induced changes in molecular markers of lung injury repair [PTHrP receptor, PPARY, ADRP, fibronectin, TGF-ß receptor (ALK5) and Smad3 activation] and lung morphology (radial alveolar count and septal thickness) were effectively blocked in the curcumin treated group.

Conclusions: Curcumin accelerates lung maturation by stimulating key alveolar epithelial-mesenchymal interactions and blocks hyperoxia-induced neonatal lung injury by blocking TGF-ß activation, suggesting it as a potential intervention against BPD. (Grant support: NIH (HL-55268, HL-075405, HD058948, and HD-051857).

394 BDNF PROTEIN ABUNDANCE IN WHITE MATTER IS DECREASED IN PRETERM LAMBS MANAGED BY MECHANICAL VENTILATION COMPARED TO NASAL VENTILATION

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Purpose of Study: Prolonged mechanical ventilation (MV) of preterm neonates is associated with white matter loss and subsequent neurodevelopmental delays or deficiencies. The molecular basis of these co-morbidities is unknown.

Methods Used: Lung tissue and bronchoalveolar lavage (BAL) were collected from wild-type and heterozygous CEABAC mice for RNA and protein analysis. Large aggregate surfactant was isolated from BAL by centrifugation. CEACAM6 was measured by Western and immunodot blot assays and normalized to total protein. Lungs were inflation fixed for immunohistochemistry (IHC). Acute lung injury was induced by intratracheal injection of bleomycin.

Summary of Results: Expression of human CEACAM3, 5, 6, and 7 in the CEABAC mouse lung is comparable to that in the human premature infant lung. CEACAM6 is up-regulated with lung injury and may play a role in cell proliferation and/or surfactant stabilization under these conditions.

Conclusions: To conclude that expression of CEACAM6 in the CEABAC mouse lung is comparable to that in the human premature infant lung. CEACAM6 is up-regulated with lung injury and may play a role in cell proliferation and/or surfactant stabilization under these conditions.

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Preterm lambs (~131d gestation; term ~150d), treated with antenatal steroids and postnatal surfactant, were managed by MV or HFNV for 3d or 21d (n=4/group). Gestation references were for the time of preterm fetal delivery (F131) and term gestation (T). Temporal lobe white matter was analyzed by immunoblot (normalized using MensCode kit).

**Summary of Results:** BDNF protein relative abundance at 3d or 21d of MV was less than HFNV at the respective times (table; *p<0.05). Pro-BDNF protein abundance, by comparison, was greater in both groups of preterm lambs (table) as compared to T.

**Conclusions:** Preterm birth followed by prolonged ventilation affect the balance of BDNF and pro-BDNF protein abundance in white matter. This shifted balance may contribute to more apoptosis in white matter that occurs in the brain of chronically ventilated preterm lambs (J Invest Med 57:141, 2010). (HLs62875, HLs6401, HD41075).

395 MATERNAL FOOD RESTRICTION ASSOCIATED INCREASE IN LUNG ELASTIN IS DUE TO PROTEIN DEFICIENCY

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**Purpose of Study:** Using a rat model of 50% maternal food restriction (MFR) during gestation, we have reported altered “lung lipid-differentiation” programming, explaining the altered pulmonary structure and function in the affected offspring. Since lung fibroblasts are the key cells that drive lung lipid-differentiation program and synthesize elastin, a major matrix protein that determines the lung structure and function, we determined the effect of MFR restriction on lung elastin gene expression. We also aimed to determine the specific nutrient component, the deficiency of which leads to altered elastin expression in the MFR offspring lung.

**Methods Used:** Pregnant dams 10 days into pregnancy were either allowed ad lib feeds or given food-restricted diet, i.e., 50% of rat chow consumed by control dams. After delivery, the pups were sacrificed at postnatal day (PND) 21 and 9M, when lungs were examined for elastin gene expression by RT-PCR and Western blotting. To determine the specific nutrient deficiency that alters elastin gene expression, primary rat lung fibroblasts were isolated. At 80-90% confluence, the cells cultured in DMEM for 24h under the following conditions: No fetal bovine serum (FBS); 1, 5, or 10% FBS; No FBS+Vitamin D (VD, 10-6M); No FBS+ arachidonic acid (ARA, 10-6M)+doxohexanoic acid (DHA, 10-6M); or No FBS+1, 5, 10% fetal bovine albumin (FBA), following which the expression of elastin-related genes was determined.

**Summary of Results:** Compared to ad lib controls, with MFR, at PND 21 and at 9M, there was significantly increased elastin gene expression, at both mRNA and protein levels (p<0.05, n=4). Culturing primary rat lung fibroblasts without FBS significantly increased the expression and protein levels of elastin and the expression of various elastin-related genes such as Lox, FGFR 3 and 4, and fibrillin 5, while the expression of fibrillin 2 was significantly decreased (all p<0.05, n=3). Furthermore, while FBS-restriction associated increase in elastin expression was only partially blocked by either VD or ARA+DHA, it was completely blocked with 10% FBA supplementation.

**Conclusions:** Elastin is significantly increased in the lung of MFR offspring and this increase appears to be largely due to protein deficiency rather than due to VD, ARA, or DHA deficiency (Grant support-HDS8948).

396 SILDENAFIL USE IN NEONATES WITH MECONIUM ASPIRATION OR SEPSIS: A RETROSPECTIVE LOOK AT HEMODYNAMIC EFFECTS

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**Purpose of Study:** Sildenafil use is increasing in the management of persistent pulmonary hypertension (PPHN) of the neonate. There are concerns that sildenafil may contribute to hemodynamic instability in these neonates. The study objective was to evaluate hemodynamic changes with sildenafil use in newborns with meconium aspiration syndrome (MAS) or sepsis and PPHN.

**Methods Used:** Late preterm and term infants with MAS or sepsis and PPHN admitted to CHLA NICU between 2004 and 2010 were identified. Patients with chromosomal abnormalities and congenital heart defects were excluded. Data on sildenafil dosing, hourly mean blood pressure (MBP), heart rate (HR), vasopressor/inotrope use were collected for 72 hours after initiation of po sildenafil. Data were compared between low-dose (<3mg/kg/day) vs. high-dose (≥3mg/kg/day) and early (<7 postnatal days) vs. late initiation (≥7 postnatal days) of sildenafil administration.

**Summary of Results:** Seventeen patients (12 MAS, 5 sepsis) were identified with mean gestational age (GA) of 39 ± 2 wks, birth weight (BW) of 3191 ± 423 g, median APGAR scores at 1, 5, and 10 minutes of 4, 7, and 7 respectively, sildenafil dose of 2.7 ±1.7 mg/kg/day, day of initiation on 8 ± 7 postnatal days, and hours on inhaled nitric oxide (INO) of 165 ± 95 hrs. Ten patients received low-dose and 7 received high-dose sildenafil. Eight and 9 patients were started on sildenafil early and late, respectively. There were no differences in GA, BW, APGAR scores, hours on INO, or mortality between the groups. After adjusting for vasopressor/inotrope use during the first 72 hours of sildenafil exposure, there were no differences from baseline in MBP and HR when comparing patients on low-dose and high-dose sildenafil. MBPs were similar between the early and late initiation group; however an increase in HR was seen in the late group.

**Conclusions:** In summary, sildenafil administration in neonates with MAS or sepsis for the management of PPHN was not associated with clinically relevant changes in MBP or HR after adjusting for vasopressor/inotropes support.

397 SILDENAFIL USE IN NEONATES WITH ISOLATED CONGENITAL DIAPHRAGMATIC HERNIA: A RETROSPECTIVE LOOK AT HEMODYNAMIC EFFECTS

Limjoco JJ, Paquette L, Ramanathan R, Seri I, Friedlich P Center for Fetal and Neonatal Medicine, USC Division of Neonatal Medicine Children’s Hospital Los Angeles and LAC+USC Medical Center; Keck School of Medicine, University of Southern California, Los Angeles, CA.

**Purpose of Study:** Sildenafil use is increasing in the management of persistent pulmonary hypertension (PPHN) of the neonate. There are concerns that sildenafil may contribute to hemodynamic instability in these neonates. The study objective was to evaluate hemodynamic changes with sildenafil use in newborns with congenital diaphragmatic hernia (CDH) and PPHN.

**Methods Used:** Late preterm and term infants with isolated CDH admitted to CHLA NICU between 2004 and 2010 were identified. Patients with chromosomal abnormalities and congenital heart defects were excluded. Data on sildenafil dosing, hourly mean blood pressure (MBP), heart rate (HR), vasopressor/inotrope use were collected for 72 hours after initiation of po sildenafil. Data were compared between low-dose (<3mg/kg/day) vs. high-dose (≥3mg/kg/day) and early (<7 postnatal days) vs. late initiation (≥7 postnatal days) of sildenafil administration.

**Summary of Results:** Sixteen patients (14 left, 2 right CDH) were identified with mean gestational age (GA) of 38 ± 1 wks, birth weight (BW) of 3343 ± 497 g, median APGAR scores at 1, 5, and 10 minutes of 5, 6, and 6 respectively, sildenafil dose of 3.8 ±1.7 mg/kg/day, day of initiation on 12 ± 28 postnatal days, and hours on inhaled nitric oxide (INO) of 442 ± 286 hrs. Five patients received low-dose and 11 high-dose sildenafil. Five and 11 patients were started on sildenafil early and late, respectively. No differences in GA, BW, APGAR scores, or hours on INO between groups existed. After adjusting for vasopressor/inotrope use during the first 72 hours of sildenafil exposure, there were no differences in MBP and HR when comparing patients on low-dose and high-dose sildenafil. MBPs were similar between the early and late initiation group; however there was an increase in HR in the late group.

**Conclusions:** In summary, sildenafil administration in neonates with CDH for the management of PPHN was not associated with clinically relevant changes in MBP or HR after adjusting for vasopressor/inotrope support.

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<tr>
<th>Protein</th>
<th>F131d</th>
<th>MV 3d</th>
<th>HFNV 3d</th>
<th>MV 21d</th>
<th>HFNV 21d</th>
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<td>BDNF</td>
<td>1.6±0.5</td>
<td>1.1±0.1*</td>
<td>2.4±1.2</td>
<td>3.6±0.4*</td>
<td>3.6±0.8</td>
<td>2.1±0.3</td>
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<td>pro-BDNF</td>
<td>2.7±0.1</td>
<td>4.2±0.6*</td>
<td>6.9±2.0</td>
<td>8.7±0.5</td>
<td>7.5±2.7</td>
<td>3.2±0.2</td>
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Neuroscience II
Concurrent Session
9:00 AM
Saturday, January 29, 2011

398 MULTIMODALITY IMAGING OF MALFORMATIONS OF CORTICAL DEVELOPMENT
Behdianian K 1, David Geffen School of Medicine at UCLA, Los Angeles, CA.

Purpose of Study: Focal cortical dysplasia (FCD), Tuberous Sclerosis Complex (TSC), and Hemimegalencephaly are malformations of cortical development (MCDs) caused by abnormal neuronal proliferation. These MCDs are often associated with early onset of seizures. Advances in imaging of these MCDs have improved the sensitivity and specificity of diagnosing subtle MCDs that are cryptic or hard to identify by MRI. PET/MRI coregistration and magnetic resonance angiography (MRA) considerably improve the localization of subtle abnormalities and Diffusion Tensor Imaging (DTI) provides information about the integrity of white matter adjacent to the lesion. It is shown that using MEG, DTI, and PET/MRI coregistration provides a non-invasive and more accurate method of diagnosing and localizing subtle MCDs which in turn results in better postoperative seizure freedom for the patients.

Methods Used: 18-fluorodeoxy glucose PET data was coregistered with 3D MRI using the commercially available “fusun” program. Fractional anisotropy (FA) and Apparent diffusion coefficient (ADC) was obtained with the program “DTI Studio”. Also, in a patient with TSC, DTI tractography map was obtained.

Summary of Results: PET/MRI coregistration and MEG localized the epileptogenic in a patient with FCD and subtle MRI abnormalities. Using PET/MRI coregistration, widespread hypometabolism was shown in the left hemisphere of a patient with HME who had subtle abnormalities on MRI. In a patient with TSC, ADC and FA were measured in the area immediately adjacent to the tuber with the largest associated hypometabolism as shown on PET/MRI coregistration. ADC=0.86 was higher than the contralateral side ADC=0.83. FA was 0.17 on the lesional side and 0.27 on the normal side.

Conclusions: Multi-modality imaging of MCDs using PET/MRI coregistration, MEG, and DTI in addition to high quality MRI considerably improves the ability to non-invasively localize and characterize the epileptogenic lesions when the MRI abnormalities are subtle.

399 SUBCLINICAL MOOD AND STRESS SYMPTOMS CORRELATED WITH REDUCED CARDIOVAGAL ACTIVITY
Hameed SR 1,2, Stains J 2, Ebrat B 2, Suyenobu B 2, Tillisch K 2,3 1University of California, Los Angeles, Los Angeles, CA and 2University of California, Los Angeles, Los Angeles, CA.

Purpose of Study: Autonomic nervous system (ANS) dysfunction is associated with chronic medical disorders such as irritable bowel syndrome, fibromyalgia, and depression, for which life stress is a risk factor. Whether ANS dysfunction is a predisposing factor or a consequence of the clinical disorders is unknown. We propose to examine the relationship between ANS function, stress, body symptoms, personality traits, and subclinical psychological symptoms in healthy women. We hypothesize that perceived stress, body symptoms, trait measures, and psychological state symptoms will correlate with increased sympathetic and decreased cardiovagal activity.

Methods Used: Healthy women aged 18–50 completed the NEO Personality Inventory, Hospital Anxiety and Depression (HAD) Questionnaire, Patient Health Questionnaire (PHQ), State-Trait Anxiety Inventory (STAI), and the Perceived Stress Scale (PSS). Resting electrocardiogram was acquired and processed for heart rate variability (HRV) with Kubios software. High frequency (HF) power represents cardiovascular tone and Low Frequency/High Frequency (LF/HF) ratio represents sympathethic function. Pearson correlations, ANOVA, and regression analysis were performed using SPSS 7 software.

Summary of Results: 41 women had complete data. HF correlated with HAD Anxiety (r=0.337, p=0.031), HAD Depression (r=0.390, p=0.012), and PSS (r=0.326, p=0.037) but not PHQ, STAI, or NEO scores. No correlations were found for LF/HF. Using the correlated variables as predictors for HF in a stepwise regression, the only significant predictor was HAD Depression (B=0.390, p=0.012).

Conclusions: These results suggest that subclinical mood symptoms and stress but not body symptoms, are linked with cardiovagal activity in healthy women. Depressive symptoms are the main predictor of decreased cardiovagal activity.

400 PERIPHERAL BLOOD LYMPHOCYTE IMMUNE CORRELATES IN GLIOBLASTOMA PATIENTS UNDERGOING DENDRITIC CELL VACCINE IMMUNOTHERAPY
Fong BM 1, Lisiero D 2,3, Odessa S 1,2, Prins R 1,2,3, Liu LM1,2,3,1 1David Geffen School of Medicine at UCLA, Los Angeles, 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: Glioblastoma is the most common and malignant primary brain cancer with a median survival of less than 1 year. At UCLA, we completed a phase 1 dose escalation study to test the safety and feasibility of autologous tumor lysate pulsed DC vaccination in malignant glioma patients. To analyze the frequency and activation status of peripheral blood lymphocyte (PBL) population in glioblastoma patients treated with anti tumor lysate pulsed DC vaccines. Data was then tested to see if a correlation with survival existed.

Methods Used: 26 patients with glioblastomas were enrolled in this study. In 13 patients, PBL from pre and post treatment, were stained with fluorescently-conjugated antibodies (CD3, CD4, CD8, CD16, CD19, CD25, CD127, CD69) and the composition of lymphocyte subsets and activation status were analyzed using multi-color flow cytometry. The lymphocyte subsets tested included CD3+CD4+ Helper T cells, CD3+CD8+ cytotoxic T cells, CD3-CD16+ classical Natural Killer (NK) cells, CD3+CD16+ NKT cells, CD3-CD19+ B cells. The activation status on these PBMC were simultaneously evaluated by measuring the median fluorescence intensity (MFI) of CD25 (High affinity IL-2 receptor) and CD69 (early lymphocyte activation marker).

Summary of Results: Initial data suggests that the degree of increase in NKT (P value=0.094, r=0.662) and CD8 (P value=0.240, r=0.414) cell populations post DC+TLR vaccination correlate with increased survival. Furthermore, there was a direct correlation with NK cell CD69 activation and survival (P value=0.022, r=0.32). In most patients, differences among CD25 and CD69 values were not statistically significant.

Conclusions: Decreased post vaccination frequencies of NKT and CD8 cell populations, along with decreased NK cell activation, were associated with shorter survival. Additional testing is needed to confirm these early findings. Further study of flow cytometry analyses of glioblastoma patient PBMC subsets as a monitoring assay to correlate immune response with survival is warranted.

401 HOW GENOTYPE INFLUENCES CLINICAL FEATURES, CELLULAR ELECTROPHYSIOLOGY, AND MICROANATOMY IN PEDIATRIC PATIENTS WITH TUBEROUS SCLEROSIS COMPLEX
Oh T 1, Mather G 1, Kwiatkowski D 2, Vinters H 1, Salamon N 1, Cepeda C 1, Qiao J 1, Chang J 1, Huynh M 1, Levine M 1, Wu J 1, Pedram K 1,2 1UCLA David Geffen School of Medicine, Los Angeles, CA and 2Harvard Medical School, Boston, MA.

Purpose of Study: Most cases of Tuberous Sclerosis Complex (TSC) have been localized to mutations in either the TSC1 or TSC2 genes. Current research has yet to establish how different types of mutations (deletions and polymorphisms) affect the variability in neurological disease phenotype. The aims of this study are to identify associations between types of genetic mutations and clinical characteristics, neuroimaging, cellular electrophysiology, brain tissue anatomy, and neuronal protein concentrations in TSC patients undergoing epilepsy neurosurgery.

Methods Used: Data were compiled for twenty pediatric patients (n=20) who developed epilepsy as a consequence of TSC. Genetic mutations were grouped into large frameshift mutations or deletions, single nucleotide polymorphisms (SNPs), and no detected TSC1 or 2 mutation. Clinical characteristics and imaging data were extracted from patient medical records and biochemical techniques, including Western blots and immunohistochemistry, were utilized on surgical brain tissue to obtain electrophysiological, anatomical, and polypeptide...
data. The correlation between these variables will be determined through the application of ANOVA statistics.

Summary of Results: Most mutations have been localized to the TSC2 gene; only 1 has been localized to TSC1. Preliminary data for clinical characteristics, cellular electrophysiology, anatomical features, protein concentrations, and neuroimaging have been compiled. However, statistical analysis remains to be conducted, as data about the patients’ developmental statuses and the EEG characterizations of their seizures must still be obtained. Due to the single-blinded design of the study, the nature of genetic mutations will be revealed and accounted for when the remaining information has been obtained.

Conclusions: We hypothesize that genotype will correlate with phenotype. More specifically, patients with the largest genetic defects in TSC mutations will present with more dysmorphic giant cells and balloon cells, increased concentrations of GABAergic post-synaptic proteins, greater abnormalities in cellular electrophysiology, and worse post-operative outcomes.

402 ORBITAL DISTANCES DO NOT CORRELATE TO CEREBELLAR VOLUME IN CHILDREN WITH AUTISM SPECTRUM DISORDERS
Camilleri K 1,2,4, Angkustsiri K 2,3, Nordahl CW 2, Lee A2, Boyd SB 2,3, Amalar DG 1, UC Davis School of Medicine, Sacramento, CA; 2 UC Davis MIND Institute, Sacramento, CA; 3 UC Davis Medical Center, Sacramento, CA; 4 WSMRF, Monterey, CA.

Purpose of Study: Abnormal cerebellar volumes have been reported in autism spectrum disorders (ASD). One of the hypothesized etiologies of ASD may be some sort of embryological insult, which could manifest as abnormal brain development and facial dysmorphisms. Orbital distances might serve as a simple, external marker for children who have abnormal brain development since the cerebellum and the orbits develop around the same time (cerebellum: beginning day 32, eyes: days 29-56). We hypothesize that orbital distances are related to cerebral volume in children with ASD.

Methods Used: 44 Caucasian, male children with ASD between the ages of 2.2 and 3.7 years old (mean age=3.0, std dev=4.2) from the UC Davis MIND Institute’s Autism Phenome Project were included in this study. Three-dimensional surface images of the children’s faces were landmarked using 3DMD software to obtain intercanthal distance, biocural width, and intercanthal index. Cerebellar volumes were obtained from structural T1 brain MRIs first using a template-based automated segmentation procedure followed by refinements done in Mayo Clinic BIR’s Analyze 10.0 software. Linear regression was used to evaluate the relationship between the individual orbital distances and cerebellar volume.

Summary of Results: Cerebellum volume does not significantly correlate to intercanthal distance ($r^2 = 0.0012, p=0.82$), biocural width ($r^2 = 0.0024, p=0.75$), or intercanthal index ($r^2 = 0.0095, p=0.53$).

Conclusions: The lack of any significant correlation between cerebellum volumes and orbital measurements suggests that the anthropometric measurements may not be good indicators of cerebellum volume in children with autism. In children with autism that may have suffered an embryological insult, cerebellum and orbital development may not be similarly affected.

403 MULTIPLE ESTROGEN RECEPTORS ARE RESPONSIBLE FOR THE ESTROGENIC ATTENUATION OF THE CANNABINOID-INDUCED INHIBITION OF GLUTAMATE RELEASE AT PROPOIOMELANOCORTIN SYNAPSES
Washburn N, Wagner E Western University, College of Osteopathic Medicine of the Pacific, Pomona, CA.

Purpose of Study: Estrogens exert far-reaching effects on mammalian biology - from reproduction to energy homeostasis. While many actions require gene transcription and protein synthesis to alter cell function via the activation of estrogen receptor subtypes ERalpha and ERbeta (which takes hours to days), it is now well recognized that estrogenic signaling can take place on a much more rapid timescale (minutes) via membrane delimited ERalpha or a Gq-coupled membrane ER (Gq-mER). We have shown previously that estrogen rapidly diminishes cannabinoid-induced hyperphagia; attributable to an attenuation in the cannabinoid-induced presynaptic inhibition of excitatory glutamate currents at anorexigenic POMC neurons of the hypothalamic arcuate nucleus. Recently, we found that activation of ERalpha and Gq-mER attenuated cannabinoid-induced hyperphagia, whereas ERbeta did not. Therefore it was the goal of the present study to further investigate whether ERalpha or Gq-mER, contribute significantly to the rapid and sustained inhibitory effect of estrogen on the cannabinoid regulation of POMC neuronal activity.

Methods Used: Voltage clamp experiments were performed in the arcuate nucleus of female Topkea guinea pigs. Once stable access to the cell was achieved either the ERalpha agonist PPT (1µM), the Gq-mER agonist STX (10µM), or their respective vehicle controls, were bath applied for 10-15 min. Miniature excitatory postsynaptic current (mEPSC) frequency and amplitude were then evaluated under both basal conditions and in the presence of the cannabinoid receptor agonist WIN 55,212-2.

Summary of Results: Transient exposure to STX diminished the decrease in mEPSC frequency caused by 1µM WIN 55,212-2, whereas PPT did not (vehicle: 49.6±9.1%; STX: 85.8±4.2%; PPT: 45.0±4.8% of baseline control). However, systemic administration of PPT (200µg; s.c.) completely blocked the cannabinoid-induced decrease in mEPSC frequency (105.8±5.4% of baseline control).

Conclusions: In conclusion, Gq-mER and ERalpha contribute to different components of estrogenic attenuation of the cannabinoid-induced decrease in glutamate release at POMC synapses: Gq-mER mediates the rapid inhibition, whereas ERalpha is involved in the sustained diminution.
epileptogenesis. GFAP (glial fibrillary acidic protein), a prototypical marker of astrocytes, is upregulated and reveal astrocytes undergo structural changes such as hypertrophy and loss of domain organization. Severe forms of astrogliosis lead to the formation of glial scars and scar-like penumbras around necrotic or ischemic areas. To further characterize astrocytes in human epilepsy, we analyzed hallmark markers of astrocytes in pediatric surgical biopsy specimens of the neocortex.

**Methods Used:** Cortical tissue were resected from pediatric patients with epilepsy disorders including Cortical Dysplasia, Rasmussen’s Encephalitis, and epilepsy due to ischemic stroke primarily from central operculum and parieto-temporal lobe regions. Specimens were vibration-sectioned and stained for GFAP and AQ4 using fluoresence immunohistochemistry and images were binarized and quantified for astrocyte abundance and coverage as a measure of reactivity.

**Summary of Results:** Our results show that abundance as well as network complexity varied in each zone. One striking finding was a noticeable sparseness, even lack GFAP fluorescence in the grey matter of infant cortical biopsies. This finding, together with the presence of immature radial glia marker expressing cells in that zone (BLBP-brain lipid binding protein) suggests that GFAP-positive astrocytes did not significantly populate the grey matter until around 4 years of age. This was found to be the case in genetic as well as acquired epilepsy etiologies, irrespective of case severity, which suggests a developmental feature of protoplasmic astrocytes rather than pathological.

**Conclusions:** These tools allow us to determine reproducible quantitative differences in astrocyte abundance, morphology and reactivity that may help define gliosis severity. These findings would help us understand the role of astrocytes in epilepsy and also be useful in histopathological diagnosis of various epilepsy syndromes.

**Pulmonary and Critical Care II**

**Concurrent Session**

8:30 AM

Saturday, January 29, 2011

**406 ACCELERATED PPARy SIGNALING IN POST PNEUMONECTOMY LUNG REPAIR**

Li Y1, Sakurai R1, Vonswinckel R2, Torday J1, Rehan V1. 1Los Angeles Biomedical Institute, Torrance, CA and 2University of Giessen, Giessen, Germany.

**Purpose of Study:** Peroxisome proliferator-activated receptor (PPAR)y, a key nuclear transcription factor, plays an essential role in lung development and repair in the newborn, but its role in pulmonary regeneration in the adult is not known. Here, we aimed to determine the role of PPARy signaling in adult lung injury/repair, as a prelude to manipulate molecular determinants for the purpose of enhancing lung regeneration in the adult.

**Methods Used:** We utilized a post-pneumonectomy (post-PNX) compensatory lung growth model. Adult C57BL/6 mice underwent left-sided PNX. The right lung was examined on days 2, 5, and 14 post-PNX. Lung volume was assessed, and PPARy signaling pathway intermediates were examined at the RNA (real time RT-PCR) and protein levels (immunohistochemistry and Western blot analysis).

**Summary of Results:** Following PNX, right-lung volume rapidly compensated for the initial volumes of the left and right lungs. Both Western blot analysis and real time RT-PCR suggest that compared to sham controls, following PNX, PPARy was up-regulated (+31% from post-PNX day 2 to day 5) and this increase was normalized by day 14 post-PNX. Qualitatively, a similar pattern was demonstrated by down-stream targets of PPARy signaling such as adipocyte differentiation-related protein (ADRP) and leptin, though the changes were more pronounced for ADRP. These data were corroborated by immunohistochemistry.

**Conclusions:** Our data suggest clear up-regulation of PPARy signaling during post-PNX lung repair, suggesting a role for this critical pathway in adult lung injury/repair. We speculate that PPARy agonists could also accelerate adult lung injury repair, similar to what we have recently shown in neonates (Dasgupta et al, Am J Physiol 2009;296:L1031-41). (Grant support: NIH-HL095326, HL075405, HD058948, HD051857 and TRDPR (15TF-0250, 17RF-0170).
Conclusions: Estimation of eNO proved to be a better objective tool for assessing asthma control than FEV1 estimation in our cohort from this pilot study. Our results indicate that eNO is an alternate, non-invasive objective tool to assess asthma control. It is a useful aid in clinical decision making process especially when history is unreliable and spirometry is either difficult or unavailable.

409 GENETIC POLYMORPHISMS OF CYTOCHROMES P450 AND PEDIATRIC ASThma CONTROL BY INHALED CORTICOSTEROIDS
Stockmann C1, Fassil B1, Nkoy F1, Stone B1, Willis L1, Gaedigk R2, Leeder S2, Ward R1,2University of Utah, Salt Lake City, UT and 1University of Kansas Children’s Hospital, Kansas City, KS

Purpose of Study: Inhaled corticosteroids (ICS) are mainline treatments for persistent asthma in children. Up to 10% of children with persistent asthma fail ICS therapy for unclear reasons. Genetic polymorphisms of cytochromes P4503A (CYP3A) have been shown to be associated with altered metabolism of ICS which might have an effect on their therapeutic effectiveness. Fluticasone, a widely used ICS, is metabolized primarily by CYP3A4 whose metabolism may be decreased by a polymorphism in intron 6. Objectives of this study are to 1) determine the frequency of genetic variants in CYP3A4 (intron 6 SNP rs5599367, C>T), in children treated with fluticasone for difficult to control asthma and 2) to describe and compare CYP450 genotypes in these children with and without previous hospital admissions for asthma.

Methods Used: We enrolled children aged 2-17 years with a diagnosis of asthma from Primary Children’s Medical Center. We collected saliva samples and analyzed for CYP450 genetic polymorphisms at the Developmental Pharmacology and Experimental Therapeutics Laboratory at Kansas City. Single nucleotide polymorphisms for 9 alleles that increase or decrease protein expression and CYP3A activity were determined along with asthma severity, preventive medication use, and number of hospitalizations for acute asthma within the preceding 12 months.

Summary of Results: Of 96 children enrolled, 23 were treated with fluticasone; all of these were classified as not-well-controlled asthmatics by NIH guidelines. Of these 23 children, 14 patients were admitted for asthma in the previous 12 months, 14/14 featured in rs5599367 C/C genotype consistent with greater CYP3A4 mRNA level and enzyme activity. Of the 9 patients not requiring hospitalization within the last 12 months, 5 (55.6%) had at least one variant rs5599367 allele, consistent with slower metabolism of fluticasone. There was no difference in the distribution of other CYP3A4, CYP3A5, and CYP3A7 polymorphisms.

Conclusions: These preliminary findings are consistent with the hypothesis that slower metabolism of ICS may improve their effectiveness for treatment of asthma. Additional patient studies are needed to confirm or refute this association.

410 INTERN NEEDS IN ULTRASOUND GUIDED CENTRAL VENous ACCESS DIDACTIC TEACHING
Abouhouli H, Bovin M, Baty G UMN, Albuquerque, NM.

Purpose of Study: Residency Training involves teaching critical procedural skills, including invasive and sometimes dangerous procedures. Central venous catheter (CVC) placement is a core procedural skill that typifies this subset. Our group sought to develop a curriculum and test different strategies to teach this multifaceted skill. There is no current standardized approach to training residents in CVC placement. Our project is designed to determine the needs within didactic training that would include theoretical and practical aspects of U/S guided CVC placement aimed toward the interns prior to their ICU rotation.

Methods Used: We have randomly selected 45 interns to participate in the above study. Out of the 45 residents 23 completed the pre-test. The questions were designed to test the theoretical knowledge, ability to assess U/S images, and the U/S general knowledge of interns at the start of their year. The questions were validated in ultrasound skilled groups prior to administration. Some of the questions had actual U/S images and the interns were asked to properly identify the marked structures. The pre-test is an online computer based test, each participant had access to the website, and they were allowed to take the test off campus, at their own pace.

Summary of Results: Out of the 23 questions, 12 questions were intended to test the resident’s theoretical knowledge, 9 questions to test their ability to assess U/S images, and 8 questions to assess their U/S practical knowledge. Their scores were as follows: 42.25% of the interns answered the questions correctly on the theoretical knowledge section vs. 26.3% on assessing U/S images, and 29.3% on the practical U/S knowledge section. The difference between theoretical knowledge was statistically significant (p=0.05), however the difference between theoretical and practical knowledge was only a trend.

Conclusions: Interns beginning their clinical rotations have greater background knowledge in theoretical concepts of U/S guided central line placement than ability to interpret U/S images. Curricula designed to teach this skill to beginning interns should place special emphasis on the development of image interpretation skills. Our presentation will also include 3-month post test data (currently being collected) to evaluate their improvement with instruction and their late retention.

411 INHIBITION OF FARNESYLTRANSFERASE WITH FTI-277 EXACERBATES EOSINOPHILIC INFLUX IN ALLERGIC AIRWAY INFLAMMATION
Zaki AA1,2, Chang KY2, Kenyon N1,2,1 U.C. Davis Medical Center, Sacramento, CA and 2Center for Comparative Respiratory Biology & Medicine (CCRBM), Davis, CA.

Purpose of Study: We have previously shown that simvastatin inhibits allergic eosinophilic inflammation and improves lung function in the ovalbumin mouse model by targeting the mevalonate (MA) pathway. Farnesyltransferase (FTase) is a downstream enzyme in the MA pathway that activates Ras, a small GTPase important in Th2-dependent eosinophilic inflammation. Thus, we hypothesized that inhibition of FTase with the drug FTI-277 would attenuate allergic airway inflammation and improve lung function.

Methods Used: BALB/c mice were sensitized to ovalbumin (OVA) over 4 weeks, then exposed to 1% OVA aerosol over 2 weeks. Mice were injected with FTI-277 (20 mg/kg/day for 14 days) intraperitoneally before each OVA exposure. Bronchoalveolar lavage fluid (BALF) total and differential cell counts, lung histology, and lung compliance and resistance were measured (using a plethysmograph for restrained animals).

Summary of Results: Control and treated animals showed no significant change in body weight. FTI-277 markedly increased total cell, eosinophil (p<0.05), and lymphocyte counts (p<0.05) in BALF. Lung histology showed marked peribronchioral and perivascular influx of eosinophils, lymphocytes, and neutrophils in FTI-277 treated mice. Systemic FTI-277 also increased airway hyperreactivity (AHR) (p<0.01) and decreased lung compliance (p<0.05).

Conclusions: Systemic treatment with FTI-277 exacerbated allergic eosinophilic inflammation in a mouse model of asthma. This is thought to occur via inhibition of Ras activity. These data suggest that inhibition of certain downstream targets in the MA pathway may be harmful, while others may have benefit.

Supported by the following grants: NIH (T32) HL07015, NCRR UL1 RR024146 (K30), HL-076415 (K08), ATS Fellows Career Development Award, and CTSC K12 Award (KL2 RR 024144).

412 HUMAN PULMONARY ARTERY ENDOTHELIAL CELL EXPOSURE TO FIBRIN(ogen) AUGMENTS INTRACELLULAR CALCIUM RESPONSES TO THROMBIN
Liang N, Firth A, Marsh I, Yuan J, Morris T University of California San Diego, San Diego, CA.

Purpose of Study: After pulmonary embolism, some patients are at risk for developing chronic thromboembolic pulmonary hypertension (CTEPH). Fibrin from patients with CTEPH is resistant to lysis, which leads to persistence within thrombi of fibrinogen, although how this phenomenon might stimulate pulmonary arterial scar formation is unknown. This study was performed to determine if exposure of human pulmonary artery endothelial cells (PAEC) to regions of fibrinogen would enhance their ability to be stimulated by subsequent exposure to thrombin.

Methods Used: The central “N-terminus disulfide knot” (NDSK), was prepared from human fibrinogen by digestion with cyanogen bromide. NDSK II (the central region of fibrin) was prepared by cleavage of NDSK with thrombin. Ca2+ was labeled using Fura-2. PAECs were exposed to NDSK,
NDK II or control then subsequently stimulated with thrombin. Thrombin-induced [Ca2+]cyt increases (i.e. Fura-2 fluorescence) were measured in peripheral cytoplasmic regions from cells by epifluorescence microscopy. Data were collected at a rate of 88 samples/minute using excitation 340 and 380 nm with xenon lamp, emission 520 nm at 20x magnification.

**Summary of Results:** Peak thrombin-induced increases in [Ca2+]cyt (F340/F360) after pre-perfusion with NDSKII (0.914 +/- 0.139) was significantly higher than after pre-perfusion with control (0.537 +/- 0.028; p < 0.001). The AUC for thrombin-induced [Ca2+]cyt increase was also higher after NDSKII (73.56 +/- 14.02) than after the control (32.49 +/- 2.49; p < 0.0001). There was a trend towards higher thrombin-induced [Ca2+]cyt increases in cells pre-perfused with NDSK (0.808 +/- 0.049) compared with control (0.716 +/- 0.051, p=0.2). The AUC for thrombin-induced [Ca2+]cyt increase was significantly higher after NDSK (78.19 +/- 6.97) than after control (35.89 +/- 4.25; p < 0.0001).

**Conclusions:** Exposure of endothelial cells to the cell signaling regions of fibrin and, to a lesser extent, fibrinogen enhanced their ability to be activated by thrombin. The enhancement may mediate the fibrin-endothelial cell interactions responsible for pulmonary embolism remodeling and persistent vascular obstruction in CTEPH.

### 413 UNDIAGNOSED AIRFLOW OBSTRUCTION, OBESITY AND RESPIRATORY SYMPTOMS: A COMPLEX INTER-RELATIONSHIP

*Zutler M, Singer J, Omachi TA, Blanc PD

**Purpose of Study:** Screening spirometry detects previously undiagnosed airflow obstruction (AO) in up to 15% of the general adult population. Although increased cough and dyspnea are associated with AO, other covariates, in particular body habits, have not been well characterized. We sought to explore the inter-relationships among AO, obesity, and self-reported dyspnea on exertion (DOE).

**Methods Used:** We analyzed the referent group of Kaiser Permanente Health Plan members (age 40-65) recruited for the Function, Living, Outcomes and Work (FLOW) study of chronic obstructive pulmonary disease (COPD). The referent group had no health utilization for COPD in the 12 months prior to recruitment. Subjects completed a structured interview (medical history, symptoms and functional status) and a clinical assessment including body mass index (BMI) and spirometry. We excluded 2 subjects with AO (FEV1/FVC ratio <0.7) who self-reported a history of physician-diagnosed COPD. We compared smoking and obesity among subjects with AO to those without AO. In multiple regression analyses (controlling for age, sex, race-ethnicity and cumulative smoking), we tested the associations of AO, obesity (BMI>30), and dyspnea on exertion.

**Summary of Results:** Of the 371 subjects studied, 69 (18.6%, 95% CI 14.6-22.6%) manifested AO. Among those with AO, 43% were never-smokers compared to 52% among those without AO (p=0.01). Among former and current smokers, those with AO had a higher cumulative smoking history than those without AO (median 27 vs. 19 pack-years, p=0.02). In multivariate analysis, obesity was associated with an increased likelihood of DOE (OR 4.0; 95% CI 2.0-7.8) as was female gender (OR 2.0; 95% CI 1.3-3.7). AO trended towards an increased likelihood of DOE (OR 1.7; 95% CI 0.7-4.6). Obesity was negatively associated with AO (OR 0.54; 95% CI 0.3-0.98).

**Conclusions:** AO is common in adults without an established COPD diagnosis. Cumulative smoking was associated with AO, but, counter to expectations, obesity was negatively associated with AO. Both female gender and obesity were linked to a higher likelihood of DOE; AO trended in the same direction. These data suggest that respiratory symptoms are related to complex inter-relationships among body habits, gender, and lung function.

### 414 SURVIVAL IN SCLERODERMA-RELATED INTERSTITIAL LUNG DISEASE FOLLOWING LUNG TRANSPLANT


**Purpose of Study:** Interstitial lung disease (ILD) is the leading cause of death in scleroderma. A diagnosis of scleroderma-related ILD (ScI-ILD) is often a contraindication for lung transplant due to concern that the extra-pulmonary organ involvement of scleroderma will result in poorer outcomes. In this study, survival following lung transplant in patients with ScI-ILD was compared to idiopathic pulmonary fibrosis (IPF), and idiopathic pulmonary hypertension (IPH).

**Methods Used:** We conducted a cohort study from 2001 to 2010. Patients transplanted for ScI-ILD were matched by age and gender to control patients transplanted for IPF; patients transplanted for IPH were also included as controls. End-stage lung disease for transplant. Demographic and physiologic data were collected. Post-transplant survival time was the primary outcome. Survival time was estimated using Kaplan-Meier methods. Secondary outcomes included episodes of acute rejection and time to development of bronchiolitis obliterans syndrome.

**Summary of Results:** Twenty-two patients with ScI-ILD, 30 patients with pulmonary fibrosis, and 11 patients with IPH who underwent lung transplant were identified. ScI-ILD and IPF patients were similar in age, gender, ethnicity, body mass index, serum creatinine, FEV1, and FVC. Most patients with ScI-ILD had esophageal dysmotility and/or gastroesophageal reflux. Patients transplanted for ScI-ILD had similar overall survival to patients transplanted for IPF and IPH (p=0.4). In the IPF group, 6, 12 and 24 month survival was 85%, 70%, and 69% in the IPF group. In IPH, 6, 12 and 24 month survival was 90%.

**Conclusions:** Patients transplanted for ScI-ILD appear to have similar survival following lung transplantation compared to patients transplanted for IPF and IPH. For patients with end-stage ScI-ILD, referral to institutions with experience in performing transplant for this disease should be considered. Future studies are needed to determine whether these similarities in survival persist, whether there are important differences in morbidity, and whether center experience and volume are important factors in ScI-ILD patient outcomes.

### 415 THE EFFECT OF INFLAMMATION AND MECHANICAL VENTILATION ON ALVEOLAR FLUID CLEARANCE IN MICE

*Loraas EK, Smith L1,2, Martin TR1,2

**University of Washington, Seattle, WA; 1VA Puget Sound Medical Center, Seattle, WA and 2Seattle Children's Hospital, Seattle, WA.

**Purpose of Study:** Acute lung injury (ALI) is a syndrome of acute hypoxic respiratory failure that affects more than 190,000 people each year in the United States, and results in over 74,000 deaths annually. Infections are the most common cause of ALI, and animal studies suggest that when infectious stimuli are combined with mechanical ventilation (MV) there are synergistic increases in the inflammatory and injury responses in the lungs. The accumulation of a protein rich alveolar edema fluid is a pathologic hallmark of ALI and resolution requires an intact alveolar epithelium to clear the fluid. Impaired alveolar fluid clearance (AFC) is associated with mortality in adult patients with ALI. Therefore, we investigated the effect of inflammation and mechanical ventilation on AFC in adult mice.

**Methods Used:** Adult (12 week) C57BL/6 mice were treated with 0.36 ng/g intratracheal lipopolysaccharide (LPS) followed by mechanical ventilation using Vt=15 ml/kg and rate=80 breaths/min for four hours (LPS+MV). Controls were untreated age-matched, spontaneously breathing mice. Animals were euthanized and fluorescent labeled albumin was instilled into the airways through a tracheotomy tube. Animals were then placed on 5 cm H2O continuous positive air pressure and 100% O2 for 30 minutes. The fluorescent labeled albumin was aspirated from the lungs and measured using fluorescent absorption spectroscopy. The difference between the initial albumin fluorescence and recovered albumin fluorescence was used to calculate AFC. Cytology was also performed on the recovered fluid.

**Summary of Results:** Treatment with LPS+MV (N=8) resulted in a significant recruitment of neutrophils (75% PMN) into the lungs as compared with untreated control mice (N=10, 0% PMN). Treatment with LPS+MV resulted in a mean AFC of 48.7  5.1, whereas the mean AFC of untreated control mice was 48.2  2.3.

**Conclusions:** The combination of LPS and mechanical ventilation causes a significant neutrophil recruitment into the lungs. Surprisingly, the inflammatory response to LPS+MV does not change AFC, suggesting that the alveolar epithelium remains functionally intact.
416 METABOLOMIC ANALYSIS OF EXHALED BREATH CONDENSATES (EBC) IN ASTHMA AND COPD

Schivo M1, Zhao W2, Akenson A2, Kenyon N1, Davis C2. UC Davis, Sacramento, CA. and Davis, CA.

Purpose of Study: A significant proportion of patients do not fit classic historical or spirometric definitions of either asthma or COPD and therefore elude a confident diagnosis. Metabolomic profiling may provide a better diagnostic strategy and may eventually elucidate specific therapeutic targets in asthma and COPD. A novel device called a differential mobility spectrometer (DMS) is a portable, highly sensitive sensor. In this study we aim to test EBC from asthma, COPD, and health subjects using a DMS. We hypothesize that EBC chemical profiles will differ between subject groups.

Methods Used: 20 subjects were assigned to an asthma, COPD, and non-asthmatic/non-smoker control group based on well-characterized clinical and spirometric criteria. Both nitric oxide and exhaled breath condensate were collected as well as a St. George’s Respiratory Questionnaire and anthropomorphic measures. The nitric oxide was measured on an analyzer, and the exhaled breath condensate was introduced into a gas chromatogram and analyzed by both a differential mobility spectrometer (DMS) and a mass spectrometer (MS). Optimization of EBC volatile compound recovery involved modifying heating temperature, sample agitation, sample volume, vial size, and background noise reduction. DMS and mass spectrometer results were compared to note if similar compounds exist, some of which can only be identified in the mass spectrometry library.

Summary of Results: Subject groups differed by age; subjects with COPD were significantly older than either those with asthma or the controls (P<0.05). DMS data is now being analyzed for peak-to-peak differences (t-testing), biomarker pattern differences (principle component analysis), and individual compound identification between groups. We anticipate that principle component analysis of the differential mobility spectrometer data will allow for accurate separation of the three groups. We also anticipate that biomarker “signatures” will emerge unique to each group from the DMS and MS data.

Conclusions: The DMS can measure large profiles of metabolites in exhaled breath condensate of subjects with asthma and COPD. PCA analyses will allow us to determine which metabolites distinguish asthma from COPD, and we plan to present these determinations. Age was a confounder in our study.

Surgery II
Concurrent Session
8:30 AM
Saturday, January 29, 2011

417 SIROLIMUS DETERS THE RATE OF HEPATITIS C PROGRESSION FOLLOWING ORTHOTOPIC LIVER TRANSPLANTATION: A SINGLE CENTER EXPERIENCE

Kelly MA, Kaplan M, Zimmerman MA, Wachs M, Bak T, Kam I. University of Colorado Health Sciences Center, Aurora, CO.

Purpose of Study: Recurrence of hepatitis C virus (HCV) infection is a foreseeable problem following orthotopic liver transplantation (OLT), which over time leads to liver fibrosis and graft loss. While several animal studies have shown that sirolimus (SRL) acts to inhibit the rate of liver fibrosis, few studies in the human population support or refute these findings. Our center has had ample experience with using SRL as an effective immunosuppressive agent. In this study we sought to determine the difference in the rate of recurrence of HCV infection as well as the progression of infection in patients who received SRL as primary immunosuppression in comparison to patients who received calcinurin inhibitors (CNIs) post-OLT.

Methods Used: Patients transplanted for end-stage liver disease (ESLD) due to HCV were identified from our transplant database. These patients were categorized into two groups: CNI immunosuppression (control group) and SRL immunosuppression group. We controlled for 3 clinopathologic variables (MELD, warm ischemic time, and cold ischemic time). Cox proportional hazards regression was used to examine the effect of Sirolimus on overall mortality as well as severe HCV recurrence (defined as a liver biopsy of stage 2 fibrosis or greater). Logistic regression analysis was used to test the association between Sirolimus and severe HCV recurrence within in the first year following OLT.

Summary of Results: From January 2000 to July 2009, 313 patients underwent OLT for HCV. After applying exclusion criteria our study population was 232 patients, of which 60 patients received SRL as primary immunosuppression therapy. Overall, no effect was detected of SRL on mortality. For severe HCV recurrence within the study period, SRL had a statistically significant reduction in risk of the outcome by 58% (p=0.011) and a trend toward significant reduction in the odds for severe HCV recurrence within the first year following OLT (p=0.072).

Conclusions: While a debate exists about the effect of SRL therapy on recurrence of HCV infection following OLT, we demonstrated a significant reduction in risk in this single-center experience. Furthermore, SRL therapy showed a trend toward significance in reducing the odds of severe HCV recurrence within the first year following OLT.

418 PRE-LIVER TRANSPLANT THERAPY FOR HEPATOCELLULAR CARCINOMA CAN ACHIEVE EQUIVALENT SURVIVAL FOR PATIENTS WITH WORSE PROGNOSIS

Klingler M, Kangus S, Maluccio M. Indiana University School of Medicine, Indianapolis, IN.

Purpose of Study: Liver transplantation is the most effective treatment for patients with cirrhosis and hepatocellular carcinoma (HCC). Patients often undergo liver-directed therapies (LDT) aimed at controlling tumor growth while they await transplantation, or to downsize tumors to meet listing criteria for transplantation. The study examines the impact of LDT on post-transplant survival.

Methods Used: Records from a prospectively collected database of 1116 consecutive adult liver transplants performed over a 9-year period were reviewed. Of these patients, 244 (22%) were diagnosed with HCC by explant pathology, with median follow-up time of 61 months. LDTs included any localized intervention made to decrease or slow the progression of the HCC.

Summary of Results: There were 124 patients (51%) who received, and 120 patients (49%) who did not receive any form of LDT. Those who received LDT had significantly larger (median 3.3cm vs 2.0cm, p <0.001) and more numerous (mean 2.5 vs 1.9, p=0.001) tumors, and were more likely to be outside Milan criteria (71% vs 29%, p <0.001) and to have neurovascular invasion on explant pathology (22% vs 14%, p=0.12). In spite of the LDT group having less favorable tumor characteristics, there was similar survival (p=0.51) over a 5-year follow up period by Cox proportional hazards regression modeling.

Conclusions: Patients with HCC who undergo LDT to slow progression or to downsize tumors prior to transplantation can achieve survival similar to that seen in patients with a much more favorable tumor status and similar to that seen for all liver transplant recipients at 5-years.
419 IN VITRO MICRODISTRACTION OF HUMAN ADIPOSE-DERIVED STEM CELLS MAY PROMOTE THEIR OSTEOGENIC POTENTIAL

Lee JC, Fan K, Sorice S, Bradley J, Zuk P David Geffen School of Medicine at UCLA, Los Angeles, CA.

Purpose of Study: Distraction osteogenesis has been used over the past 15 years to induce bone formation through the application of gradual, outward linear forces. The molecular mechanisms in which these forces promote bone formation in the field of craniofacial reconstruction are not well understood. In an effort to study the response of osteoblasts to compressive and distractive forces in a 3D environment, we have developed an in vitro model utilizing a microdistraction (MD) system capable of subjecting cells to distractive and compressive forces. Using the MD system, our aim is to characterize the effect of distraction forces on the expression of osteogenic, cytoskeletal, and cell cycle-related genes in adipose-derived stem cells (ASCs).

Methods Used: Adipose-derived stem cells were isolated from raw human lipoaspirates, cultured, and suspended in a type-I collagen gel fitted to the MD system. ASC-collagen 1 gels were subjected to 1) outward linear distraction forces (distraction gels), 2) static forces (isometric controls), and 3) no forces (free-floating). At designated lengths, all ASCs were harvested and gene expression for markers of osteogenesis, cell cycle progression, and cytoskeletal changes were measured using RT-PCR.

Summary of Results: Distraction over four days produced fluctuating temporal expression patterns in cytoskeletal and cell-cycle markers. While short-term distraction over four days demonstrated inhibition of osteogenic Cbfα-1, AP, OP, and OC expression, long-term distraction up to two weeks resulted in increased levels of Cbfα-1, AP, and ON expression compared to isometric and free-floating controls.

Conclusions: The application of linear forces on ASCs has complex temporal effects on many markers associated with cell cycle progression and cytoskeletal changes. Linear stress is capable of promoting the expression of several key osteogenic genes in our in vitro model. The osteogenic response to these forces appears to be complex, dependent on the type and timing of linear stress. Further study is focused on more precisely examining the response of ASCs to distractive manipulation and the roles of the various intracellular signaling pathways involved.

420 NOVEL RISK FACTORS FOR ANTERIOR CRUCIATE LIGAMENT INJURY: SHORT LATERAL TibIAL PLATEAU LENGTH AND DECREASED LATERAL TibIAL PLATEAU RADIUS OF CURVATURE


Purpose of Study: Attributes of femoral condyle and tibial plateau geometry may play a role in the stability of the knee. We studied differences in lateral knee geometry in ACL-injured and uninjured cohorts.

Methods Used: 185 age and activity-matched uninjured and non-contact unilateral (uACL-) and bilateral (bACL-) injured groups were included. MRI measurements of the articular cartilage surfaces in the mid-weight-bearing sagittal plane of the lateral compartment evaluated tibial plateau radius of curvature (TPr) and distal femoral radius of curvature (Fr). Femoral condyle (FAP) and tibial plateau (TPAP) lengths were also measured. Measurements were made by 3 observers blinded to gender and injury group.

Summary of Results: The mean lateral plateau radius of curvature, femoral radius, and TPr were all significantly smaller in the ACL-injured versus non-injured groups (33.6 vs 37.4mm, p=0.003; 25.1 vs 24.2mm, p=0.01; 31.4 vs 33.2mm, p=0.004, respectively). Femoral length was not different between ACL-injured and uninjured groups (p=0.19).

Gender Groups: Compared to uninjured males, ACL-injured males demonstrated significantly smaller tibial plateau radius (41.1 vs 35.3mm, p=0.001), femoral radius (26.7 vs 25.5mm, p=0.002), and tibial plateau length (35.5 vs 33.1mm, p=0.001). No differences in geometry were noted between female groups.

Conclusions: Our data suggest that all females (injured or uninjured) and ACL-injured males share a common lateral knee geometry characterized by a shorter tibial plateau length relative to the femur and smaller (more convex) articulating surfaces of both the proximal tibia and distal femur. It is possible that the opposition of two highly-convex articulating surfaces may be inherently less stable to anterior tibial translation and rotation. These findings also may help to explain the apparent female predilection for ACL-injury compared to males, but suggest that this may be as much driven by geometry as it is by gender.

421 PREOPERATIVE CT IMAGING IN REOPERATIVE CARDIAC SURGERY

Chen WS, Wu J, Johnstone S Cardiothoracic Surgery, Kaiser Permanente, Honolulu, HI, USA, Honolulu, HI.

Purpose of Study: Preoperative imaging is being utilized with increasing frequency in the planning of reoperative cardiac surgery cases. This study sought to evaluate the use of preoperative CT imaging in our reoperative cases and its impact on operative approach and surgical outcome.

Methods Used: This is a retrospective review of all patients who underwent reoperative cardiac surgery from 2000–2009 at a single health maintenance organization hospital. We evaluated operative reports of all reoperative cases to determine if there was significant modification of surgical approach. Modification of surgical approach was defined as incision other than median sternotomy, femoral or axillary exposure prior to sternotomy, initiation of cardiopulmonary bypass before sternotomy, or use of circulatory arrest. The rate of modification of surgical approach was compared between the groups of patients with preoperative CT imaging and without imaging. A comparison of the observed-to-expected mortality rates was made using the Euroscore prediction model.

Summary of Results: A total of 128 reoperative cardiac surgical cases were performed from 2000–2009. Preoperative CT imaging was used in 49 patients. Surgical approach was modified in 49% (24) of patients with preoperative CT imaging and only 34% (27) of patients without preoperative CT imaging (p = 0.17). Observed-to-expected mortality rates were 1.5 and 1.28 respectively for the group with preoperative CT imaging and the group without.

Conclusions: Patients undergoing preoperative CT imaging had a greater rate of modification of surgical approach than those who didn’t. However, this did not have a significant impact on the observed-to-expected mortality ratio. While additional studies are necessary, our results indicate that the use of preoperative CT imaging could be useful in the planning of reoperative cardiac surgery. Preoperative CT imaging may provide information resulting in the modification of surgical approach, possibly leading to safer surgery. We believe it is worthwhile to consider preoperative CT imaging in high-risk patients undergoing reoperative cardiac surgery.

422 GHRELIN ADMINISTRATION ATTENUATES THE MYOCARDIAL INFLAMMATORY RESPONSE TO HYPOTHERMIC ISCHEMIA/REPERFUSION AND AFFORDS CARDIOPROTECTION

Austin EW, Ghaly Yousif N, Ao L, Fullerton D, Meng X University of Colorado School of Medicine, Aurora, CO.

Purpose of Study: During most cardiac surgical operations, the heart must endure the injuries of hypothermic ischemia followed by blood reperfusion. Mechanisms of inflammation are recognized to contribute to injuries of ischemia/reperfusion (I/R). Ghrelin is an endogenous peptide, principally produced by the gastric mucosa, and has recently been shown to have anti-inflammatory actions. We hypothesized that exogenous administration of ghrelin would reduce the myocardial injury associated with hypothermic ischemia and blood bypass. In a heterotopic heart transplant model, the purpose of this study was to examine the anti-inflammatory actions of ghrelin in the myocardium.

Methods Used: Syngeneic mice (C3H/HeN strain) underwent heterotopic heart transplantation. Donor hearts were stored in cold, cardioplegic solution for 4 hours. Recipient mice received synthetic ghrelin (100 μg/kg iv) prior to cervical implantation of the heart. Donor hearts were reperfused by the recipient’s blood for 4 hours, and then harvested for analysis. Myocardial injury was evaluated by serum cardiac troponin-I (cTn-I) levels. Levels of monocyte chemotactic protein-1 (MCP-1), intercellular adhesion molecule-1 (ICAM-1) and monocyte infiltration were assessed by ELISA, immunoblotting and immunofluorescence, respectively. Controls underwent the same procedure without ghrelin. Statistical analysis was performed using Student’s t-test (p<0.05 significant).
Ghrelin was cardioprotective, and this protection was associated with significant anti-inflammatory actions. Ghrelin treated animals exhibited myocardial MCP-1 and ICAM-1 by 68.3% and 61.3% (both p<0.05), respectively. These findings correlated with a 58.3% reduction of mononuclear infiltrates in the myocardium (p<0.05). These anti-inflammatory actions were associated with significantly reduced circulating levels of CRP (p<0.05). Conclusions: Exogenous administration of the anti-inflammatory protein, ghrelin, was cardioprotective. It suppressed myocardial expression of MCP-1 and ICAM-1, and reduced mononuclear cell infiltration and myocardial damage. Based on these novel findings, we conclude that ghrelin is a potent suppressor of the myocardial inflammatory response and protects the myocardium against I/R injury.

**423 AUGMENTED TENDON ACHILLES REPAIR USING A TISSUE REINFORCEMENT SCAFFOLD: A BIOMECHANICAL STUDY**

Frizzell L, Giza E, Farac R, Williams J. UC Davis, Sacramento, CA.

**Purpose of Study:** Mixed or chronic Achilles tendon ruptures may have muscle atrophy, tendon retraction, and a defect that must be augmented with endogenous or exogenous materials. The Artelon® Tissue Reinforcement (ATR) scaffold is a readily available synthetic degradable polyurethane (urea) material used to augment tendon repair. The objective of this study was to compare load to failure of human cadaveric Achilles tendon repairs with and without ATR.

**Methods Used:** Eighteen fresh frozen human cadaveric Achilles tendons were dissected and a rupture was simulated using a cam proximal to the calcaneal insertion. The control group of nine specimens was repaired with sutures, while the experimental group was repaired with sutures and reinforced with a tubularized patch of ATR. Specimens were tested using a load to failure test on an Instron machine after preloading to 10 N followed by cyclic loading for 20 cycles from two to 30 N.

**Summary of Results:** The ultimate load to failure in the control group was a mean of 248.1 N ± 19.6 N (202 to 293 at 95% CI) versus 370.4 N ± 25.2 N (312 to 428 at 95% CI) in the ATR group. The ultimate load to failure was 370.4 ± 25.2 N (312 to 428 at 95% CI) and 248.1 ± 19.6 N (202 to 293 at 95% CI) in the experimental and control groups, respectively (p=0.0015). Creep of the ATR augment group was 2.051 ± 0.517 mm, compared to 3.126 ± 1.141 mm for the control group (p=0.026).

**Conclusions:** ATR is a readily available material that can be used to augment chronic or acute Achilles tendon ruptures. It is less bulky than currently available allograft or xenograft alternatives and provides a statistically considerable improvement in load to failure when compared to control specimens in a cadaver model. This finding may allow for development of more aggressive rehabilitation techniques following chronic Achilles tendon repairs.

MATLAB Accuracy: Calculation of relative marker motion yielded nonsense data with very large noise signal at small ranges of motion (~6300% at 0.005°), but became much more accurate at large displacements (1.3% at 1.500°). Accuracy remained poor even at large displacements in the Y-axis (A/P), with ~13% error, but was comparable between X- and Z-axes. Signal to noise exceeded 4:1 in the axis of interest at 0.020°, but noise in other axes was still significant.

**425 SEVERE PRE-LIVER TRANSPLANT CORONARY ARTERY DISEASE ASSOCIATED WITH SIGNIFICANT WORSENSING OF LATE POST-TRANSPLANT SURVIVAL**

Simon N1, Mangus S2, Kinsella S1, Fredell J1, Vianna R2, Quirk C1, Wilkes K1, Tector J1. Indiana University School of Medicine, Indianapolis, IN and 2Indiana University School of Medicine, Indianapolis, IN.

**Purpose of Study:** Coronary artery disease (CAD) is an important risk factor for perioperative complications and worse clinical outcomes in patients undergoing liver transplantation (LT). This study reviews the pre transplant cardiac history of a large number of LT recipients and evaluates post transplant outcomes for those with a history of severe CAD.

**Methods Used:** A complete cardiac history was available for 1122 of 1170 LT recipients (96%) from 2001 to 2010. Patients were categorized into 3 study groups, based upon a history of (1) no previous cardiac intervention, (2) a “remote” (>12 months pre transplant) intervention, or (3) “recent” (within 12 months of transplant) cardiac intervention. Intervention included either surgical revascularization, angioplasty or placement of a coronary artery stent. A Cox proportional hazards model was constructed using direct variable entry to evaluate long-term post-transplant patient survival.

**Summary of Results:** There were 59 patients with a history of pre transplant cardiac intervention (5.2%), 3.7% recent and 1.5% remote. Median follow up for this cohort was 59 months. Rates of 90-day post LT MI, stroke and DVT did not differ among the 3 study groups. The Cox patient survival curve is shown in Figure-1.

**Conclusions:** Patients with previous cardiac intervention, both recent and remote, had significantly worse long term post LT survival. However, these groups did not have significantly worse perioperative morbidity.

**424 VALIDATION OF REAL-TIME, LOW-COST STEREO VIDEOGRAPHY IN MEASURING 3D SPINE MOTION**

Namperumal S1, Leung E2, Curtiss S1, Roberto R1. UC Davis School of Medicine, Sacramento, CA and 2UC Davis, Davis, CA.

**Purpose of Study:** A low-cost spine motion measurement system is validated for accuracy and resolution to aid in the development of therapeutic, motion-sparing fixation hardware.

**Methods Used:** Stereo high-resolution digital video was taken of precise linear motions of LED triad markers in a 3D measurement space. Calculation of marker positions was performed using SIMI motion analysis software (SIMI Reality Motion Systems, Germany) using both automatic and manual tracking of marker motions. A previously validated MATLAB algorithm was then used to calculate the relative motion between markers. Error calculations were made for both SIMI and MATLAB displacement data to validate the accuracy of 3D motion measurement in the proposed system.

**Summary of Results:** Tracking: Noise generated at rest in automatic tracking (29%-81%, mean position of 0.0019°) was comparable to that of manual tracking (38%-87% of 0.0026°), and accuracy of automatic tracking was comparable to or exceeded that of manual tracking (mean error of 0.0003° to 0.0032°) over absolute displacements from 0.005° to 1.000°. Accuracy was comparable in all axes of 3D motion.

**Figure 1.** Cox proportional hazards model for post LT survival.
SONOGRAPHIC EVALUATION OF THE PERONEUS LONGUS AND BREVIS TENDONS: ANATOMICAL RELATIONSHIPS AT REST AND UNDER STRESS
Chen LE¹, Hager NA²,³ University of Washington School of Medicine, Seattle, WA; University of Washington Medical Center, Seattle, WA and University of Washington Medical Center, Seattle, WA.

Purpose of Study: Ultrasound has been considered a valuable imaging modality to assess superficial tendons due to its high resolution, noninvasiveness, and cost effectiveness. The normal locations of the peroneus longus and brevis tendons at rest and under stress have not been well characterized in the literature. It is hypothesized that the tendon position changes are correlated with the various provocative maneuvers and the stress applied to the tendons. The goal of the study is to identify the particular provocative maneuver of an ankle that demonstrates the greatest change in tendon positions. Practitioners could then rely on this maneuver as their primary assessment method.

Methods Used: The sample population consisted of 17 asymptomatic subjects (34 ankles) with no history of ankle pathology. Inversion, eversion, plantar flexion and dorsiflexion were performed, first passively without resistance then actively against resistance. Transverse dimensions were imaged.

The changes in the positions of the peroneal tendons at rest and under stress were determined by the displacement angle of the peroneus brevis tendon with respect to the peroneus longus tendon.

Summary of Results: At the resting position, 65% of the samples showed the peroneus longus tendon off-center lateral to the peroneus brevis tendon. No statistically significant difference was seen in the tendon displacements among four passive motions. Under stress, eversion combined with dorsiflexion demonstrated an averaged displacement of 66° ± 12° compared with the resting position, and this result does not show statistically significant differences from pure eversion (65° ± 6.9°). Inversion against resistance showed the least position change (25° ± 3.5°) from the tendons at rest.

Conclusions: Without resistance, the tendons do not show significant differences in positions among various passive maneuvers. The greatest displacements of the relative tendon positions were noted during eversion combined with dorsiflexion against resistance. These findings suggest that the optimal provocative test maneuver for assessing the status of the peroneal tendons is eversion and dorsiflexion of the foot against resistance.

PREDICTORS OF COMPLICATIONS IN CHILDREN WITH DUPLICATED RENAL COLLECTING SYSTEMS
Spelliscy C, Kiddoo D University of Alberta, Edmonton, AB, Canada.

Purpose of Study: 1. To identify which factors are correlated with urinary tract infections (UTIs) and decreased renal function in children with antenatally diagnosed duplicated renal collecting systems (DRCS).

Methods Used: EMRs were searched for patients diagnosed with hydronephrosis. The 217 patients identified were narrowed to 26 with DRCS. Data was collected on factors which may predispose to infection including gender, presence of vesicoureteral reflux, obstruction, ureteroceles and/or ectopic ureteric orifice. Other data collected included presence of foreskin, sidedness, constipation, infrequent voiding, surgical history, antibiotic use, family history, and past medical history. The primary and secondary outcomes were UTI and new renal scarring.

Summary of Results: 26 patients had DRCS diagnosed antenatally (12 males and 14 females between 6 months and 8 years old); 15/26(57%) had febrile UTIs. Of the male patients, 42% developed UTIs versus 64% of females. Within the group with vesicoureteral reflux alone 1/2(50%) developed an infection. Patients with both obstruction and reflux developed infections in 3/3(100%). Finally 10/14(71%) patients with obstruction alone developed infections. Regression analysis did not identify an association between the independent anatomic variables and UTIs (Ch2= 6.3,DF=3,p=0.10). No children developed progressive renal scarring.

Conclusions: Since the advent of pregnancy ultrasounds, children are identified early with collecting system abnormalities. Some children go on to have UTIs and/or renal damage; however, at this point we do not know which patients require early intervention. We found that children with DRCS have an extremely high rate of UTIs. We did not identify an association between UTIs and specific variables. While these infections may not cause ongoing scarring, they do contribute to morbidity. Antibiotic prophylaxis in all children with duplicated systems may warrant consideration in the first year of life. We are currently expanding our patient data base.

RAISING AWARENESS OF ELDERLY SUICIDE RISK IN SANDPOINT, IDAHO
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Purpose of Study: Idaho’s five most northern counties, known as Region 1, have the highest rate of suicide in Idaho. The age group at highest risk of suicide is age 65 and older, but this information has been mostly unknown among local organizations serving older adults. The Region 1 community of Sandpoint, Idaho includes over 1,000 elderly residents in this unique risk category. The purpose of this project was to raise awareness of this public health problem and catalyze a local prevention effort.

Methods Used: Initial surveillance began by collecting information from census and vital statistics reports, speaking with clinicians and community organizations, and observing elderly mental health counseling in a community health center. Other investigation included meeting with the Region 1 suicide prevention network chairperson and examining the State of Idaho Suicide Prevention Plan. A professional literature review helped define specific risk factors and evidence of effective community suicide prevention efforts. The project culminated in a community roundtable discussion among public agency representatives. Prior to discussion, attendees received a summary hand-out and presentation of the scope of the elderly suicide problem and ideas for community interventions. Sandpoint Community Resource Guide and Suicide Prevention Action Network brochures were also distributed.

Summary of Results: Nine community agency representatives attended the roundtable, including: Chief of Police, director of the Senior Center, president of the local Vietnam Veterans of America chapter, elder advocate columnist from the local newspaper, and North Idaho’s suicide prevention network chairperson. Attendees discussed the lack of community awareness of the elderly suicide problem, shared information about their agency resources, and agreed that prevention training should be publicized and widely available beyond medical and mental health providers. A closing survey revealed that all attendees were more aware of the elderly risk and motivated to work with each other to make a positive impact on elderly mental health.

Conclusions: Raising awareness among public service agencies provided the first step toward community suicide prevention efforts. Networking at the roundtable provided support for existing suicide prevention action networks and foundation for agency collaboration.

HORTICULTURAL THERAPY FOR PERSONS WITH SEVERE DEMENTIA
Bomalaski MN University of Washington, Seattle, WA.

Purpose of Study: It is estimated that around 7% of persons over the age of 65 have diagnosable dementia, with the risk of dementia doubling every five years. With 23% of residents in Lewistown, MT over the age of 65, the prevalence of dementia is projected to continue rising, along with costs and challenges to caregivers. The Montana Mental Health Nursing Care Center (MMHNCC) in Lewistown, MT is currently home to 90 inpatients, including 14 residents with severe dementia. Horticultural therapy (HT) has been shown to improve emotional well-being and social functioning, while reducing confusion, agitation, and anxiety. The purpose of this project was to design a program of HT for the dementia-affected residents of MMHNCC, providing opportunities for constructive engagement through a culturally familiar activity.

Methods Used: Interviews with staff at the MMHNCC revealed challenges to constructively engaging residents with severe dementia. Because of the cultural significance of agriculture to this region, HT was identified as an appropriate intervention. A review of the literature was performed to assess the efficacy of HT, and to determine HT methods appropriate for those with cultural backgrounds in agriculture.
dementia. An HT activity was conducted with dementia patients at the MMHNC, and a multisensory garden was designed in collaboration with local garden and landscape architects.

**Summary of Results:** An activity of HT was conducted with four residents with severe dementia. Participants were encouraged to appreciate the sensory qualities of each plant and to recall pleasant memories associated with horticulture. Participants were observed to have improved mood and constructive engagement throughout the activity. A document was created with plans for a multisensory garden, including specific design considerations, suggested plantings, ideas for accommodating persons of different abilities, and relevant literature and community resources. This was distributed to recreation staff of the MMHNC, as well as local arts and gardening organizations.

**Conclusions:** Creating spaces for personal enrichment is vital for improving the quality of life of those suffering from severe dementia. The positive effects of the HT activity and community support for designing a therapeutic garden indicate the potential for the future use of HT at the MMHNC and other health care settings.

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**430 ALTERNATIVE INTERVENTIONS IN A STATE WITHOUT NEEDLE EXCHANGE TO REDUCE INFECTION AMONG INJECTION DRUG USERS IN BUTTE, MT**

Campbell CJ; University of Washington School of Medicine, Seattle, WA.

**Purpose of Study:** Butte, Montana is known for its high incidence of IDU drug use and associated diseases. Despite increases in the incidence of HCV and HIV in a state where 43% of methamphetamine users inject for drug delivery, the State of Montana does not have any Needle Exchange Programs. Without access to sterile needles, alternative interventions for infection risk reduction must be promoted. Bleach-mediated disinfection, while not completely eliminating infection risk, has been shown to be the most effective needle disinfection method when properly utilized. This project aims to increase awareness of needle disinfection techniques within the Butte IDU community.

**Methods Used:** The need for IDU educational-based intervention was supported by consultation with the Butte-Silverbow Health Department. A review of the professional literature provided background for educational materials aimed at IDU’s. An evidence-based protocol was developed for bleach-mediated disinfection, and an educational brochure was developed specifically for IDU’s. The disinfection protocol and brochure were presented to the Butte-Silverbow Health Department and five local social agencies that are highly visited by IDU’s. Lastly, a presentation on needle disinfection and safer drug use was given to the residents of a local transitional housing facility.

**Summary of Results:** The comprehensive brochure was developed to inform the IDU community regarding the proper disinfection protocol, misconceptions about “safe” injection, locations for free disease testing, facts about HCV, HBV and HIV, and local resources for quitting drug use. To ensure maximum sustainability, the brochure and disinfection protocol were incorporated into the health department’s HIV, HCV, and injection drug use educational initiatives. Lastly, this project used harm reduction principles to raise awareness of safer drug use among a high-risk population.

**Conclusions:** While the bleach-media method is the most effective method for reducing infection risk, proper bleach-mediated disinfection of needles can be a highly effective alternative intervention. Until the IDU’s of Butte have access to sterile equipment, education regarding proper disinfection must continue to ensure an optimal reduction in disease transmission.

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**431 ADDRESSING INTIMATE PARTNER VIOLENCE IN A BLACKFEET COMMUNITY**

Clark EA; University of Washington, Seattle, WA.

**Purpose of Study:** Intimate partner violence (IPV) poses a disproportionate risk to American Indian and Alaska Native (AI/AN) populations. About 1/3 of AI/AN women experience rape and 2/3 experience physical abuse. IPV is harmful to all women, and pregnant women face greater risks. The Blackfeet community in Browning has identified IPV as an issue; last year, law enforcement officers received 329 reports of domestic violence. The purpose of this project was to educate community members about the health consequences of IPV and increase awareness of resources available to address this issue.

**Methods Used:** The actions of this project were: 1) a 1/2 hr educational program was designed and implemented in cooperation with the Blackfeet Domestic Violence Program (BDVP) for three prenatal care groups, reflecting current health concerns for pregnant women. 2) a 1 hr educational session on traumatic brain injury was presented to a men’s batterer’s group showing neurological and cognitive effects of even “mild” head injuries. 3) resource lists were compiled for both with contact information for local, state and national agencies dealing with domestic violence. 4) a meeting with Blackfeet Community Hospital health care providers and staff of the BDVP was facilitated to strengthen inter-agency relationships.

**Summary of Results:** 15 patients and 10 companions attended the prenatal educational sessions. Two men attended the batterer’s support group; both had either experienced head injury themselves or knew someone who had. Patient and provider resource lists were distributed to each provider and were requested by three community groups. The meeting with providers and the BDVP was well received, and BDVP plans to do more educational sessions with providers and patients.

**Conclusions:** The project’s multifaceted approach allowed the complex issue of IPV to be explored from many angles. The survivors, perpetrators and care providers were each addressed separately, but in a way that strengthened inter-community relationships, with agencies sharing responsibility. Browning faces significant domestic violence, and one of the main project objectives was to increase community awareness of available resources. The cooperation between the hospital and the BDVP suggests a future of coordinated efforts in providing care and recovery to survivors of IPV.

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**432 PEER-SUPPORT IN THE MANAGEMENT OF DIABETES IN THE URBAN AMERICAN INDIAN POPULATION OF SEATTLE, WASHINGTON**

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**Purpose of Study:** The prevalence of diabetes mellitus amongst American Indian/Alaska Native (AI/AN) adults is estimated to be 16.5%, the highest of any ethnic group worldwide. The Seattle Indian Health Board (SIHB) is one of 34 urban affiliates of the Indian Health Service (IHS) serving 7201 patients, 18.1% of whom are diabetic. The purpose of this project was to improve the quality of care for the diabetic patient population served by the SIHB by addressing the need for low cost and culturally relevant diabetes education and management.

**Methods Used:** Consultation with the SIHB agencies helped in recognizing the diabetic population as an appropriate target for a community-based intervention. Discussions with providers, patients, and community elders were critical in revealing areas of universal misunderstanding about diabetes. Research was conducted to produce a comprehensive list of accurate, relevant, and cost-free resources for diabetes information and support. An informational handout addressing diabetes myths common to the AI/AN community were generated for SIHB patients. A survey was developed in order to quantify interest of diabetic patients in becoming peer-support educators. Groundwork was laid to establish a longitudinal program for peer-support and diabetes counseling.

**Summary of Results:** A round-table discussion including 13 diabetic patients, three members of the diabetes team, and one physician was successfully facilitated. All feedback regarding the session and the informational handout was positive. Providers, staff, and community elders all agreed that peer-support and mentoring is a culturally appropriate and effective method for low cost diabetes prevention. Collaboration between the SIHB diabetes team and key AI/AN community members was established in order to move forward with the development of a peer-support training program.

**Conclusions:** Culturally relevant methods are critical to the successful prevention and management of chronic illness. Implementation of peer-support has been shown to be an effective method of raising awareness and compliance in various ethnic communities, both rural and urban. Although it is a relatively new approach in the treatment of diabetes, peer-education promises to be a useful tool in the promotion of education and trust in the AI/AN diabetic population.

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**433 OCCUPATIONAL HEALTH STATUS AND NEEDS OF KOREAN IMMIGRANT WORKERS IN LOS ANGELES COUNTY**

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**Purpose of Study:** Los Angeles County has the largest population of Koreans outside of Korea, comprising approximately 2.1% of the County’s
population (207,000). Our objective was to conduct an exploratory study on the work-related health needs of Korean immigrants in the Los Angeles area.

Methods Used: We partnered with UCLA’s Koreatown Community Health Center that routinely conducts medical and social assessments at local community venues. We extracted occupational health information from charts of 132 patients who presented to community health outreach events at two Korean churches. Univariate and bivariate analyses were conducted using Stata 9.

Summary of Results: Of the 132 patients, 65.2% were working and 10.5% were unemployed. Twenty-three percent of the working population was self-employed, compared to 7.5% of the average American worker. The average hours worked per week was 45.6 hours for all of the workers and 54.7 hours for full-time workers. Fifteen percent were working at jobs that required education above the level they had attained and 40% were working at jobs below the level of their education. Almost half (48.2%) reported working at least six days per week. Approximately 35 (34.0%) people reported having some type of health problem as a consequence of their work. The common listed occupational health problems were various body pains, organ-based problems, and “stress”.

Conclusions: In our small community sample of Korean immigrants, we found a greater prevalence to work long hours, and many suffer from various work-related health problems. Our results highlight the importance of addressing occupational health among the Korean immigrant patients in clinical settings.

434 DIABETIC FOOT CHECKS AND EDUCATION IN THE HISPANIC COMMUNITY OF BREWSTER, WASHINGTON

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Purpose of Study: The Hispanic community is the fastest growing minority group in the United States, and now comprises nearly 16% of the total population. Since Hispanics have a higher risk for developing type 2 diabetes and are most likely to be uninsured, early intervention is important. In Brewster, Hispanics make up over half of the local population and diabetes is endemic. The purpose of this project was to provide microfilament foot exams for Hispanic patients with diabetes, to educate patients, and to improve self-foot care within the community.

Methods Used: Demographic analysis of the local population, discussions with local providers, and clinical experience highlighted the need for diabetes education. A literature review revealed the efficiency/efficacy of foot education for amputation prevention and provided information for the educational packets. The Brewster Sacred Heart Catholic Parish was used to access the potential patients because church attendance is a community expectation for the local Hispanic population. Six services were attended over 3 days, 5 of which were in Spanish. The project was announced during each service, and afterwards educational information was distributed and diabetic parishioners could participate in the microfilament exam. A Bible verse about foot washing was provided in the packet to connect the shared community experience with self-foot care. Throughout the foot exam, an oral survey was taken to gauge the intervention’s effectiveness. The priest translated.

Summary of Results: The 6 services drew more than 500 people, of whom 40 people requested information and 60 received exams. 15% stated that they already performed foot checks at home, but all except for one were English speakers. 77% stated that they would begin performing regular foot checks after this event. 17 foot exam participants thought the Bible verse was a good reminder for foot checks. Everyone stated that they would attend the microfilament exam again if offered.

Conclusions: To access the Hispanic community it was necessary to attend established events to have support from community leaders. Material must be available in Spanish and contain images, due to limits of English proficiency and literacy.

435 THE EDUCATION OF WOMEN ON SMOKING CESSION AND LIMITING SECONDHAND SMOKE EXPOSURE IN WEISER, IDAHO

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Purpose of Study: Cigarette smoking and secondhand smoke exposure is quite prevalent in the city of Weiser and its neighboring communities. Every year over 1,400 Idahoans die from smoking-related diseases according to the Behavioral Risk Factor Surveillance System. Currently about 12-20% of pregnant women are smokers and approximately 27% of children under the age of 6 live with a caregiver who smokes. In the past, efforts have been made to promote smoking cessation for the general population, but no local programming has been done to educate pregnant women. The purpose of this project was to educate pregnant women about the adverse effects of smoking and secondhand smoke exposure and in addition provide them with resources for cessation or to limit their exposure.

Methods Used: Patients of the target group encountered in a clinical setting were polled on their thoughts on smoking cessation as a means of evaluating the need in the community. In addition, clinicians, medical staff, smoking cessation instructors and WIC educators were interviewed to identify resources and challenges in programming for pregnant women. A literature review was done for validation of chosen methods and creation of educational materials. A brief informational session on smoking cessation and secondhand smoke exposure was conducted as part of a breastfeeding class series at the Southwest Health District. In addition, an educational brochure was created and distributed at the informational session.

Summary of Results: About eight to ten pregnant women attended the first class which is the average class size for this venue. The intent was to continue to incorporate this informational session in future class series and to disseminate brochures through local medical facilities. The purpose of this project was to educate pregnant women on the adverse effects of smoking and secondhand smoke exposure, programming must be focused on resources and information specific to pregnant women. This approach also integrated into other already existing programs offered to pregnant women, could be largely successful in addressing this health issue in the community.

436 EPIDEMIOLOGY OF COMPLEX REGIONAL PAIN SYNDROME

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Purpose of Study: Complex regional pain syndrome (CRPS) is a painful progressive neurologic condition, usually developing secondary to an inciting traumatic event. It is understudied and poorly understood with most data investigating treatment modalities and coming from hospital based settings, with minimal epidemiological data. The purpose of this study was to obtain further epidemiological information on CRPS.

Methods Used: Billing codes for CRPS 1 were used to identify 148 patients treated from January 2005 through August 2010 at a rural outpatient pain clinic in south east New Mexico. Charts were reviewed and 117 patients satisfied the current IASP diagnostic criteria for CRPS 1. Patient demographics, injury profile, relationship to work, type of referring physician, and length of time from injury to referral were analyzed via the SAS computer program and reported as means +/- the standard deviations or using standard statistics to attain frequency distributions and percentages.

Summary of Results: Of the 117, there was an equal percentage of males and females, the average age was 48+/−12.5) and the ethnicity profile was 59% Caucasian, 33% Hispanic, 3% Native American, and 6% unrecorded. 29% of the initiating injuries involved surgery; 28% were associated with crush/contusion injuries. 75% of the patients had their employment change secondary to this condition with 60% losing their benefits. The median number of days from onset of symptoms to referral to the pain clinic by a primary care provider was 1226 days in contrast to 292 days for surgeons, which was a significant (p-value=0.046) difference in referral patterns.

Conclusions: This data shows CRPS has no gender preference and affects some type of health problem as a consequence of their work. The common listed occupational health problems were various body pains, organ-based problems, and “stress”.

437 BUPRENORPHINE THERAPY FOR OPIOID ADDICTION IN RURAL WASHINGTON: THE EARLY ADOPTERS

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Purpose of Study: Rates of opioid abuse in the United States have reached unprecedented levels. In Washington State, opioid overdoses result in more...
deaths than car accidents. Rural providers face many challenges addressing this issue, ranging from limited local resources to the inconvenience or impossibility of accessing methadone clinics. Buprenorphine is a partial opioid agonist-antagonist approved in 2001 for the use of treating opioid dependence and addiction. This therapy is particularly useful in the treatment of opioid addicted patients in rural settings. Since 2001, little is known about the impact of buprenorphine on rural physicians and their experiences with treatment. This study seeks to find and share the stories of early buprenorphine adopters in rural Washington and aid providers in their treatment of addiction.

**Methods Used:** This investigation surveyed all rural physicians certified to use buprenorphine and recognized by the State of Washington as practicing in a rural county. Physicians were contacted and consent was obtained to participate in an interview based on an established questionnaire. This questionnaire elicited discussion on a wide range of issues concerning their rural experiences, efficacy, and recommendations, as well as provided numerous opportunities to collect data and analyze results in a qualitative and quantitative fashion. The primary outcome measures for the study were the extent to which certified physicians were using buprenorphine in their practices, the methods they were using to manage patients in rural settings while on opioid replacement therapy, and their recommendations on the efficacy of this intervention.

**Summary of Results:** While most physicians are overwhelmingly satisfied with the impact of buprenorphine on their practice, these same physicians have encountered numerous barriers and provided specific suggestions as to what additional resources are needed to improve rural treatment of opioid addiction, particularly with regard to counseling services.

**Conclusions:** Buprenorphine is a valuable treatment in rural settings, but physicians need more behavioral health resources to address the problem of opioid addiction and dependence.

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**438 IS PHYSICIAN NON-PROFESSIONAL EXPERIENCE WITH CANCER ASSOCIATED WITH OVARIAN CANCER SCREENING PRACTICES?**

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**Purpose of Study:** No professional organization recommends routinely providing ovarian cancer screening to asymptomatic patients. However, many physicians recommend screening for patients, unnecessarily exposing them to screening harms. Though many patient and physician variables have been associated with cancer screening, little research exists on the influence of personal experience with cancer on physicians’ screening practices. This study assesses whether physician non-professional experience with cancer influences adherence to ovarian cancer screening guidelines.

**Methods Used:** A mail survey with a female annual exam vignette and questions about recommendations for cancer screening tests, including transvaginal ultrasound and cancer antigen 125, was sent to a random sample of 3200 U.S. family physicians, general internists, and obstetrician-gynecologists. This analysis included 503 physicians representing 48,067 physicians nationally.

**Summary of Results:** An unadjusted analyses, 86% of physicians without non-professional cancer experience reported adherence to ovarian cancer screening guidelines (never recommending screening to an asymptomatic patient at average risk of having ovarian cancer).

**Conclusions:** Despite recommendations to the contrary, many physicians recommend ovarian cancer screening in low-risk women. Physicians without their own or familial exposure to cancer are more likely to recommend unnecessary screening than physicians without this experience.

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**439 IMPACT OF ANKLE BRACING ON SKILL PERFORMANCE OF RECREATIONAL SOCCER PLAYERS**

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**Purpose of Study:** In many sports, ankle injuries are common and often very minor. While most athletes can return to play soon after the injury, some choose to wear ankle braces to prevent re-injury. A number of past studies have shown that ankle braces are effective as prophylactics to injury, however, there are conflicting studies relating to the impact of braces on performance. The purpose of the study is to determine the effect of ankle bracing on skill performance in healthy recreational soccer players. It was our hypothesis that ankle bracing would have a negative effect on performance initially, but not after a period of acclimation.

**Methods Used:** A prospective randomized study conducted with twenty healthy recreational adult soccer players (males=5, females=15) of average age 23 years (16–40 years old) participated in the prospective randomized study. Each subject completed two testing sessions (S1 and S2) one week apart on a turf soccer field. During each testing session, subjects completed a set of performance measures (40-yard sprint, T-test, S180 and a kicking accuracy test) with and without an ankle brace (McDavid 199 Lightweight). Between testing sessions, subjects wore the ankle brace to ensure acclimation.

**Summary of Results:** There was no significant difference in performance of the 40-yard sprint, T-test, S180 and kicking accuracy test (P>0.05) with and without use of an ankle brace during a specific testing session (S1 or S2). There was no significant difference in performance of the 40-yard sprint, S180 and kicking accuracy test (P>0.05) with and without use of an ankle brace when comparing testing sessions (S1 vs S2). There was a significant difference between performance of the T-test (P<0.05) between testing sessions (S1 vs S2) for subjects without an ankle brace, but not with use of the ankle brace.

**Conclusions:** Ankle bracing does not appear to significantly affect performance in speed, agility, or kicking accuracy in healthy, competitive, recreational soccer players. Future studies are needed to assess the impact of ankle bracing on skill performance in elite soccer players and athletes with a history of ankle sprains, or acute ankle sprains.

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**440 LOW CARDIAC INDEX IN ASYMPTOMATIC HEART TRANSPLANT PATIENTS AT ONE YEAR POST TRANSPLANT DOES NOT LEAD TO POOR LONG TERM OUTCOME**

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**Purpose of Study:** The denervated heart after heart transplant exhibits different physiology. Heart transplant patients appear to have smaller stroke volume, and therefore a lower cardiac index (CI) despite faster heart rate due to the lack of vagal tone in the denervated heart. Some asymptomatic heart transplant patients have excessively low CI found at the time of routine annual heart catheterization, which may be due to a smaller donor heart or to a significant increase in weight after heart transplant, resulting in a lower CI. It is not clear whether this low CI is a marker for poor outcome long term after heart transplantation.

**Methods Used:** We evaluated 892 patients transplanted between 1994 and 2010, and assessed those patients that survived 1 year post transplant who were evaluated with routine protocol annual cardiac catheterization. Patients were divided into 2 groups, those with CI<2.0 L/min/m2 (n=20) and those with CI≥2.0 L/min/m2 (n=339), and then assessed for subsequent 5-year outcomes, including actuarial survival, freedom from cardiac allograft vasculopathy (CAV, angiographic stenosis ≥30%), and freedom from non-fatal major adverse cardiac events (NF-MACE, MI, CHF, PTCA, pacemaker, stroke, new peripheral vascular disease). We also looked at 1st-year freedom from any treated rejection in these patients.

**Summary of Results:** All patients were asymptomatic and were noted to have normal cardiac function by left ventriculogram at the time of heart catheterization. Patients with CI<2.0 L/min/m2 were found to have similar subsequent outcomes compared to those patients with CI≥2.0 L/min/m2, including survival (75% vs. 85%, p=0.16), freedom from CAV (65% vs. 78%, p=0.15), and freedom from NF-MACE (89% vs. 94%, p=0.53). 1st-year any-treated rejection was also found to be similar between the two groups (85% vs. 91%).
p=0.38). In the low CI group, only 5/20 patients had a BMI >30, and no patient had a BMI >33. The mean BMI in the low CI group was 26 ± 5. Of the 20 patients, there was no significant donor-recipient size mismatch.

**Conclusions:** Low CI <2.0 L/min/m² does not appear to be an important factor for poor long term outcome after heart transplantation. The denervated heart model may result in decreased cardiac index, but does not appear to not impact long term outcome.

441 ADDRESSING MEASLES VACCINATION REFUSAL IN THE UKRAINIAN COMMUNITY OF MOSES LAKE, WASHINGTON

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**Purpose of Study:** Moses Lake has one of the highest percentages of residents born in Ukraine of all U.S. communities. The Ukrainian community of Moses Lake is a highly religious Christian group that has consistently refused to immunize their children. According to physicians at Moses Lake Community Health Center, an outbreak of measles occurred in 2008 where about a dozen individuals became ill. The purpose of this project was to 1) listen to concerns of the Ukrainian community regarding vaccination, 2) create a positive interaction between the community and a healthcare provider, 3) increase public awareness of the need for measles immunization while addressing concerns of community members.

**Methods Used:** A literature review was performed to investigate effective communication methods with the Ukrainian community and to guide creation of educational material. A discussion in Russian regarding vaccination concerns was held with community members at the end of Sunday church service. Conversation topics included 1) willingness of members to vaccinate their children and 2) concerns about vaccination and in particular, the MMR vaccine. A pamphlet in Russian and English was subsequently created to provide education about measles and the MMR vaccine while addressing common vaccination concerns.

**Summary of Results:** Seven female church members chose to stay after Sunday service to discuss vaccination concerns. All participants were firmly against vaccination. Common reasons included 1) a belief that vaccines cause illnesses such as autism, paralysis, tremors, and a “vegetative state,” 2) safety concerns about preservatives used in vaccines, 3) a belief that real diseases develop the immune system “naturally” while vaccines “slow it down.” These issues were addressed in the educational pamphlet which was distributed to three family physicians and one pediatrician at Moses Lake Community Health Center for patient education.

**Conclusions:** Fear of vaccination is deeply rooted in the Ukrainian community of Moses Lake. This project was only a first step in providing immunization education while addressing fears of mothers in a culturally sensitive manner. To increase vaccination rates in the long term, it is crucial for healthcare providers to continue building a trusting relationship with the Ukrainian community.

442 PNEUMOCOCCAL DISEASE VACCINATION AND PREVENTION EDUCATION FOR ALASKA NATIVE ADULTS IN THE BRISTOL BAY REGION

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**Purpose of Study:** Incidence rates of Invasive Pneumococcal Disease (IPD) among Alaska Natives are 2-3 times the rates for non-native Alaskans, and Alaska natives are experiencing IPD at younger ages compared to non-native Alaskans. This is of serious concern because IPD is often fatal. The purpose of this project was to educate high risk Alaska Native adults in specific communities in the Bristol Bay region of Alaska about IPD prevention. Vaccination with the pneumococcal polysaccharide vaccine (PPSV) and smoking cessation programs were stressed because of the proven efficacy of vaccination and smoking cessation in preventing IPD.

**Methods Used:** At the beginning of this project information was gathered from local health officials and state epidemiologists to identify groups of Alaska Native adults in the Bristol Bay region at particularly high risk for IPD. Once a subpopulation was identified, a multistep approach was taken to educate this group about IPD, the PPSV and smoking cessation programs available in the region. In order to gauge the level of understanding about IPD in the community, an initial education campaign was performed using CDC fliers and IPD fliers specific for the Alaska Native population. Once the baseline level of understanding about IPD was assessed for the target population, posters, fliers, and public service announcements were created and used to spread information about local PPSV vaccination and local smoking cessation programs. The specific communities targeted in this phase of the project included Dillingham, Naknek, South Naknek and Egegik.

**Summary of Results:** A population of Alaska Native adults in the Bristol Bay region of Alaska was identified and evaluated for targeted educational intervention on IPD. Population sensitive educational materials about locally available PPSV vaccination and smoking cessation programs were created and distributed specifically targeting this high risk group.

**Conclusions:** This project strives to increase public understanding for IPD and IPD prevention strategies among high risk Alaska Native adults in specific communities in the Bristol Bay region of Alaska. This project was successful in its goal. In addition, the educational materials created through this project will be used in the future to educate high risk Alaska Native adults in the Bristol Bay region about IPD prevention.

443 DISPELLING MYTHS AND PROVIDING FACTS ABOUT CHILDHOOD IMMUNIZATIONS TO THE EASTERN EUROPEAN COMMUNITY OF AUBURN, WASHINGTON

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**Purpose of Study:** Individuals from the former Soviet Union make up a large portion of the immigrant population in Washington. Over 135,930 people immigrated to Washington State between 1990 and 2000. Currently, the majority of the Ukrainian population lives in south King County including Auburn. Since the communities are close knit, people often seek advice within the community. Over the past couple of years, vaccination rates of children have dropped due to parent refusal. This is largely due to myths of children being debilitated by vaccines. The purpose of this project is to inform the Ukrainian and Russian community in the Auburn area about the benefits of childhood vaccination and dispel popular myths.

**Methods Used:** In order to develop a curriculum that focused on the most prevalent myths and misunderstandings about childhood vaccinations, patients were contacted via phone and also at the HealthPoint of Auburn Clinic. The clinic identified the target population and provided phone numbers. After an informal conversation with patients, a list of top vaccination concerns was compiled. Each of these concerns was thoroughly researched by reviewing relevant studies and Power Point presentation was developed. Parents were invited via flyers and email. The presentation was conducted in Ukrainian in August. Parents had the opportunity to ask a panel of physicians’ questions after the presentation.

**Summary of Results:** While the majority of parents do vaccinate their children, many still have concerns about vaccine safety and effectiveness. Top concerns that parents expressed included: vaccines cause autism, vaccines are not safe, vaccines are not effective and harmful chemicals are injected. This was based on 20 people interviewed. Eight people attended the presentation. Many of those who could not attend had questions answered via telephone. The success of this project spurred the clinic to adopt more culturally sensitive ways of increasing immunization rates in this population. A link to the presentation will be available on the clinic’s website.

**Conclusions:** More work needs to be done to provide parents accurate information about childhood vaccines. Much of this can be done by improving patient trust of the providers and providing forums in which parent’s concerns can be addressed. Literature in the native languages is also helpful.

444 COMBATTING TICKS IN COLUMBUS: EDUCATING THE BOY SCOUTS IN TICK AWARENESS

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**Purpose of Study:** In comparison to most of Montana, Stillwater County’s climate is mild and relatively moist. This unique climate, combined with the county’s location along the Yellowstone and Stillwater Rivers sets the stage for an environment rich in plant life that hosts ticks not endemic to the majority of Montana. Many outdoorsmen, including kids, are exposed to ticks on an almost daily basis as their activities lead them through the underbrush that surrounds the rivers. Although actual tick-borne illness incidence is low in Montana, physicians report encountering erythema migrans in the population and several cases of relapsing fever have been documented. By educating young leaders of the outdoors, the local Boy Scout troop, on the
risks associated with tick bites and tick-borne infections, this project aims to create a more tick-savvy culture among the youth and limit the incidence of tick bites and associated complications.

Methods Used: The Scouts were taught a set of risk-limiting steps as published by the Infectious Disease Society of America. This was accomplished through an interactive workshop addressing each step individually. The steps corresponded to specific problems at different stages along the exposure timeline, beginning with pre-exposure preparedness and continuing through bite-wound complications such as infection. The steps were taught in a chronological order: identification of problem habitats, avoidance, prevention/protection, self-examination, removal, and red flags of infection.

Summary of Results: As part of the presentation, students learned a hands-on method of correct tick removal technique, became proficient at identifying the duration of tick attachment, and came to understand the importance of prompt removal in minimizing the transmission of tick-borne disease.

Conclusions: One of the main goals in choosing a Boy Scout audience for this project was to help disseminate information on tick awareness throughout the community. To conclude the session, the Boy Scouts were reminded of their role as leaders in their peer groups and their call to educate their peers in the knowledge they find so integral to outdoor activities. The long-term goal of this project could take years to develop, but the desired result is a more tick-conscious youth in Columbus, who understand the risks ticks impose and are better able to minimize the risk of infection.

446 SMALL PLATELET CATIONIC PEPTIDES DEMONSTRATE MICROBICIDAL ACTIVITY AGAINST MYCOBACTERIUM SMEGMATIS

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Purpose of Study: Mycobacterium tuberculosis is the causative agent of Tuberculosis which affects 1/3 of the total world population. Effective drug therapies have been hindered by development of resistance to antibiotics and other multi-drug therapies. Studies have shown that small platelet cationic peptides have had antimicrobial effects on many microbes including but not limited to Staphylococcus aureus. We hypothesized that these same synthetic peptides would exhibit microbicidal activity against other gram positive bacteria such as those in the genus Mycobacterium. To accomplish this we mixed known dilutions of Mycobacterium smegmatis, a less pathogenic yet rapidly growing mycobacterial species, with 5 novel synthetic peptides. Following an incubation period, we plated the serial dilutions on agar plates. These plates were compared to control plates to determine if any microbicidal activity had occurred.

Methods Used: Mycobacterium smegmatis was grown to mid logarithmic phase to an O.D. 600 of 0.7. Cells were diluted to 1,000,000 CFU/ml and specific synthetic peptides were added to concentrations between 0.5 and 8 micrograms/ml. Following an incubation period, serial dilutions at 0, 3, 6, and 24 hours were plated on 7H10 agar plates. Different concentrations of the synthetic peptides were used for efficiency purposes. Plates that received exposure to the synthetic peptides were compared to a control group of plates that received no exposure to peptides.

Summary of Results: Results revealed microbicidal activity against Mycobacterium smegmatis at the 24 hour mark for most of the 5 synthetic peptides at a buffer pH of 7.3 and 3 out of 3 peptides assayed at pH 5.5.

Conclusions: Typically, Mycobacterium tuberculosis can resist lower pH levels that exist within phagocytic compartments of activated macrophages. Because some peptides have microbicidal activity at both pH 7.3 and pH 5.5, these peptides are predicted to have increased antimycobacterial activity in the human body as the peptides can attack microbes both in areas of neutral pH and areas of acidic pH where many mycobacterial bacilli are thought to reside.

447 INVERSE CORRELATION OF INITIAL CD8 LYMPHOCYTE COUNT AND CD4 LYMPHOCYTE RESPONSE TO COMBINATION ANTIRETROVIRAL THERAPY IN TREATMENT-NAIVE VETERANS

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Purpose of Study: Determining which clinical factors are most important in predicting immune response to antiretroviral therapy, as measured by increase in CD4 lymphocyte count, is of great clinical importance.

Methods Used: We reviewed electronic medical records of 146 veterans previously naive to any antiretroviral therapy who started combination antiretroviral therapy (cART) from 1995 to 2010 to determine the association of the following clinical factors (determined within 6 months of starting cART) with immune response to treatment, as measured by CD4 area under the curve above initial CD4 count (CD4AUC) at 6 months (6m), 1 year (1y), and 3 years (3y) following cART initiation: age, calendar year of cART initiation, initial CD4 and CD8 lymphocyte counts, log(HIV RNA), hemoglobin, estimated glomerular filtration rate, a modified FIB-4 index indicating liver function, hepatitis C antibody, and components of initial cART regimen. Multivariate regression models were constructed, with backwards elimination used to exclude variables with p-value > 0.20.

Summary of Results: 131, 120, and 67 patients had CD4AUC data available at 6m, 1y, and 3y, respectively. Higher CD8 count was most strongly associated with lower CD4AUC at all time points (6m p=0.001, 1y p=0.013, 3y p=0.003). Older age was also associated with lower CD4AUC at 6m (p=0.026). Presence of ritonavir was associated with higher CD4AUC at 1y (p=0.039). Log(HIV RNA) was associated with higher CD4AUC at 3y (p=0.021).

Conclusions: A high initial CD8 count was the strongest predictor for decreased CD4 response to cART. Further study of the role of CD8 presence, activation, and apoptosis in CD4 response to cART is suggested.
in organ transplant recipients is high. Treatment of LTBI during transplant candidacy is recommended but is often avoided during liver transplant candidacy due to concerns of hepatotoxicity associated with isoniazid (INH). Treatment of LTBI with rifampin (RIF) is an option per American Thoracic Society guidelines. Treatment with RIF in the candidacy period may cause less hepatotoxicity to the failing liver than INH but has not been adequately studied. We retrospectively reviewed patients with LTBI who underwent liver transplantation at UCLA to assess strategies and outcomes of treatment.

Methods Used: Patients with LTBI (defined as PPD >5mm and no prior treatment) who underwent liver transplantation from 2005-2010 were identified from transplant databases. Type of LTBI treatment, lab data, complications, and clinical history were assessed via medical record review. Microbiology records were reviewed to identify all patients with cultures positive for Mycobacterium tuberculosis.

Summary of Results: 31 patients were identified with LTBI in the transplant candidacy period, of which 22 (71%) were male. Underlying diseases include hepatitis B (42%), hepatitis C (48%), alcoholic liver disease (10%), and non-alcoholic steatohepatitis (5%). Six patients (20%) received complete treatment during candidacy, 4 with INH and 2 with RIF. Treatment was initiated in the post-transplant period. The remainder were observed without LTBI treatment. None of those treated developed hepatic dysfunction or adverse effects attributable to their LTBI. None of the 31 patients developed active TB during the period of follow-up. One patient identified from microbiology records died of complications of extrapulmonary TB post-liver transplant; this patient had not undergone PPD testing or received prophylaxis prior to undergoing transplant.

Conclusions: Treatment of LTBI in the liver candidacy period was not associated with differences in hepatic function or outcome as compared with patients who were observed, although the small number of treated patients precludes any definitive conclusions. The single patient with active TB had not been evaluated for LTBI and died of complications of his infection.

449 EVALUATION OF EXHALED NITRIC OXIDE AS A SURROGATE FOR AIRWAYS HYPERRESPONSIVENESS

Larson JL, Zeidler M, Kleerup E, Tashkin D. David Geffen School of Medicine at UCLA, Los Angeles, CA.

Purpose of Study: Invasive procedures such as bronchoscopy or induced sputum are required to determine the degree of the inflammatory response. Surrogate measures of inflammation, such as the methacholine challenge test (MCT), are less invasive but still time consuming and difficult for the patient to tolerate. Exhaled nitric oxide (eNO) is a non-invasive measure of asthma inflammation which can be performed easily and quickly with minimal discomfort to the patient. While studies have evaluated the changes of eNO with asthma treatment and exacerbations, none have yet evaluated the correlation between exhaled NO and the MCT.

The purpose of this study was to evaluate the changes in eNO after a naturalistic cat challenge and also to determine if there was a correlation of eNO with the PC20 obtained from a MCT. It is hypothesized that changes in eNO will correlate with the change in PC20 obtained from the MCT.

Methods Used: Thirty mild to moderate steroid naive asthmatic subjects were studied at baseline and then 24 and 72 hours after exposure to a naturalistic cat antigen challenge. Pulmonary function tests, MCT, and eNO were evaluated and measured at baseline and after the cat challenge. The data was then extracted from the database, the MCT data was logarithmically transformed, and plateau and “best test” were confirmed prior to statistical analysis.

Summary of Results: There was a significant increase in eNO at 2, 4, and 6 hours at 50 ml/sec, and also at 2 and 4 hours at 150 ml/sec following the cat challenge (Tables 1 & 2). Additionally, there was a significant inverse correlation (p=0.0443) between eNO and the PC20 obtained from the MCT performed three days following the cat challenge, but not at 24 hours post cat challenge.

Conclusions: eNO measurements can serve as possible markers of airway inflammation as there are significant increases in eNO following the cat challenge. Furthermore, there may be an inverse correlation between eNO and the PC20 obtained from a MCT, but further studies are needed to confirm this correlation.

Residents’ Forum

8:30 AM Saturday, January 29, 2011

450 UTILITY OF SERUM TOTAL IgE ESTIMATION AND ALLERGY TESTING IN CHILDREN WITH PERSISTENT ASTHMA. A RETROSPECTIVE STUDY FROM THE CENTRAL VALLEY OF CALIFORNIA

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Purpose of Study: Inhaled allergens play a major role as triggers in asthmatic children. Although allergy testing has been recommended in all children with persistent asthma, there is still considerable reluctance in doing so. The aim of the study was to (a) compare reported environmental asthma triggers with actual inhalant allergen sensitivities via allergy testing in children with persistent asthma and (b) to study the accuracy of serum total IgE in predicting atopy vs. non-atopy. This was done in an urban, pediatric asthma clinic in Fresno, CA.

Methods Used: A retrospective chart review of children with persistent asthma ages 2-18 years who had allergy testing during the past 3 years was done. We used the immunocap method for allergy testing. Asthmatics were considered non-atopic if allergen specific IgE responses were <0.35 kIU/L and atopic if 1 or more were >0.35 kIU/L. Accuracy of total serum IgE level in predicting atopy vs. non-atopy was analyzed using ROC curve. Area under the curve and confidence interval were calculated using non-parametric methods.

Summary of Results: Data from 136 cases (78 males and 58 females) was analyzed. Environmental asthma triggers were reported in 87.5% (n=119). The majority were non-allergic environmental triggers: colds in 77%, weather in 66%, exercise in 53%, dust/pollen in 35%, emotions in 27%, and ETS exposure in 23%. Only 52/38% identified inhalant allergens as triggers. Allergy testing showed atopy in 49% (n=67) and non-atopy in 51% (n=69). The area under the curve for ROC analysis for total IgE was 0.84 (95% CI, 0.77–0.90; p<0.001). Using the upper limit of normal level for serum total IgE at 10 years of age (195 IU/mL) as the cut off, the specificity in predicting atopic status was 90% and the sensitivity was 50%.

Conclusions: Only 38% of our cohort identified inhalant allergens as asthma triggers. However, nearly half of our asthmatics were found atopic by allergy testing. Mold, dust mite, grass, tree pollen and weed sensitization were little recognized by asthmatic patients and families as their asthma triggers. Estimation of serum total IgE level showed specificity in ruling out atopy but was not found sensitive enough to predict atopy in our cohort. Based on our results, we recommend allergy testing in all children with persistent asthma.

451 APNEA AND MORTALITY IN CHILDREN WITH CEREBRAL PALSY

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Purpose of Study: Children with cerebral palsy (CP) experience increased mortality rates in comparison to the general population. Apnea is also known to be associated with excess mortality. No studies have quantified the effect of apnea on mortality in children with cerebral palsy. Our objective is to determine the effect of apnea on mortality of children with CP, and to assess whether the effect of apnea on mortality is modified by tracheostomy presence.

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Methods Used: The population-based cohort included individuals aged 2 to 20 years who received services for CP from the California Department of Developmental Services between January 1988 and December 2002. Logistic regression was used in a person-month approach to determine the all-cause mortality risk of apnea and tracheostomy, controlling for possible confounders (i.e. gender, age, mobility, and feeding ability).

Summary of Results: 27,149 individuals with CP were identified, contributing a total of 2,047,068 person-months to the analysis. 287 children had a history of apnea. Of these, 40% also required a tracheostomy. The frequency of apnea was considerably higher among children with CP who required tube feeding (7.5% vs. 0.3%, p-value=0.0001). All-cause mortality in children with CP and apnea was significantly higher than in those without apnea (RR=2.38, 95% CI 1.74–3.25). Amongst children with apnea, those with a tracheostomy experienced a significantly lower mortality rate (p-value=0.04). Furthermore, the mortality rate in children without tracheostomy was still considerably higher than in children without apnea (RR=1.45, 95% CI 0.99–2.11).

Conclusions: Children with cerebral palsy who experience apnea have over a two-fold increased mortality risk compared to those without apnea. Children who are tube fed are at a remarkably higher risk of having apnea. The presence of a tracheostomy appears to have a protective effect in children with apnea. This may be due to prevention of airway blockage due to obstructive apnea.

452 DEVELOPMENT OF AN INTERNATIONAL ELECTIVE FOR PEDIATRIC RESIDENTS: BRINGING CAMBODIA TO FRESNO

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Purpose of Study: With migration, intercontinental travel, and international adoption on the rise, U.S. physicians increasingly are caring for more patients from developing countries. Thus, physicians today must understand the global burden of diseases, inequities in global health systems, and importance of cultural sensitivity. The Angkor Hospital for Children (AHC) elective was developed to provide a unique opportunity for pediatric residents to (1) compare and contrast health disparities in the U.S. vs. a developing country, (2) foster skills in teamwork, communication, cultural sensitivity, and leadership, (3) improve global health knowledge and clinical skills, and (4) develop skills in providing cost-effective care and resource allocation in a setting of limited resources and technologies.

Methods Used: The curriculum for the AHC elective in Siem Reap, Cambodia was developed within the ACGME competencies framework. Educational tools developed included a detailed handbook of the rotation’s key cultural, medical, and travel points; and an internet blog for learners to reflect on experiences and for mentors to provide timely feedback.

Summary of Results: The AHC elective provided a venue to learn about comprehensive care in a children’s hospital and community settings. Experiences ranged from daily ICU rounds to visiting homes. The first resident who piloted the rotation reflected that: (1) she enhanced her clinical knowledge and skills, as well as her understanding of cultural health beliefs, (2) she discovered wide health disparities in a developing country, (3) she changed to more international medical education and teamwork, and (4) she grew professionally by having the lead role in the development and piloting of the rotation curriculum, handbook, and internet blog.

Conclusions: An international elective can greatly augment a pediatric residency curriculum. Training abroad in a developing country can play a pivotal role in expanding one’s understanding of global health systems, honing of clinical diagnostic skills, and professional development. Moreover, such an experience can provide a rich, holistic perspective about life, culture, and medicine that would otherwise be difficult to obtain during traditional clinical rotations in the U.S.

453 THE USE OF VERBAL AUTOPSY TO DETERMINE LEADING CAUSES OF NEONATAL DEATH IN RURAL TIBET

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Purpose of Study: Tibet has one of the highest neonatal mortality rates in the People’s Republic of China. No information exists about specific causes and contributing conditions to newborn deaths especially in rural areas of Tibet. In order to develop goal directed maternal-child health programs aimed at preventing neonatal mortality, more information regarding causes of neonatal death is needed. Objectives of this study are to 1) determine the leading causes of neonatal death in one county of rural Tibet, 2) describe common contributing factors and associated conditions of neonatal mortality.

Methods Used: Using available death records for a rural Tibetan county, we identified 200 neonatal deaths that were recorded from 2000–2002. From this sample, we conducted 82 verbal autopsies (VA) that were available and accessible at the time of the interview (2003), using Tibetan translators proficient in medical translation. The VA questionnaire used was based on the United Nations Children’s Fund and World Health Organization standard VA form adapted for use in Tibet. The cause of death was assigned by 2 independent reviewers based on clinical knowledge and judgment utilizing standard medical definitions.

Summary of Results: Verbal autopsies for 82 neonatal deaths were completed. 50/82 infants (61%) were male, and 51/82 (62%) of the infants died on the first day of life. The leading direct cause of death was birth asphyxia (49%; 40/82), followed by infections (18%; 15/82), prematurity (16%; 13/82), intraterine death (2.5%; 2/82) and unknown/other causes (15%; 12/82). Prolonged labor (33%; 27/82), maternal hemorrhage (15%; 12/82) and mal-presentation (15%; 12/82) were the most common contributing complications. The majority of neonatal deaths occurred at home (82%; 68/82) without a skilled birth attendant (68%; 60/82).

Conclusions: Birth asphyxia is the single most predominant cause of death in children in rural Tibet. Proportional mortality due to asphyxia in this county appears significantly higher than in other comparable areas of southeast Asia. Public health interventions that target management of birth and newborn resuscitation are urgently needed. More studies are needed to examine the contribution of other factors such as high altitude to the increased birth asphyxia rate.

454 AN UNUSUAL CASE OF THYROID CARCINOMA

Castillo J, Kapsner P, Garimella M, Colleran K, University of New Mexico, Albuquerque, NM.

Case Report: The solid variant of papillary thyroid cancer (PTC) is a rare, poorly characterized type of PTC dominated by solid sheets of tumor cells. There is disagreement on its behavior. In one-third of cases, vascular invasion and extra-thyroidal extension is present at diagnosis. It is associated with a higher frequency of distant metastases and a less favorable prognosis. Poorly differentiated thyroid carcinoma is a very aggressive tumor with a poor prognosis and few treatment options. We present a case of PTC, solid variant with an intra-thyroidal focus of poorly differentiated carcinoma.

A 52 year old female patient with hypothyroidism presented with 6 months of worsening dyspnea, dysphagia, hoarseness, weight loss and a large thyroid (11–12 cm transversely) with multiple surrounding hard, enlarged, non-tender lymph nodes. TSH was 111 IU/mL (normal 0.4–4.5 IU/mL). CT scan showed diffuse enlargement of the thyroid with several calcified masses within the gland, the largest measuring 1.8 cm, numerous bilateral level 2 and 3 lymph nodes, the largest being 2.8 cm, and multiple small bilateral lung nodules. FNA cytology of the dominant thyroid nodule showed probable poorly differentiated carcinoma of the thyroid with a recommendation for tissue sampling. Open biopsy showed PTC, solid variant with enlarged overlapping nuclei and immunostaining positive for TTF-1 and thyroglobulin (Tg); negative for calcitonin, chromogranin, synaptophysin, Melan-A, and HMB45. Patient subsequently underwent a thyroidectomy with a total neck dissection followed by 200 millicuries of radioactive iodine therapy. Pathology revealed PTC, solid variant with nodal metastases and a small intra-thyroidal focus of poorly differentiated carcinoma. Post treatment scan showed hilar, mediastinal and diffuse lung uptake with a question of focal uptake in the liver. Tg antibodies were present and Tg undetectable after stimulation.

This case illustrates a case of PTC, solid variant, with an intra-thyroidal focus of poorly differentiated carcinoma, initially diagnosed as probable poorly differentiated carcinoma of the thyroid. As therapeutic options differ between these entities, it is important correct diagnosis is made. We review the relevant literature regarding the diagnosis and management of PTC, solid variant and poorly differentiated carcinoma of the thyroid.

455 LEARN PEDIATRICS: CREATING INSTRUCTIONAL VIDEOS FOR MEDICAL STUDENTS LEARNING PEDIATRIC CLINICAL SKILLS - DIFFICULTIES ENCOUNTERED

Pediatric residents wrote scripts for system-based videos. Hageman J. et al Severe Community-acquired Pneumonia Due to Volume 59, Number 1, January 2011

A 51 year-old male presented to a community clinic with Instructional videos are invaluable in teaching clinical skills to Difficulties encountered can be divided into pre-production, production, and post-production problems. The main pre-production difficulty was in creating video scripts that included an optimal amount of detail. With an overly detailed script, visuals tended to become static. A solution was to use text slides, graphics or photos to supplement and complement visuals. When the script was too short, audio collected from the examiner performing the physical exam was used. Filming before writing a script was attempted but this lead to important aspects of the demonstration being missed. Recruitment of actors was also difficult. Personal contacts were asked to volunteer as patient actors and physicians as examiners. The production phase required close cooperation between media- and medical- staff. Logistics of setting up the scene and time constraints of all parties involved were the main hurdles. The post-production phase had several challenges. Medical participation in video editing was essential but time consuming. Using graphics raised issues of consent, creating the need to create original material. The need to avoid publishing sensitive material on a free access web page required the use of graphics with limited detail rather than video for the demonstration of genital exams. Voice recording was complicated by inexperience.

Conclusions: Instructional videos are invaluable in teaching clinical skills to medical students. Difficulties encountered were discussed and some solutions proposed so to improve future endeavors at creating similar resources.

456 ADULT PULMONARY LANGERHANS’ CELL HISTIOCYTOSIS: A CASE REPORT AND REVIEW OF THE LITERATURE
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Case Report: Adult Pulmonary Langerhans Cell Histiocytosis (PLCH) is a rare interstitial lung disease, affect young to middle-age adults, and associated with relatively high mortality and morbidity. Nearly all cases are associated with history of cigarettes smoking. A 20 year old healthy male, with two year history of smoking, presented to the ED with sudden severe pain in the left chest wall along with cough and difficulty breathing. On presentation the patient was in respiratory distress and tachycardic, physical examination showed mild tracheal deviation to the right, decreased breathing sounds on right side and hyperresonance to percussion. A chest x-ray revealed a moderate volume left pneumothorax, diffuse coarse interstitial markings and scattered cystic lucencies. Chest tube was placed immediately after which patient’ s symptoms improved. Chest CT scan was done and showed diffuse cystic change in the upper and lower lobes with relative sparing of the costophrenic angles. Transbronchial biopsy was obtained and the pathology showed cellular infiltrate composed of histiocytic cells with indented nuclei. Three days later CXR showed a complete resolution of the pneumothorax, chest tube was removed and the patient was discharged on the following day.

PLCH occurs in about 1:500,000, it occurs predominantly in young smokers, with an incidence peak at 20–40 yrs of age. Patients usually present with respiratory symptoms, mainly a dry cough and dyspnea on exertion. Spontaneous pneumothorax occurs in 15–25% and considered the most significant and life threatening complication. It may occur at any time during the course of the disease and may be bilateral and/or recurrent, raising difficult therapeutic challenges. The diagnosis is usually made by radiography; the combination of multiple cysts and reticulonodular opacities in the upper lungs lobes in a young smoker is characteristic. But the definite diagnosis is confirmed by lung biopsy. As compared to an excellent prognosis in single foci disease, prognosis with multifocal disease is relatively poor; 60% have a chronic course & mortality is around 10%. The initial focus in treatment should be cessation of smoking. Immunosuppressive therapies are of limited value, and lung transplantation should be considered in the patients with advanced progressive disease.

457 AUTOFLUORESCENCE BRONCHOSCOPY IS ESSENTIAL IN THE EVALUATION AND TREATMENT OF MYOFIBROBLASTIC TUMORS IN THE AIRWAY
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Case Report: The inflammatory myofibroblastic tumor is a relatively rare spindle cell tumor. Although they are reported in various sites, these tumors are rarely found in the airway. Because surgical resection can be curable, accurate delineation of the tumor margins is paramount. We describe a case of inflammatory myofibroblastic tumor involving the trachea and bronchi in a 37 year old male presenting with exertional dyspnea. Rigid Bronchoscopy and subsequent Autofluorescence bronchoscopy was utilized to map the surgical margins of the disease and prepare the patient for resection. This is the first time Autofluorescence bronchoscopy (AFB) has been used to delineate tumor margins for surgical planning and for surveillance for recurrence. A review of the literature focusing on diagnostic considerations, surgical planning and surveillance for recurrence is discussed.

458 COMMUNITY-ACQUIRED METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS CAUSING NECROTIZING TRACHEOBRONCHITIS
Reagle Z, Patel J, Evans T UCSF Fresno, Fresno, CA.

Case Report: A 51 year-old male presented to a community clinic with three weeks of flu like illness. He was diagnosed with pneumonia and initially prescribed levofloxacin. He subsequently returned to the clinic experiencing respiratory failure. He was transported to our emergency department where he presented with hemoptysis, and was found to be febrile, tachycardic and tachypneic. Chest radiography demonstrated bilateral infiltrates. Despite aggressive treatment, he decompensated. He was intubated and fiberoptic bronchoscopy was performed. Findings included extensive hemorrhagic mucosa from the trachea down both endobronchial trees. Washings were taken and sent for appropriate studies.

The patient was treated with vancomycin, ceftriaxone, and azithromycin. Respiratory culture grew Panton-Valentine Leukocidin positive (PVL+) Methicillin-Resistant Staphylococcus Aureus (MRSA.) The patient developed acute respiratory distress syndrome & multi-organ failure. Despite all aggressive measures, the patient significantly deteriorated & expired.

While infections with MRSA were once considered to be a nosocomial infection, the incidence of Community-Acquired MRSA (CA-MRSA) infections is increasing. Community acquired strains of MRSA differ from those found in nosocomial infections due to the presence of the PVL toxin that is produced by the staphylococcal cassette chromosome mec.2 Francis reported what is believed to be the first case of necrotizing pneumonia caused by PVL+ MRSA.2 To the knowledge of the authors, we present the first case of necrotizing tracheobronchitis caused by PVL+ MRSA.

It is well established that PVL+ CA-MRSA can cause pneumonias. One manifestation of PVL+ CA-MRSA airway infections that is less well established may be soft tissue infections of the trachea and bronchus. Here we present a case of necrotizing tracheobronchitis that may have been caused by PVL+ CA-MRSA, possibly represented a new manifestation of CA-MRSA infections.


459 DEVELOPMENT OF A CYANIDE TOXICITY LETHAL MODEL AND MEASUREMENT OF PHYSIOLOGIC AND METABOLIC EFFECTS IN RABBITS USING NON-INVASIVE DIFFUSE OPTICAL SPECTROSCOPY MONITORING
Thomas J1,2,3, Patino R1,2,3, Bremer M1,2,3, Kim JG1,2, Mahon SL1,2, Mukai D1,2, Sato R1,2,3 UCI, Irvine, CA; UCI, Irvine, CA; and 3UCI, Irvine, CA.

Development of a cyanide toxicity lethal model and measurement of physiologic and metabolic effects in rabbits using non-invasive diffuse optical spectroscopy monitoring Thomas J1,2,3, Patino R1,2,3, Bremer M1,2,3, Kim JG1,2, Mahon SL1,2, Mukai D1,2, Sato R1,2,3 UCI, Irvine, CA; UCI, Irvine, CA; and 3UCI, Irvine, CA.
Purpose of Study: Cyanide (CN) poisoning presents a major threat worldwide. CN toxicity results from a diverse range of both civilian and military exposures, including fire smoke inhalation and terrorist acts. The onset of cyanide poisoning is rapid and the presence of cyanide in blood is not easily measured by any current rapid assay technology. Toxicity effects do not correlate closely with blood cyanide levels. Therefore, methods for assessing the physiologic effects of cyanide may be more clinically relevant as indicators of cyanide toxicity and response to treatment.

Methods Used: A lethal cyanide toxicity model was developed in anesthetized and ventilated New Zealand white rabbits. 20mg of sodium cyanide in 60cc normal saline was infused through the femoral vein at a rate of 1cc/min. After 30 mins, inspired O₂ was reduced from 100% to 21%. Diffuse optical spectroscopy (DOS) and continuous-wave near-infrared spectroscopy (CWNIRS) were used to non-invasively measure tissue oxy (OHb)-and deoxy (RHb) hemoglobin tissue concentration changes, and quantitative metabolic and gas exchange parameters were measured by standard means.

Summary of Results: Toxic effects of cyanide were clearly evident by substantial decreases in blood pressure and marked increases in OHb and decreases in RHb concentrations seen by non-invasive DOS and CWNIRS measurements. Lethal levels of cyanide were demonstrated by decreased O₂ elimination and increased CO₂ elimination in exhaled gas analysis, decreases in bicarbonate representing an increasing metabolic acidosis as well as decreases in venous carbon dioxide and oxygen levels representing a failure of aerobic respiration.

Conclusions: DOS and CWNIRS in combination with respiratory and metabolic data allow more precise assessment and monitoring in cyanide toxicity in a lethal animal model.