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MWSPR Plenary Session I

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DNA METHYLTRANSFERASE 3B TRUNCATED PROTEIN (DNMT3B7) IS INVOLVED IN DECREASED TUMOR PROGRESSION IN HUMAN NEUROBLASTOMA

Q Yang[1], KR. Ostler[2], S Gu[3], Y Tian[3], A Chlenski[3], HR Salwen[3], A Gill[3], B Blumenschein[3], LA Godley[2], and SL Cohn[3] [1]Department of Pediatrics, University of Illinois at Chicago, Chicago, IL; [2] Department of Medicine, University of Chicago, Chicago, IL; [3]Department of Pediatrics, University of Chicago, Chicago, IL

Background: A CpG island methylator phenotype is associated with aggressive neuroblastoma (NB), a common pediatric cancer. However, the molecular basis for abnormal DNA methylation patterns in NB remains unclear. In this study, we characterized the effects of DNMT3B7, a truncated DNMT3B protein, on NB tumor phenotype. **Methods:** A Tet-off inducible system was established to express DNMT3B7. Transduction was performed in LA1-55n NB cells. Gene expression was examined by quantitative PCR. The level of global and gene-specific DNA methylation was determined by liquid chromatography-mass spectrometry and quantitative methylation-specific PCR. A Tet-off inducible NB xenograft model was used to determine the effects of DNMT3B7 on tumor growth and angiogenesis. **Results:** *In vitro*, inducible expression of DNMT3B7 in NB cells resulted in decreased proliferation ($P<0.001$). The global methylation level is higher in DNMT3B7 cells than in control cells. *In vivo*, tumor growth was markedly inhibited in NB xenografts with inducible expression of DNMT3B7 ($p<0.01$). The global level of methylcytosine is higher in DNMT3B7 tumors than in control tumors. *HIC-1* expression was increased in DNMT3B7 xenografts in relation to decreased methylation level around its promoter region. Ki-67 staining demonstrated significantly decrease in NB cell proliferation in xenografts with forced DNMT3B7 expression ($p<0.01$), In addition, angiogenesis was inhibited in the DNMT3B7-positive xenografts, with significantly decreased numbers of CD-31 positive cells compared to controls. **Conclusion:** Our results suggest that aberrant DNMT3B transcripts drive NB phenotype by regulating DNA methylation and gene expression. Further knowledge of the mechanisms by which DNMT3B7 inhibits NB tumor growth may provide insight for the development of new therapeutic strategies.

2

AGE-DEPENDENT MICROGLIAL RESPONSES IN HYPOXIA-ISCHEMIA

Ferrazzano P, Chanana V, Barthakur S, Uluc K, Akture E, Cengiz P, Sun D.

Cerebral ischemia affects over 20,000 infants and children every year in the US, and treatment options are limited. Therapies that mitigate the neuroinflammatory response to ischemia have been recognized as a promising neuroprotective strategy. However, the microglia-mediated inflammatory response to ischemia in the developing brain is not well understood. In the immature brain, microglia are activated for phagocytosis of cellular debris during synaptogenesis, and it is unknown how this predominance of activated microglia impacts ischemic injury. **Hypothesis:** Ongoing differentiation and activation of microglia in the immature brain predisposes it to a more vigorous pro-inflammatory response to HI than juvenile brains. To address this hypothesis, we determined age- and region-dependent differences in the microglial response to cerebral ischemia. **Methods:** Hypoxia-ischemia (HI) was induced in postnatal day 9 (P9) and P30 mice by unilateral carotid artery ligation and subsequent exposure to 10% O2 for 50 minutes. Flow cytometry, immunostaining and ELISA were performed at 0-17 days after HI to characterize microglia proliferation, neuronal injury, and cytokine expression in the hippocampus, cortex, and striatum. **Results:** Flow cytometric analysis revealed an increase in $CD11b^+/CD45^+$ cells (microglia) in ipsilateral brain regions after HI. P9 mice exhibited an early increase in reactive microglia and proinflammatory cytokines in the ipsilateral hippocampus that peaked 2 days after HI, and a more delayed microglial response in the cortex and striatum that peaked 9-10 days post-HI. In contrast, P30 brains demonstrated a 50-70% reduction in microglial counts in each sub-region and time-point after HI, and an increase in anti-inflammatory cytokine IL-10 expression. Interestingly, microglia in the hippocampus of P9 and P30 mice demonstrated increased CD45 expression *under normoxic conditions*, suggesting a more activated microglia phenotype in the hippocampus of the developing brain. **Conclusion:** We found both regional and age related differences in the microglial response to ischemia. P9 mice demonstrated a more vigorous and pro-inflammatory microglia response compared to P30 mice after HI. Additionally, a baseline increase in microglial activation state was seen in the hippocampus, corresponding to the early and intense microglial response in this brain region after HI. Ongoing studies will determine whether age- and region-dependent differences in microglial responses to HI result in different profiles of neuronal injury.

3

INTERLEUKIN 13 RECEPTOR ALPHA 1 KNOCKOUT NONOBESE DIABETIC MICE ARE PROTECTED FROM TYPE 1 DIABETES

A. VanMorlan, L. Rowland, X. Wan, and H. Zaghouani. The University of Missouri, Columbia, MO.

Background: Type 1 diabetes (T1D) is an autoimmune disease in which the insulin-producing beta (β) cells of the pancreas are destroyed by cells of the immune system. $CD4^+$T lymphocytes specific for β cell- associated antigens are thought to play a major role in the pathogenesis of T1D. It has been shown that Th17 cells as well as antigen presenting cells (APC) contribute to disease development. Interestingly, the cytokine interleukin (IL)-13 seems to have an inhibitory effect on both Th17 cells and APC and it has been shown that rIL-13 delays T1D in NOD mice. However, the mechanism by which IL-13 suppresses T1D has yet to be defined and understanding this process may lead to new treatment options. **Methods:** To investigate the role IL-13 and its receptor, IL-13Rα1, play in T1D, we generated IL-13Rα1-deficient (IL-13R$\alpha1^{-/-}$) nonobese diabetic (NOD) mice. The goal is to utilize these mice to determine how IL-13/IL-13Rα1 regulates T cells and APC to suppress diabetes. **Results:** We used flow cytometric analysis to determine whether IL-13Rα1-deficiency influences the number of $CD4^+$ and $CD8^+$ T cells, T regulatory cells, and dendritic cells prior to disease onset. There were no major differences observed between the IL-13Rα1 sufficient (IL-13R$\alpha1^{+/+}$) and the IL-13R$\alpha1^{-/-}$ mice. To examine the effect IL-13Rα1 deficiency has on disease development we monitored the blood glucose levels of 62 individual IL-13R$\alpha1^{+/+}$ and IL-13R$\alpha1^{-/-}$ mice . Blood glucose levels were monitored weekly, from week 10 of age, and mice with a blood glucose level greater than 300 mg/dl for two consecutive weeks were considered diabetic. We found that the IL-13R$\alpha1^{-/-}$mice are more resistant to diabetes as compared to IL-13R$\alpha1^{+/+}$ mice. Histologic analysis was performed on pancreatic islets of 5 mice from each group at 14 weeks of age, a time point directly before a difference develops in disease incidence, to determine the level of insulitis. We found that the IL-13R$\alpha1^{-/-}$ mice have a higher percentage of both healthy islets (no evidence of lymphocytic infiltration) and islets with peri-insulitis (infiltration restricted to the periphery of the islets). In addition, IL-13R$\alpha1^{-/-}$ mice showed a decrease in the production of the proinflammatory cytokine IL-6 by antigen presenting cells. **Conclusion:** These data suggest that the lack of IL-13Rα1 results in a decrease in proinflammatory cytokine production and consequently a less inflammatory environment which protects against diabetes.

4

NEONATAL SERTRALINE EXPOSURE ALTERS ADULT ENERGY BALANCE IN MICE

G Kummet, S Haskell, A Younes, V Zhu, G Hermann, and R Roghair, Department of Pediatrics, Carver College of Medicine, University of Iowa, Iowa City, Iowa, USA

Purpose: Up to 10% of all pregnancies are complicated by selective serotonin reuptake inhibitor (SSRI) therapy. We have administered the most commonly prescribed SSRI, sertraline, to neonatal mice to model third trimester human exposure. Exposed male mice had adult serotonin deficiency with phenotypes including hyperphagia, hyperactivity, tachycardia and increased metabolic rates. Because estrogen increases tryptophan hydroxylase, the rate limiting enzyme in serotonin production, we hypothesized that intact ovarian function would protect female mice from the effects of neonatal SSRI exposure. **Methods:** C57BL/6 pups were randomized to intraperitoneal injections of saline or sertraline (5mg/kg/d) on days 1 to 14 of life. At 6 months, female mice underwent either sham surgery or bilateral paravertebral ovariectomy (OVX). Adult phenotypes were assessed after recovery. Open field testing assessed locomotor activity, hemodynamics were measured by tail cuff, and resting metabolic rates were measured by indirect calorimetry. Fear conditioning assessed extinction of anxiety from a non-desirable stimulus. Post-mortem mass of major internal organs were collected. Data were compared by 2-way ANOVA. Bonferroni post-hoc testing was performed if significant differences were detected ($P < 0.05$). **Results:** Neonatal SSRI exposure increased adult basal metabolic rate ($F(1,26)=5.94$, $P=0.02$) with post-hoc analysis isolating the effect to SSRI exposed non-OVX mice ($P < 0.01$). Independent of OVX status, SSRI exposure led to increased adult feed intake ($F(1,21)=6.75$, $P=0.02$), decreased body weight ($F(1,25)=5.8$, $P=0.02$), and increased relative brain weight ($F(1,25)=6.1$, $P=0.02$). No significant differences were noted in tail cuff blood pressures, fear conditioning, locomotor activity, or both absolute and relative gonadal fat mass. **Conclusion:** As in males, neonatal SSRI exposure alters adult female phenotypes even in the absence of ovarian function. Consistent with their known neurotrophic effects, SSRI exposure increased relative brain weight and altered centrally-mediated phenotypes including the regulation of energy balance. Ongoing investigations are assessing the roles of altered neurodevelopment and programmed central serotonin deficiency in these phenotypes. Further basic and clinical research is needed into the long-term effects of SSRI exposure during vulnerable windows of development.

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TREATMENT WITH TRKB AGONIST 7,8 DHF ATTENUATES HIPPOCAMPAL DAMAGE AND IMPROVES LONG-TERM FUNCTIONAL OUTCOME AT P90 AFTER PERINATAL HI

Cengiz P[1], Uluc K[1,2,3], Akture E[1,2], Zhang L[3], Kendigelen P[1], Song C[4], Johnson-Bice S[1], Ye K[5], Ferrazzano P [1,3], Sun D [2,3] [1]Dept. of Pediatrics, [2]Neurosurgery, [3]Waisman Center, [4]Medical Physics, University of Wisconsin, Madison, WI, [5]Dept. of Pathology and Laboratory Medicine, Emory University School of Medicine, Atlanta, GA

Background: Hypoxia ischemia (HI) is a common cause of mortality and chronic morbidity in neonates. Hippocampus is selectively injured after neonatal HI resulting in learning and memory deficits. In this study, we investigated neuroprotective effects of a bioactive high-affinity tyrosine kinase B (TrkB) receptor agonist 7,8-dihydroxyflavone (7,8 DHF) in mouse model of neonatal HI. 7,8-DHF has been shown to reduce neuronal apoptosis, inhibit kainic acid-induced toxicity, decrease infarct volumes in cerebral ischemia in a TrkB-dependent manner. **Methods:** HI was induced by using Vanucci-Rice method in P9 mice (C57/Black6) and exposing animals to 10% O_2 for 50 min at 37°C. Initial dose of 7,8 DHF (5mg/kg, i.p.) was given as 10 min after HI and subsequently followed at 24, 48 h or daily up to 7 days. Vehicle control groups received an equal volume of PBS. FJ-C staining, Microtubule associated protein 2 (MAP2), glial fibrillary acidic protein (GFAP), myelin basic protein (MBP), neurofilament (NF) immunofluorescence stainings were performed either at 3 days or 3-months post-HI (after Rotarod and Morris Water Maze (MWM) testing performed). **Results:** TrkB agonist 7,8 DHF reduced hippocampal neurodegeneration, loss of MAP2 expression, reactive astrogliosis at P12 and attenuated corpus callosal white matter injury at P90 post-HI. 7,8 DHF-treated female mice not only exhibited less ipsilateral hippocampal lesion at 3 days post HI but also demonstrated better learning and memory in the Rotarod and MWM test, respectively ($p<0.05$). **Conclusion:** Activation of the TrkB receptors via brain-derived neurotrophic factor promotes neuronal survival, differentiation, synaptic plasticity and neurogenesis. In this study, we showed that application of a bioactive high-affinity TrkB agonist 7,8-DHF was neuroprotective in neonatal mice after HI. Stimulating TrkB receptor activity in female mice has more profound effects on decreasing delayed hippocampal neurodegeneration and improving spatial learning after neonatal HI. Supported by Univ. of Wisconsin Dept. of Pediatrics R & D Grant (Cengiz P), 1UL1RR025011 from the CTS award program of NCRR and NIH (Ferrazzano P), NIH grants RO1NS38118 and RO1NS48216 (Sun D), and NIH P30 HD03352 (Waisman Center).

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LONG-TERM EFFECTS OF RECURRENT HYPOGLYCEMIA ON THE DEVELOPING RAT HIPPOCAMPUS

KM Czerniak and R Rao Department of Pediatrics, Neonatology Division, University of Minnesota, Minneapolis, MN

Background: Long-term hippocampal dysfunction is a sequela of recurrent hypoglycemia (RH). In adult rats hippocampal dysfunction due to RH is present during hypoglycemia (H) but not at euglycemia. The molecular mechanisms of this differential response are not known. **Objective:** To investigate the effects of RH during development on the glucose transporters, NMDA receptors and synaptogenesis in the rat hippocampus at adulthood. **Design/Methods:** Insulin-induced H (blood glucose, 30mg/dl for 120 min) was induced, once daily from postnatal day (P) 24 to P28 (RH group) in male rats. The control group was administered normal saline. On P65, glucose transporters (Glut) 1 and 3, NMDA receptors (Nr1, Nr2a, and Nr2b) and profilin (Pfn, marker of synaptogenesis) 1 and 2 mRNA expressions in the hippocampus were determined using qRT-PCR (n=8-12). **Results:** Relative to the control, Glut 1 (22%) and Glut 3 (35%) were increased in the RH group ($p<0.0001$). NR2a (37%) and NR2b (30%) were increased ($p<0.03$), and Pfn1 (22%) and Pfn2 (27%) transcripts were decreased. NR1 expression was unaffected. **Conclusions:** RH during development altered glucose transporters, NMDA receptors and synaptogenesis markers in the rat hippocampus at adulthood. Glut1 and Glut3 mRNA upregulation suggests enhanced glucose transport and is likely responsible for the preserved cognitive function under euglycemic conditions. The alterations in NMDA receptors, Pfn1 and Pfn2 reflect altered synaptogenesis and may explain hippocampal dysfunction during subsequent hypoglycemia. These results may explain the cognitive deficits due to RH during development.

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DISCOVERY OF OXYTOCIN RECEPTORS IN RETINA PIGMENT EPITHELIAL CELLS: DOES IT CONTRIBUTE TO PROGRESSION OF ROP?

M. P. Asuma[1], W. Luo[1], D. M. Pillers[1, 2], B. R. Pattnaik[1, 2, 3], Departments of 1) Pediatrics, 2) Eye Research Institute, 3) Ophthalmology and Visual Sciences, University Wisconsin, Madison, WI.

Background: Retinopathy of prematurity (ROP) is known to alter development of the central retina the site affected in many inherited and age-related eye diseases. The central retina is characterized by an absence of retinal vasculature and is the last retinal region to mature. Retinal Pigment Epithelial (RPE) cells are located between the choroidal vasculature and the neural retina. RPE cells develop early during retinogenesis, secrete vascular endothelial growth factor (VEGF) and pigment epithelium-derived factor (PEDF) to regulate retinal vasculature, and provide a cellular environment that provides continued nourishment for retinal neurons. Oxytocin, a uterotonic agent used to induce labor, binds to cell-surface receptors to activate complex intracellular signaling pathways. Preterm infants by virtue of their early birth are deprived of exposure to the usual tightly controlled regulation of birth hormone levels, including oxytocin. **Objective:** Since oxytocin has been detected at the protein level in posterior retina, we sought to define the oxytocin signaling mechanism in human RPE cells as a potential contributor to the bio-pathology associated with ROP. **Methods:** Human RPE cells were maintained using optimized growth medium. Ionic currents were recorded from single cells using the whole cell configuration of the patch clamp technique. Single cell RT-PCR was performed by harvesting cellular content after each electrophysiology recording. Transcripts for the oxytocin receptor (OTR) and a panel of RPE-specific markers were amplified (RPE65, bestrophin, ezrin). **Results:** RPE cells in culture showed Kir7.1 current and the presence of Kir7.1 transcript. Results of single cell RT-PCR amplification showed presence of an appropriate size band for OTR. Confirmation that the cells were RPE cells was shown by amplification of RPE65, bestrophin, Na-K-ATPase and ezrin. **Conclusions:** We have shown that the oxytocin receptor is expressed in RPE cells. The finding of the hormone oxytocin in the posterior retina where RPE cells are localized suggests that oxytocin plays a role in regulating RPE function via activation of the oxytocin receptor. Premature infants who are born without appropriate exposure to oxytocin during their birth process may have risks for abnormal retinal development due to unusual regulation of RPE cell function including altered sub- retinal space as well as abnormal secretion of angiogenic factors. Both of these aspects of RPE function are important contributors to the development of ROP.

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THE EFFECT OF ERYTHROPOIETIN AND IRON TREATMENT IN BRAIN DEVELOPMENT IN NEWBORN RATS WITH DIFFERING IRON STATUS

KP Flores, SE Blohowiak, PJ Kling. UW Dept. Pediatrics and Meriter Hospital, Madison, WI.

Background: Nutritional iron deficiency (ID) during fetal or early postnatal development can alter brain growth and function, especially in the hippocampus. With both rapid growth and erythropoietic need, iron stores in premature infants may be depleted. The drug Erythropoietin (Epo) stimulates erythropoiesis and is used clinically, but Epo shifts body iron utilization to erythrocytes. Epo receptors are present on neurons and immature brain oligodendrocytes, but Epo treatment may preferentially withhold iron from the developing brain. Conversely, Epo also contributes to brain development by promoting neuron proliferation or oligodendrocyte differentiation to myelin-producing cells. **Objective:** To determine whether injections of Epo at erythropoiesis-stimulating doses affect brain development, especially in ID, with or without adequate iron (Fe) treatment. **Methods:** Sprague-Dawley newborn rats modeled premature newborns from P4-P12: dam fed-control (iron-sufficient, IS) or ID (artificial ID milk via gastronomy). Eight randomly assigned groups were studied; IS, IS+Epo, ID and ID+Epo; with each group ± Fe. Fe groups were given ferrous sulfate 6 mg/kg/d and parenteral Epo groups given 425 U/kg/d SQ daily. Tissue Fe was measured. Brains were fixed and stained with enhanced Prussian Blue (PBR) Fe stain, Luxol Fast Blue and myelin basic protein (MBP). On photomicrographs, neuronal density (cell counts per unit area) was measured in the hippocampal CA1 area and cerebellar and fimbriae myelination was estimated. **Results:** Brain Fe content in ID groups was lower than IS groups, $p<0.05$, with neither improvement nor worsening with addition of Epo, ±Fe. PBR showed greater cerebellar Fe storage sites in the IS+Fe than IS+Epo+Fe group, but with virtually no staining in other groups. Neuronal density in the CA1 area of the hippocampus was stepwise higher in IS, IS+Fe, IS+Epo and IS+Epo+Fe ($p<0.05$ for all), but density in ID+Fe, ID+Epo and ID+Epo+Fe was similar in all three, but was higher than in ID, $p<0.05$. Myelination was stepwise higher in ID+Fe, ID+Epo+Fe, IS+Fe, IS+Epo+Fe groups, but each of these groups without Fe exhibited relatively less myelination. **Conclusions:** The ID rats exhibited impaired brain Fe content, but adding either Fe or Epo increased neuronal density without further inhibiting brain Fe content. Previously, we found brain Fe content was directly related to plasma Epo levels, with the new data providing a potential mechanism; Epo may improve Fe utilization for both neuronal proliferation and oligodendrocyte differentiation. As in erythrocyte precursors, Epo appears to work in conjunction with Fe in neurons and oligodendrocytes.

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ACETYLATION AND LYSINE METHYLATION OF HISTONES ARE INVOLVED IN FETAL PULMONARY ARTERIAL SMOOTH MUSCLE CELL PROLIFERATION AND CONTRACTILE PHENOTYPE

Qiwei Yang, Dev Singh, Ziyan Lu, Ramaswamy Ramchandran, J. Usha Raj Department of Pediatrics, University of Illinois at Chicago, Chicago, IL

Background: The proliferation of vascular smooth muscle cells can contribute to a variety of pathological states, including vascular remodeling. Recent studies have implicated an important role for epigenetic-mediated responses in various cellular processes. However, the role of epigenetic alterations for proliferative vascular disease remains elusive. **Materials and Methods:** chronic hypoxia sheep model was used to determine the epigenetic alteration *in vivo*. Global DNA methylation level was determined by liquid chromatography-mass spectrometry. Three small molecular inhibitors (i) of protein arginine methyltransferase (PRMT), histone lysine methyltransferase (HMT), and histone deacetylase (HDAC) were used to investigate the role of epigenetic alterations in cell proliferation and contractile phenotype. Gel contraction assay was performed to determine the effect of epigenetic modifier on gel contractile force. **Results:** Global DNA methylation level in chronic artery was significantly lower than in nomoxia artery. CNKN1A mRNA expression was also significantly decreased in hypoxia artery in relation to decreased level of acetyl H4. To characterize the relation between epigenetics and cell proliferation, FPASMCs were isolated from chronic artery tissue and treated with HDACi, HMTi, and PRMTi respectively. Both HMTi and HDACi resulted in decrease in FPASMC proliferation and exhibited cell cycle arrest at G1 phase. The PRMTi exhibited no effect in cell proliferation. Moreover CDKN1A mRNA expression was increased by ~ 3.7-fold and 4-fold without inducing P53 in FPASMCs treated with HMTi and HDACi respectively. Interestingly, both HMT1 and HDACi were capable of increasing the level of 5-methylcytosine level. Knockdown of CDKN1A with siRNA significantly restored the proliferation of FPASMCs. HDACi mediated- upregulation of CDKN1A is associated with increase of histone acetylation. However, change of histone acetylation by HMTi was not observed. In addition, both HDACi and HMTi altered the contractile properties in FPASMC. **Conclusion:** our findings demonstrate a genome-wide modulation of DNA methylation and histone acetylation in chronic hypoxia sheep model, and epigenetics plays a significant role in fetal pulmonary vascular smooth muscle cell proliferation and the contractile phenotype.

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DENDRITIC C-JUN N-TERMINAL KINASE IS NESSESARY AND SUFFICIENT FOR METABOTROPIC GLUTAMATE RECEPTOR REGULATED AMYLOID PROTEIN EXPRESSION

TL Schmit, M Maes, M Wilhelm, Department of Pediatrics, University of Wisconsin-Madison

Background: Local protein translation is required for proper metabotropic glutamate receptor (mGluR) regulated synaptic plasticity. Amyloid precursor protein (APP) is one protein translationally regulated by mGluR signaling. APP is abnormally expressed in a variety of neurological disorders, including Fragile-X syndrome (FXS). Aberrant APP expression is thought to play a role in the pathophysiology of FXS; however the signaling mechanisms regulating APP are not fully understood. **Hypothesis:** The c-Jun N-terminal kinase (JNK) pathway is activated in response to mGluR stimulation and has been implicated in regulating **nuclear** transcription of several genes. We hypothesized that mGluR stimulation regulates localized JNK activation in *dendritic spines* and that elevated JNK activity directly contributes to the aberrant APP expression in the FXS mouse model, *fmr-1* knockout. **Methods:** We used wildtype (WT) and *fmr-1* KO synaptoneurosomes (SNs) and primary cortical neuron cultures to examine the effects of mGluR stimulation on JNK activity and JNK's role in the regulation of dendritic APP expression. Immunofluorescence of cortical neurons was used to examine levels of APP in dendritic spines. Western-immunblots were used to determine total APP and JNK activity in SNs. DHPG was used to activate group 1 mGluRs and JNK was inhibited with SP600125. Cell permeable MKK7 (activator of JNK) was used to determine if JNK activity was sufficient to promote APP expression. **Results:** Basal JNK activity is elevated in *fmr-1*KO SNs when compared to WT. Further, treatment with DHPG activates JNK in WT SNs, whereas no additional JNK activation occurred in *fmr-1* KO SNs. Both DHPG treatment and forced JNK activation increased APP levels in neurons and SNs, respectively. Finally, mGluR stimulation in the presence of JNK inhibition abrogated the mGluR dependent APP expression. **Conclusion:** Our data indicate that JNK activity is dysregulated in *fmr-1* KO mice. Further, JNK is both necessary and sufficient to drive synaptic APP expression. However, if JNK is regulating APP translation, degradation, or both is unknown.

MWSPR Plenary Session II

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RESTING ENERGY EXPENDITURE IS NOT ELEVATED IN INFANTS WITH SHORT BOWEL SYNDROME

Rachel A. Umoren, MD, MS[1], Edward A. Liechty, MD[1], Wendy Cruse, MMSc, RD, CNSD[1], Elaina Szeszycki, PharmD, BCNSP[1], Karen W. West, MD[2] and Scott C. Denne, MD[1] (1)Department of Pediatrics, Section of Neonatal-Perinatal Medicine, Indiana University School of Medicine, James Whitcomb Riley Hospital for Children, Indianapolis, IN, (2)Department of Pediatric Surgery, Indiana University School of Medicine, James Whitcomb Riley Hospital for Children, Indianapolis, IN

Background: Short bowel syndrome (SBS) is a malabsorptive state resulting from congenital malformation or extensive resection of the small intestine. Electrolyte imbalances, nutrient deficiencies, and parenteral nutrition (PN) associated liver disease are common complications. Energy expenditure (EE) was decreased in a mouse model of SBS, but adults with SBS have shown significantly elevated values. Infants with SBS are also assumed to have increased caloric needs. It is essential to determine the resting energy expenditure (REE) in infants with SBS order to accurately meet their nutritional needs. **Methods:** We hypothesized that REE would be elevated in infants with SBS when compared with matched healthy controls. Infants at 3-12 months corrected gestational age (GA) with PN-dependent (> 2 months) SBS were enrolled. Infants in the immediate post-resection period, with clinical evidence of infection, antibiotic therapy within the last 7 days, oxygen requirement, diagnosed chromosomal anomaly, or congenital neuromuscular intestinal disease were excluded. The control group consisted of healthy infants at 3-12 months, matched for age and sex. Open circuit indirect calorimetry was performed. O2 consumption (VO2) and CO2 production (VCO2) were determined during a steady state period of at least 30 minutes. The REE and respiratory quotient (RQ) were calculated. Data was presented as mean ± standard deviation and compared using the student's t-test. **Results:** Data was obtained on 8 (6 male, 2 female) infants with a mean GA of 36 ± 0.7 weeks with PN-dependent SBS. 8 healthy term infant controls were matched for age and sex. Anthropometric measures, caloric intake goals, VCO2, VO2, and RQ were compared between groups. The mean REE was 37 ± 10 kcal/kg/d in infants with SBS compared to 39 ± 9 kcal/kg/d in matched healthy controls. **Conclusion:** Data collection are ongoing. REE in infants with SBS does not appear to differ significantly from healthy controls. These results are significant because they depart from previous findings in adults with SBS and may guide recommendations for caloric goals in this patient population. Other implications for clinical care are a potential reduction in the costs and complications of prolonged parenteral nutrition.

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COULD RETINOIC ACID PROTECT AGAINST NEONATAL NECROTIZING ENTEROCOLITIS?

N Kopperuncholan, M Krishnan, A Rahman, D Arbach, P Barodiya, R Jagadeeswaran, A Maheshwari; Pediatrics (Div. of Neonatology & Center for Neonatal/Pediatric Gastrointestinal Disease), University of Illinois, Chicago, IL

Background: We have shown recently that a developmental deficiency of transforming growth factor (TGF)-beta2 predisposes premature neonates to NEC by promoting macrophage cytokine production and inflammatory responses in the developing intestine. In this study, we used in-vitro and in-vivo models to investigate whether retinoic acid, a known inducer of TGF-beta, can protect against NEC-like injury. **Objective:** To determine (1) the kinetics and mechanisms involved in retinoic acid induction of TGF-beta$_2$ in intestinal epithelial cells (IECs), (2) whether retinoic acid can sensitize intestinal macrophages, which are located in sub-epithelial lamina propria, to epithelial-derived TGF-beta$_2$, and (2) whether enteral administration of all-trans retinoic acid can protect murine pups against NEC-like injury. **Design/Methods:** IEC-6 epithelial cells and RAW246.7 macrophages were treated with all-*trans* retinoic acid *in vitro*. TGF-beta expression and involved signaling pathways were investigated by qPCR, ELISA, western blots, bioassays, a phospho-MAPK array, and transfection studies. TGF-beta receptor expression on macrophages was measured by PCR, western blots and flow cytometry. To investigate whether enteral retinoic acid can protect mice against NEC-like injury, we administered platelet-activating factor (PAF; 50 μg/kg) and LPS (1 mg/kg) intraperitoneally in 10- to 12-day old mouse pups. **Results:** All-*trans* retinoic acid induced TGF-beta2 in IEC6 cells in a time- and dose-dependent fashion, through activation of rho A and p38α MAPK. Retinoic acid induced the expression of the splice variant A, which encodes the full-length, secreted isoform. Enteral administration of retinoic acid also increased epithelial expression of TGF-beta$_2$ *in vivo*. Retinoic acid treatment of macrophages increased the expression of TGF-beta receptors and sensitized these cells to TGF-β_2. Finally, enteral administration of TGF-beta$_2$ in murine pups for seven days reduced the severity of NEC-like injury by 45% ($p<0.05$). **Conclusions:** All-trans retinoic acid (1) induces TGF-beta$_2$ in IEC6 cells via a rhoA-p38α mediated pathway; (2) sensitizes macrophages to TGF-beta$_2$ through the induction of TGF-β receptors on these cells; and (3) protects mouse pups against NEC-like injury induced by intraperitoneal administration of platelet-activating factor and LPS. Further studies are indicated to investigate these effects in pre-clinical and clinical settings.

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FUNCTIONAL METAGENOMIC CHARACTERIZATION OF ANTIBIOTIC RESISTANCE IN PEDIATRIC FECAL MICROBIOTA

Moore AM, Tarr PI, Dantas G. St Louis Children's Hospital Department of Pediatrics/Division of Newborn Medicine and Center for Genome Sciences and Systems Biology, Washington University School of Medicine, St Louis, MO.

Background: Lateral transfer of mobile genetic elements is an important mechanism for bacterial evolution of antibiotic resistance; it is therefore essential to accurately characterize genetic reservoirs of resistance (resistomes) accessible to pathogens. The human gut flora is one such reservoir whose genetic diversity has been underestimated by traditional analytic methods relying on stool culture. Since the infant gut microflora is largely determined by the maternal gut microflora, even young infants are expected to harbor resistance genes. **Objective:** To characterize the antibiotic resistome in the fecal microflora of healthy infants and children with a novel culture-independent functional metagenomic technique, and to test the hypothesis that a diverse resistome is present early in life. **Methods:** Genomic DNA was extracted from frozen stool samples collected from 22 healthy children ages 0-19y for a prior study. The DNA was sheared into ~2kb fragments, ligated into an expression vector, and transformed into E. coli to create a metagenomic library. The libraries were plated on antibiotic-containing media to functionally select genomic inserts conferring resistance in the previously susceptible host. **Results:** Metagenomic libraries representing 0.35–4.6 gigabases (mean 1.5 GB) were screened against 15 antibiotics from 7 classes (aminoglycosides, beta-lactams +/- beta-lactamase inhibitors, quinolones, amphenicols, tetracyclines, pyrimidine derivatives, and sulfonamides). All age groups from 0-19 years were represented; 28% of the libraries came from infants <12mo old. All libraries yielded clones resistant to 8-13 agents irrespective of donor age; no trend toward increased resistance genes in older subjects was observed. Pediatric fecal microbiota were a particularly rich source of resistance genes to beta-lactam and sulfonamide antibiotics with some samples containing an estimated >100 resistance genes per gigabase. **Conclusion:** Functional metagenomics is a novel method for culture-independent characterization of antibiotic resistomes in infants and children. The fecal microflora of even young infants is a substantial genetic reservoir of antibiotic resistance, especially to beta-lactam and sulfonamide antibiotics. Sequencing these functionally selected resistance genes will yield key information on the diversity of the pediatric fecal resistome.

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ROLE OF BONE MARROW DERIVED ENDOGENOUS PROGENITOR CELLS IN GUT MUCOSAL REPAIR AND REGENERATION

D Slagle, C Sodhi, M Good, T Prindle, M Branca, A Russo, C Ma, D Hackam, Children's Hospital of Pittsburgh and University of Pittsburgh, Pittsburgh, PA.

Mucosal damage, from such an insult as necrotizing enterocolitis (NEC), is the leading cause of gastrointestinal death in preterm infants. There is currently no definitive treatment for NEC, or its associated mucosal injury. However, stem cell lineages, of vast plasticity, have demonstrated their ability in prior research studies to promote healing at sites of mucosal injury and hence, a potential therapeutic role. We sought to test the hypothesis that murine bone marrow derived progenitor cells can provide therapeutic mucosal healing and intestinal epithelial regeneration. Bone marrow derived cells (BMDCs) were isolated from the femur and tibia of wild type or from double-fluorescent Cre reporter strain mT/mG mice. BMDCs were also labeled with Vybrant CM-DiI Cell Labeling Solution for lineage tracing. BMDCs were either cultured alone in growth media or cultured on an Intestinal epithelial cell (IEC-6) feeder layer. Differentiations of BMDCs into epithelia lineage were studied by immunofluorescence staining using antibodies against intestinal epithelial markers. mRNA expression of intestinal epithelial genes was studied using quantitative real-time PCR. Brdu-labeled BMDCs were also injected in Diphtheria toxin-induced gut epithelial injured (iDTR-villin-cre) mice to study mucosal healing. Results indicated that BMDCs, when co-plated with IEC-6 cells, appear to transform and differentiate to a cell resembling that of an IEC-6 cell and have the ability to provide restoration in an area of artificial damage. This was verified by increased mRNA expression of intestinal epithelial marker genes for enterocytes (sucrase-isomaltase), goblet cells (MUC2), and paneth cells (lysozyme) in BMDCs and IEC-6 co-culture compared to BMDCs cultured alone. Furthermore, cells resembling differentiated BMDCs appeared to have migrated and accumulated in areas of artificial damage (wound-scratch assay). Immunohistochemistry analysis revealed the presence of Brdu-labeled BMDCs in intestinal epithelium. In summary, co-culturing of BMDCs on IEC-6 feeder layer induces differentiation into an epithelial phenotype. Also, the presence of donor BMDCs at the site of intestinal injury suggests a role in intestinal healing.

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LOSS OF TGF-BETA RECEPTORS DURING NEC SENSITIZES INTESTINAL MACROPHAGES TO BACTERIAL PRODUCTS AND PROMOTES MUCOSAL INFLAMMATION

M Krishnan, N Kopperuncholan, R Jagadeeswaran, P Barodiya, D Arbach, S Garzon, A Maheshwari; Pediatrics (Division of Neonatology & Center for Neonatal/Pediatric Gastrointestinal Disease), University of Illinois, Chicago, IL.

Background: We have shown recently that enterally-administered TGF-beta protects against NEC by suppressing macrophage cytokine production. To determine whether protective effects of TGF-beta against NEC can be obtained with physiological levels of TGF-beta or require pharmacological doses, we now investigated changes in TGF-beta receptors and downstream signaling during NEC. **Objective:** To determine whether (1) NEC is associated with decreased expression of TGF-beta receptors (TBR), particularly TBRII, and (2) loss of TGF-beta receptor expression during NEC sensitizes intestinal macrophages to bacterial products. **Design/Methods:** TBR II expression was measured in human NEC and a murine neonatal model of gut mucosal inflammation (enterally-administered TNBS). To investigate the effects of decreased TBRII, we used a transgenic mouse where Zn supplementation blocks native TBR II expression and all TGF-beta signaling. LPS-induced cytokine production and TLR4- and NF-kB-activated pathways were investigated in marrow-derived macrophages using focused qPCR arrays and in vivo after intraperitoneal LPS (1 mg/kg) administration. **Results:** Human NEC and murine mucosal inflammation was associated with decreased TBR II expression in macrophages. In mice, mucosal inflammation was associated with an altered pattern of mRNA splicing during inflammation with a shift from full-length isoforms 'a' and 'b' to a shorter, kinase-deficient transcript 'e'. We detected increased expression of microRNA 23a (miR23a), which is expressed in myeloid cells and is predicted to interrupt TBRII mRNA stability, in inflamed tissues. By co-transfecting with a TBRII 3'UTR-luciferase plasmid and miR23a, we confirmed direct binding of miR23a to the 3'UTR and loss of TBRII expression in RAW264.7 cells. Finally, bone marrow macrophages from transgenic DNIIR mice showed a 2-15x greater IL-6 and MIP-2/CXCL2 response following LPS stimulation than cells from WT mice. These effects were most prominent for. Macrophages in DNIIR mice showed increased expression of TLR4, Myd88, IRAK1, TAK1, IKKalpha, andN FKB2 in the TLR4- and NF-kB-activated signaling pathways. **Conclusions:** (1) Decreased TBR II expression during NEC results from a shift from full-length transcripts to a shorter, kinase-deficient isoform; and (2) Decreased TBR II expression sensitizes macrophages to LPS by upregulating key rate-limiting mediators in the TLR4- and NF-kB-activated signaling pathways.

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REGENERATING ISLET-DERIVED 3-ALPHA IS A BIOMARKER OF GASTROINTESTINAL GRAFT-VERSUS-HOST DISEASE

AC Harris[1], JLM Ferrara[1], JK Greenson[1],TM Braun[1], E Holler[2], T Teshima[3], JE Levine[1], SWJ Choi[1], E Huber[2], K Landfried[2], M Vander Lugt[1], P Reddy[1], Q Zhang[4], S Hanash[4], S Paczesny[1]. [1]University of Michigan, Ann Arbor, MI. [2]University Medical Center, Regensburg, Germany. [3]Kyushu University, Fukuoka, Japan. [3]Fred Hutchinson Cancer Research Center, Seattle WA.

Background: There are no plasma biomarkers specific for GVHD of the gastrointestinal (GI) tract, the GVHD target organ most associated with non-relapse mortality (NRM) following hematopoietic cell transplantation (HCT). **Methods:** We have previously identified elafin as a skin-specific biomarker of graft-versus-host disease (GVHD) (Science TM, 2010). We used the same unbiased, large-scale, quantitative proteomic discovery approach to identify candidate biomarkers that were increased in plasma from HCT patients with GI GVHD. 5 of the 74 proteins that were increased at least 2-fold were of GI origin. We validated the lead candidate, REG3α, by ELISA in samples from 1014 HCT patients from three transplant centers: 197 with GI GVHD, 63 with non-GVHD enteritis, 339 with isolated skin GVHD, and 415 without GVHD. We generated logistic and Cox regression models to test the association of REG3α concentrations with response to treatment and overall survival, respectively. **Results:** Plasma REG3α concentrations were 3-fold higher in patients at GI GVHD onset than in all other patients. Elevated REG3α concentrations correlated most closely with lower GI GVHD and histologic severity (grade 1-3 versus 4, $p=0.03$). REG3α identified GVHD as the cause for diarrhea with better sensitivity and specificity (receiver operating characteristic (ROC) area under the curve (AUC) $=0.80$) than all 5 other biomarkers of GVHD, which added little when combined with REG3α in a 6 biomarker panel (AUC$=0.81$). REG3α concentrations at GVHD onset predicted response to therapy at 4 weeks, NRM, and 1-year survival ($p\leq0.001$). Patients with high REG3α concentrations were more likely to not respond to GVHD therapy (odds ratio$=5.8$) and to die within 1 year of transplant (hazard ratio$=2.1$) when modeled simultaneously with GVHD stage and histologic grade and adjusted for other known risk factors. **Conclusion:** REG3α is a plasma biomarker of GI GVHD with important diagnostic and prognostic value.

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ANALYSIS OF THE HUMAN AMYLASE LOCUS REVEALED EXTENSIVE COPY NUMBER VARIATION OF SALIVARY AND PANCREATIC AMYLASE GENES IN TYPE 1 DIABETES PATIENTS.

A Rutherford, B Zhou, YL Wu, SE Kingery, J Germak, S Bowden, RP Hoffman and CY Yu. Nationwide Children's Hospital, Columbus, OH.

Amylase (AMY) is a digestive enzyme that hydrolyzes the α1,4-glycosidic bonds in polysaccharides. The precursor protein for salivary amylase AMY1 and pancreatic amylase AMY2 share 97% sequence identities, and their genes are located at chromosome 1p21 of the human genome. Multiallelic, continuous copy-number variation (CNV) of AMY1 genes from 2 to 10 copies on a chromosome (haplotype) have been demonstrated by fluorescent *in situ* hybridization. Human populations with traditionally high starch diets were shown to have higher copy-number of AMY1 genes. Autoantibodies against pancreatic AMY2 are a novel biomarker for human acute pancreatitis and fulminant type 1 diabetes (T1D). The objectives of this study are to characterize the specific patterns of AMY1 and AMY2 genes variations among different individuals, and determine if CNVs of AMY1 and AMY2 play a role in genetic susceptibility of T1D and diabetic complications. Our current study population includes 112 T1D patients of European ancestry recruited at the Nationwide Children's Hospital, Ohio, plus 280 race-matched healthy controls. Pulsed field gel electrophoresis (PFGE) of *Pme*I digested genomic DNA was applied to determine segmental duplications of the AMY locus. Genomic Southern blot analyses of *Taq*I and *Pvu*II restriction fragment length polymorphisms (RFLPs) using a 320-bp 3' AMY cDNA probe were performed to distinguish among AMY2 and AMY1 genes. Dotplot analyses (window size$=30$) of the AMY locus revealed duplications of two 50-kb segments spanning from AMY2A to AMY1A and AMYP1 to AMY1C, which are separated by a 46-kb region with similar sequences but configured in the reverse orientation and consists of the AMY1B gene. Southern blot analyses of *Pme*I-digested genomic DNA resolved by PFGE displayed multiple AMY haplotypes with *Pme*I fragments varying from 250 kb to 610 kb. The simplest haplotype is represented by a ~ 250 kb *Pme*I fragment characterized by the presence of AMY2B, AMY2A and AMY1C genes, as revealed by *Taq*I and *Pvu*II RFLP. The most common *Pme*I-haplotype is represented by a 354 kb fragment similar to that of the Human Reference Sequence with six AMY genes, and is present in two-thirds of our samples. *Taq*I RFLP revealed that the pancreatic AMY2B gene also varies in copy number between 1 and 4 copies in a diploid genome. Comparisons between T1D and control samples reveal that there is no difference in the copy number of the salivary AMY1 genes, but having a higher copy number of AMY2B (>2 genes) is protective against T1D ($p=0.045$). In addition, a polymorphism at the 3' region of AMY2B characterized by the presence of a 4.5kb *Taq*I fragment in Southern blot appears to be highly protective against T1D ($p=0.0027$). Further experiments are in progress to define the roles of AMY2 in T1D.

MWSPR Plenary Session III

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REGULATION OF OVINE FETAL RENAL IRON, NEPHROGENESIS AND ENOS IN UTERINE SPACE RESTRICTION (USR)

MY Sun[1], KM Meyer[1], JM Koch[1], RR Magness[1] and PJ Kling, [1] [1]Ob/Gyn, Pediatrics, Nutritional Sciences, University of Wisconsin, Madison, WI.

Aims: Intrauterine growth restriction (IUGR) deriving from many etiologies disrupts iron (Fe) transport and impairs renal function in rodent models; Fe status has not been studied in IUGR sheep. Transferrin receptor (TfR) is the major placental cell-surface protein transporting Fe. TfR expression is controlled by iron regulatory proteins (IRP) modulated by nitric oxide (NO) via endothelial nitric oxide synthase (eNOS). We hypothesized that kidneys from a USR model of IUGR will exhibit impaired development, in association with negative Fe status and reduced eNOS expression. **Methods:** We used an ovine IUGR model of USR (Meyer et al. 2010) via single unilateral uterine horn ligation before breeding for 1-3 fetuses. These were compared to sheep fetuses of nonspace restricted (NSR) controls at gestation day (GD) 120 and GD130 (term$=147$). Blood total iron binding capacity (TIBC), plasma transferrin (Tf), Tf saturation, and fetal kidney and liver non-heme Fe were quantified. Immunoblotting was performed for TfR and eNOS expression. Cessation of nephrogenesis was determined by identifying an active or inactive nephrogenic zone. **Results:** Compared to NSR, USR fetal TIBC, [Tf] was increased, and Tf saturation decreased at GD130, but not at GD120. Total fetal kidney Fe content (μg) was similar in NSR and USR at GD120 and 130. However Fe proportionate to fetal wt (μg/kg) fell from GD120 to 130. Because fetuses were smaller in USR, proportional Fe levels were greater in USR at GD130. Liver [Fe] and total Fe appeared to be increased between NSR 120 and NSR 130, but were significantly lower in USR 130; no differences were observed across all groups when expressed μg/kg of fetal wt. There was no difference in renal TfR expression between groups, while renal eNOS expression trended higher in GD130 USR vs both GD120 USR and GD130 NSR. The nephrogenic zone had matured at GD120 in NSR, but matured later in USR. **Conclusions:** Contrary to our hypothesis, the IUGR fetuses adapted to USR by delivering more Fe via the blood and accretion by the kidney. There was no association between renal TfR and eNOS. The non-heme Fe assay does not measure red cell Fe, so greater renal Fe is not derived from eNOS-mediated renal vascular dilation. Interestingly, fetal liver and kidney Fe appear to be regulated differently. Greater renal Fe may be due to the tight regulation of fetal Fe homeostasis during the delay in active nephrogenesis and Fe needs for ongoing cell proliferation during nephrogenesis. NIH HL49210, HD38843, HL87144

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DIFFERENTIAL SEX-SPECIFIC EFFECTS OF HYPERGLYCEMIA DURING PREGNANCY ON OFFSPRING KIDNEY DEVELOPMENT AND RENAL HISTOLOGY

RS Lundberg[a], JL Segar[b], TD Scholz[b], PJ Kling[a] [a]University of Wisconsin School of Medicine and Public Health, Department of Pediatrics, Madison, WI; [b]University of Iowa College of Medicine and University of Iowa Children's Hospital, Iowa City Iowa.

Background: Offspring of Diabetic Mothers (ODM) are at risk for adverse conditions due to changes in postnatal physiology and metabolism, for example, hypertension as a young adult. The mechanistic reason for this association is unknown. The maculae densa (MD) are specialized distal tubules that regulate salt and water balance and control blood pressure at rest. **Objective:** To determine whether ODM rats develop hypertension due to developmental alterations in microscopic kidney architecture. **Methods:** Sprague-Dawley rats were made diabetic on day 13 of gestation by injection of 50 mg/kg streptozotocin (STZ) to study effects on offspring (ODM). Blood glucose levels were monitored and SQ insulin maintained levels between 150-400 mg/dL. After birth pups from STZ dams were cross-fostered to non-diabetic dams and weaned normally. Controls were also studied. Developmental study included kidneys from P1 and P21 ODM and controls. At 7 months of age, before sacrifice, blood pressure was determined by surgically placed telemetry probes. Kidneys were harvested, weighed, fixed and sections made. Slides were blindly examined for appearance of MD, with # nuclei in the largest MD recorded and hyperplasia of MD, defined as >10 cells/MD. **Results:** The male ODM were hypertensive at rest, compared to the female ODM, or control, $P<0.05$. As in controls, male ODM were larger than female ODM, $P<0.001$. Kidneys were proportionate to body size in controls, but kidney wt/body wt was lower in male ODM than female ODM, $P<0.006$. Male ODM kidney wt/body wt was also lower than male controls, $P=0.05$. At P1 and P21, MD appearance between ODM and control, and between sexes were similar. At 7 months, median # of cells/MD was similar between ODM and controls, and between sexes. At 7 months, MD in controls were similar in appearance, but # nuclei in the largest MD was greater in ODM males than ODM females, $P=0.04$. Additionally % of MD with hyperplasia trended higher in male ODM than female ODM ($P=0.1$). **Conclusion:** Sex differences in kidney wt and MD appearance at 7 months were seen in the ODM but not control. The MD are specialized distal tubular cells that sense sodium chloride to regulate GFR and renin release from its cellular stores. Hypertrophy of MD in male ODM could alter gene expression and function, increasing salt and water retention and permanently programming higher blood pressures at rest.

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ANGIOTENSIN II (ANG II) MODULATION OF CARDIAC REMODELING IN THE IMMATURE FETAL HEART IS DEPENDENT UPON AFTERLOAD EFFECTS

JA Sandgren TD Scholz, JL Segar. University of Iowa Children's Hospital, Iowa City, IA.

Background: In the postnatal heart, ANG II stimulates cardiomyocyte hypertrophy. *In vitro* ANG II stimulates hyperplasia of fetal mononucleated cardiomyocytes. However, *In vivo* effects of ANG II on the immature fetal heart have not been studied. **Objective:** To determine whether ANG II stimulates heart and cardiomyocyte growth in the immature fetal heart *in vivo* independent of the effects on cardiac afterload. **Methods:** Twin gestation fetal sheep were studied at ~100 d gestation, when cardiomyocytes are predominantly mononucleated. One fetus was catheterized and received a continuous infusion of ANG II alone (50ug/kg/min) or ANG II plus nitroprusside (NTP) to attenuate the increase in blood pressure. The second twin remained a non-catheterized control. After 6 d hearts were removed and either perfused for myocyte isolation or weighed and tissue frozen for later analysis. **Results (see table):** ANG II produced a significantly greater increase in mean BP than ANG II + NTP. ANG II, but not ANG II + NTP resulted in a significant increase in heart mass (left and right ventricle + septum, corrected for body weight) compared to controls. Preliminary, ANG II but not ANG II + NTP increased LV cardiomyocyte area above control. No differences in nucleation or RV cardiomyocyte area or were identified among groups. **Conclusion:** *In vivo*, ANG II increases fetal cardiac mass via cardiomyocyte hypertrophy and likely hyperplasia. The effects of ANG II appear dependent upon a co-existing increase in afterload. Effects of ANG II and ANG II + NTP on expression of proteins involved in cardiomyocyte growth and cell cycle activity are ongoing.

Group	Mean BP, mmHg (day 0)	Mean BP, mmHg (day 6)	Heart mass/fetal body weight (g/kg)	LV cardiomyocyte area (um^2)
ANG II	37±1 (n = 9)	50 ± 1*#	7.26±0.21* (n = 5)	364 ± 12
Control for ANG II			5.27 ± 0.20 (n = 5)	323 ± 15
ANG II+NTP	34±1 (n = 9)	40 ± 1*	6.24±0.15 (n = 6)	300 ± 5
Control for ANG II+NTP			5.67 ± 0.18 (n = 6)	306 ± 6

* $p<0.05$ compared to relative control. # $p<0.05$ compared to ANG II + NTP. Mean ± SE.

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HUMAN ENGINEERED CARDIAC TISSUE MODEL OF POMPE'S DISEASE

K Raval, A Grimes, WJ de Lange, TJ Kamp, JC Ralphe University of Wisconsin School of Medicine and Public Health, Madison, WI.

Infantile-onset Pompe disease is a lethal autosomal recessive lysosomal storage disorder caused by the complete loss of acid α-glucosidase activity resulting in glycogen accumulation within lysosomes. Skeletal and cardiac muscle pathology develop due to aberrant glycogen metabolism and lysosomal dysfunction. This work presents the first in vitro human cardiac tissue model of Pompe disease documenting the classic Pompe cellular phenotype within a multicellular engineered cardiac tissue preparation. Methods:Pompe disease-specific human induced pluripotent stem (iPS) cell derived cardiomyocyte (iPS-CMs) were seeded into a polymerizing fibrin mixture to form mechanically integrated multicellular engineered cardiac tissue (ECT) constructs that were evaluated over a one-month period in parallel with healthy human iPS cell derived ECTs. Weekly measurements of isometric force and intracellular calcium transients were recorded under varying work load imposed by electrical pacing and β_1 adrenergic stimulation. Structural assessment was performed using electron microscopy and immunohistochemistry. Results: Healthy and Pompe iPS-CMs form cylindrically shaped ECTs of 1-2 mm diameter and 1.5-2 cm in length composed of coupled CMs that demonstrate calcium transients and twitch force. We report structural changes in the ECT model consistent with the hallmarks of Pompe disease including progressive intracellular glycogen accumulation, lysosomal disruption, and myofibrillar disarray. Both healthy and Pompe ECTs generate higher twitch forces in response to mechanical stretch, have a negative force-frequency relationship characteristic of immature myocardium, and have an inotropic response to dobutamine stimulation. Conclusion: Human iPS-CM ECTs obey the Frank-Starling law and produce calcium cycling and force kinetics that respond to β_1 adrenergic stimulation. The Pompe ECT provides a novel cardiac tool in which to further study the pathophysiology of this disease and test novel therapeutic approaches.

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NATIONAL VARIANCE IN INFANT MORTALITY FOLLOWING EXTREMELY PRETERM BIRTH

R Nubani,[1] C Burnett,[1] S Verhulst,[2] D Batton,[1] B Batton[1] [1]Department of Pediatrics and [2]Department of Statistics and Epidemiology, Southern Illinois University School of Medicine, Springfield, IL

Background: Although extremely preterm infants comprise a disproportionally high percentage of infant deaths, contemporary infant mortality rates (IMR) and the impact of different epidemiologic data on these rates specific to this population are lacking. **Objectives:** Identify national differences in the extremely preterm IMR based on location of birth and maternal demographics. **Methods:** Data from the birth-death linked database from the National Center for Health Statistics was used to determine the IMR for infants born alive in the United States at $20^{0/7}$– $27^{6/7}$ weeks gestation from 1/1/1995 – 12/31/2005. Multi variable analysis was used to assess the impact of different factors on IMR. **Results:** The IMR for the 306,502 extremely preterm infants born alive during the study years was 392/1,000 live births. The IMR varied considerably across states (309 – 477), urban counties (223 – 548), and rural counties (309 – 469), but the IMR did not vary significantly across geographic regions. There was not a statistically significant relationship between state IMR and population or extremely preterm birth rate. Urban counties with higher extremely preterm infant birth rates had lower IMR ($p=0.02$). On univariate analysis, significant differences in IMR were noted across different maternal races and education levels as well as urban versus rural place of birth, but on multi-variable analysis, maternal race had the largest impact on IMR. **Conclusions:** Although IMR have declined substantially over the years, disparities continue to exist between different maternal races and levels of education, as well as location of birth. The single largest impact on IMR in urban versus rural residence was maternal race.

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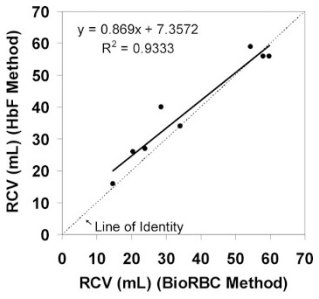
RED CELL VOLUME CAN BE MEASURED IN VERY LOW BIRTH WEIGHT INFANTS USING DILUTION METHOD OF HEMOGLOBIN F CELLS BY ALLOGENEIC ADULT RED BLOOD CELL TRANSFUSION

D Nalbant[1], N Matthews[3], P Bhandary[2], A Bogusiewicz[3], R Schmidt[2], D Mock[3,4], J Widness[2], [1]Pharmacology, [2]Pediatrics, U Iowa, Iowa City, IA; [3]Biochemistry & Molecular Biology, [4]Pediatrics, U Arkansas Med Sciences, Little Rock, AR

Background and Objective: Anemia is a common medical problem in very low birth weight (VLBW), preterm infants necessitating multiple red blood cell (RBC) transfusions due to physiologic and non-physiologic factors associated with prematurity. To further elucidate pathophysiology of anemia and the responses to RBC transfusion in preterm infants, the development of a non-invasive, accurate, and repeatable method for measuring RBC volume (RCV) using small quantities of blood would be useful. In the current study we sought to compare a newly developed method for RCV based on the dilution principle and flow cytometric measurement of fetal hemoglobin (HbF) against RCV determined using the established biotinylated RBC (BioRBC) method. **Study design and Methods:** 8 preterm infants with gestational age 26-30 weeks and weight 0.37-1.96 kg were studied. RBCs (1-2 μL) from pre- and post-clinical transfusion blood samples were incubated with a fluorescent-tagged antibody against HbF; HbF containing recipient RBCs (HbF+) and donor RBCs without HbF (HbF-) were enumerated by flow cytometry. 2-3 % of donor RBCs were labeled with biotin and infused at the end of the clinical RBC transfusion. Flow cytometric enumeration of fluorescent-tagged BioRBCs was used to determine the percentage dilution in the total RBC population, and RCV was determined by the dilution method. Paired *t* test was used to determine the significance of a difference between the means. **Results:** The mean±SD of RCV values for HbF method was 39±16 mL and for BioRBC method was 37±18 mL (p=NS, paired *t* test). The 2 methods were highly correlated with the slope of regression line similar to the line of identity (Fig). **Conclusions:** Detection of HbF by flow cytometry is a safe and accurate method requiring μL amounts of blood for determination of circulating RCV in VLBW, premature infants that does not require any additional labeling of RBCs. We speculate that the HbF flow cytometry method can also be used for repeated measurements of RCV in premature infants in conjunction with a substantial transfusion of RBCs from adults.



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POSH: A POTENTIAL MEDIATOR BETWEEN SARCOMERIC DYSFUNCTION AND HYPERTROPHY IN A MYBPC-NULL MODEL OF HCM

Adrian C. Grimes, Willem J. DeLange, Travis L. Schmidt, Michael Wilhelm, J. Carter Ralphe. University of Wisconsin Madison, Department of Pediatrics, 601 Science Drive, Madison, WI 53711

Affecting approximately one in every 500 people, familial hypertrophic cardiomyopathy (HCM) is a leading cause of sudden cardiac death in young adults. The majority of HCM-causing mutations identified to date encode sarcomeric contractile proteins and among the most prevalent are those that affect cardiac myosin binding protein C (*MYBPC*). While much research has led to a greater understanding of how such mutations impair sarcomeric function, it is still not clear how dysfunction causes progression through compensated hypertrophy to dilated failing cardiomyopathy. In a mouse model of HCM in which *MYBPC* has been genetically ablated and causes impaired sarcomeric function, we observe a severe hypertrophy between birth and 10 days. This can be seen most obviously by histology, heart weight to body weight ratios, and increased expression of hypertrophic markers such as *Nppa* & *Nppb*. We also find that POSH (Plenty of SH3s), a scaffold protein involved in the c-Jun N-terminal kinase (JNK) and nuclear factor (NF)-κB pathways, undergoes a 5-fold upregulation coincident with the onset of the development of hypertrophy. Both p-JNK (activated) and total JNK are also upregulated. In addition to its role in the JNK and NF-κB pathways, POSH is known to interact with the proapoptotic proteins BNip3 and Nix, which are implicated in the hypertrophic response in cases of ischemia reperfusion and chronic pressure overload, respectively. We propose that POSH is likely a mediator of the disease progression from the primary impairment of sarcomeric function through compensated hypertrophy and cardiac failure.

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THE RELATIONSHIP OF FOLATE AND METHOTREXATE WITH DRUG RESPONSE IN JUVENILE IDIOPATHIC ARTHRITIS

D Patel[1], L vanHaandel[2], A Lasky[2], MF Hoeltzel[2], JS Leeder[2], ML Becker[2] [1] University of Missouri Kansas City School of Medicine, Kansas City, MO; [2] Children's Mercy Hospital and Clinics, Kansas City, MO

Background: Methotrexate (MTX) is an effective drug for the treatment of many adult and childhood diseases. This folate antagonist is bioactivated by polyglutamation (MTXGlu). "Long chain" $MTXGlu_n$ have been associated with improved response to MTX in Rheumatoid Arthritis. However, studies in the pediatric population have been contradictory. We investigated the relationship between intracellular folate and MTXGlu concentrations and clinical response to MTX in JIA. **Methods:** This was a cross sectional single center study of JIA patients (n=97) receiving stable doses of MTX. After obtaining informed consent, blood was obtained during routine lab monitoring. Demographic characteristics, efficacy and toxicity outcomes were collected by chart review. $MTXGlu_{1-7}$in red blood cell (RBC) lysates were quantitated using an innovative ion-pairing chromatographic procedure with mass spectrometric detection. Concentrations of 5-methyl-tetrahydrofolate (5-CH_3-THF) and 5,10-methenyl-THF (5,10-MTHF) in whole blood, plasma and erythrocytes were deconjugated and analyzed via reversed phase separation and stable isotope dilution tandem mass spectrometry. **Results:** The majority of RBC folate was present as 5-CH_3-THF (669.0 ± 276.4 nmol/L, range 164.5 to 1,448.8 nmol/L). The distribution of 5,10-methenyl-THF was right-skewed with a median concentration of 46.4 nmol/L (range 9.7 to 667.7 nmol/L). $MTXGlu_{TOT}$ concentrations were corrected for MTX dose administered ($cMTXGlu_{TOT}$): median 166.6 nmol/L (range 12.2 to 865.9 nmol/L). The relationship of folate to MTX represented as the ratio of sum RBC folate/ log $cMTXGlu_{TOT}$ was much higher in subjects with persistent disease (p=0.005), indicating less folate inhibition by MTX. Mean ratios were lower in subjects with GI side effects and liver toxicity, but not statistically significant in the small number of affected patients. **Conclusion:** In JIA, measurement of MTXGlu and folate together may be more meaningful in relation to drug response than either variable alone. JIA patients with active arthritis had higher folate/MTX ratios compared to asymptomatic subjects. Subjects with side effects may have an opposite effect; however, we could not prove this statistically in the small group of affected patients. The prospective evaluation of folate patterns in relation to MTX will be necessary to fully elucidate the intracellular effect of this drug and any relationship with outcome.

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NOVEL ECHOCARDIOGRAPHIC MEASURES OF RIGHT VENTRICULAR PERFORMANCE DISCRIMINATE NEED FOR RESPIRATORY SUPPORT IN PREMATURE INFANTS

Philip T. Levy, Mark R. Holland, Gautam K. Singh, Aaron Hamvas. Departments of Physics and Pediatrics, Washington University School of Medicine, St Louis, MO.

Background: Pulmonary morbidity in infants born prematurely is reflective of a reduction in both alveolar growth and structural changes in the lung vasculature. Arrest of vascular development results in a decrease in vascular surface area and, thus, an increased pulmonary resistance that can adversely affect right ventricular (RV) cardiac structure and function. Novel echocardiographic measurements of myocardial strain, a measure of myocardial deformation or stretching over the heart cycle, and strain rate, the rate of this myocardial deformation, may provide insight into pulmonary vascular development. **Objective:** To determine if novel echocardiographic measurements of right ventricular strain correlate with need for respiratory support at 36 weeks corrected gestational age (CGA). **Methods:** Premature infants (n=22) born at 27 ± 2 weeks gestational age, underwent echocardiographic studies at 33 ± 1 weeks of age, and at 38 ± 2 weeks post-conceptional age We used 2D speckle tracking echocardiography to measure peak global longitudinal strain (GS) and strain rate (GSR) in the RV free wall (RVFW) and LV free wall (LVFW). Lower strain and strain rate indicate less myocardial deformation (contractility). We then compared LV and RV GS and GSR in infants with and without respiratory support (supplemental flow via nasal cannula or CPAP) at 36 weeks CGA. **Results:** LV-GS and LV-GSR did not change between the initial and follow-up echocardiographic examinations in either group. Initial RV-GS and RV-GSR were also not different between the two groups at the 1st measurement; however, on the follow-up exam, RV-GS (p = 0.02) and RV-GSR (p = 0.008) were lower for the group who required respiratory support at 36 weeks. Longer duration of ventilation correlated with lower RV-GS (r = 0.86, p= 0.003) and lower RV-GSR (r = 0.81, p= 0.008) at the follow-up exam. **Conclusion:** Our study suggests that premature infants who require respiratory support at 36 weeks CGA demonstrate reduced RV myocardial function. This observed decrease in RV deformation (strain), coupled with the decrease in the rate of RV deformation (strain rate), may provide objective markers that lend insight into pulmonary vascular development in premature infants with adverse pulmonary outcomes. (This study was supported, in part, by NIH 1U01 HL101465.)

Poster Session

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PREMEDICATION WITH PROPOFOL VERSUS ATROPINE, MIDAZOLAM, AND SUCCINYLCHOLINE FOR NON-EMERGENT NEONATAL INTUBATION

M. Moallem, A. Shah, E. Ambrecht, M. Al-Hosni Department of Pediatrics, Division of Neonatal-Perinatal Medicine, Saint Louis University SOM, St Louis, MO 63104

Background: Endotracheal intubation is a stressful and painful procedure. However, in neonates, less than 50% of the non-emergent endotracheal intubations (NEI) are preceded by premedication. In this study, we evaluated the use of premedication for neonatal NEI using propofol versus a regimen of atropine, midazolam, and succinylcholine. **Methods:** A randomized, controlled clinical trial, which includes all patients admitted to our Neonatal Intensive Care Unit and requiring NEI. Patients with cardiac, airway or major congenital anomalies are excluded. At time of intubation, infants are randomized to receive either propofol (P group) or the regimen of atropine, midalzolam, and succinycholine (R group). All intubation procedures are recorded at the bedside using a portable video camera. All recorded videos are reviewed to assess medication infusion time, total procedure time (start of medication infusion to confirmation of tube placement), and vital signs throughout the procedure. **Results:** To date, 19 neonates are enrolled in the study (R group 11, P group 8). There was no statistical difference between the two groups in medication infusion time in seconds (R group 288 vs P group 179, P =0.34) or in the total procedure time in seconds (R group 502 vs P group 378, P= 0.39). Percentage change in mean blood pressure from baseline at 6 minutes from the start of medication infusion, was noted between the two groups (R group +27%,vs P group −11% P = 0.04). **Conclusion:** Propofol is a valid alternative to a combination of sedative and paralytic premedication to achieve successful neonatal NEI. Tendency for lower blood pressure after propofol infusion has been noted.

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ONLINE SAFETY EDUCATION: A PILOT INTERVENTION

Bare KF, Moreno MA, University of Wisconsin-Madison, Madison, WI

Purpose: Today's youth are vulnerable to the potential risks of online activity, including cyberbullying and online predation. Little research has examined the effectiveness of intervention materials to promote discussions between parents and their children about online safety. The purpose of this study was to determine the feasibility of distributing an intervention booklet within a general pediatrics clinic and if it is successful in promoting parent-child discussions about online safety. **Methods:** An online safety booklet was developed through a review of scientific literature followed by examination of current online educational materials. Marketing principles were applied to design and content to enhance appeal and readability. The booklet was piloted at a university-based general pediatrics clinic to parents with children ages 7-12. The parents were asked to complete a baseline survey about the frequency and content of online safety discussions with their child. The child received the booklet during the visit. The parents were contacted two weeks later to complete a follow-up survey and provide feedback on the booklet. Descriptive statistics were calculated. **Results:** A total of 12 parent-child dyads were recruited. At baseline, 10 parents reported previous discussions with their child about online safety issues. Of these parents, 25% had monthly discussions and 33% had discussions more often than monthly. Privacy protection and cyberbullying were the most commonly discussed topics. A majority of parents reported consulting the internet (50%) or newspapers and magazines (50%) as sources of information for their discussions. In the follow-up survey, 92% of parents reported that they kept the booklet. Of these parents, 91.1% had at least one internet safety conversation in the two weeks following the clinic visit. Other children in the family looked at the booklet in 36.6% of households. Of the two parents that had not previously engaged in discussions with their child, both reported at least one discussion within the two weeks following the clinic visit. Suggestions for improvement included making content easier to read for younger teens and children, however 63% of parents felt that no improvements were needed. **Conclusion:** The results of this study demonstrate that a physician-distributed booklet to children would be a feasible and successful tool for promoting parent-child discussions concerning online safety. Future plans include modifying the booklet and testing in a larger randomized controlled trial.

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BIOCHEMICAL EFFECTS OF ACTIN MUTATIONS ASSOCIATED WITH VASCULAR DISEASE

SE Bergeron, LE Malloy, ND Vanderpool, AR Pierick, EW Wedemeyer, KK Wen, M McKane, PA Rubenstein, *HL Bartlett* University of Iowa Carver College of Medicine, Iowa City, Iowa

Approximately 20% of thoracic aortic aneurysm and dissection result from inherited disorders and is the 15th leading cause of death in the United States. The vascular smooth muscle cell specific isoform of alpha-actin (*ACTA2*) is a major component of the contractile apparatus. Twenty-two missense mutations in *ACTA2* have been identified to cause autosomal dominant thoracic aortic aneurysms. The biochemical basis for the actin mediated aortopathy is still unknown due to limited access to diseased tissue, the presence of multiple unresolvable actin isoforms in the cell, and lack of an animal model. We hypothesize that *ACTA2* mutations contribute to aortic disease by inducing actin filament instability or altered actin-binding protein interaction. We have engineered several human mutations into yeast actin, which is 86% similar to human alpha-smooth muscle actin to investigate their effect on *in vivo* and *in vitro* actin function. The current study focuses on two mutations of the same amino acid, R256C and R256H. The human phenotype includes aortic aneurysm, occlusive stroke and patent ductus arteriosus. Cells expressing these mutant actins as the sole actin in the cell were viable but exhibited reduced ability to grow under a variety of stress conditions. Cell cytology was altered with abnormalities found in the morphology of the cytoskeleton, mitochondria and vacuoles. *In vitro*, we assessed biochemical changes in the actin monomer. Mutant actins exhibited altered decreased thermostability indicating altered monomer conformation but maintained normal nucleotide exchange rates. Mutations displayed different polymerization kinetics despite altering the same residue. R256H decreased filament stability compared to R256C. Preliminary data on the ability of mutant actin to interact with myosin shows no deficits. Actin-dependent myosin ATPase was normal for both mutant isoforms and no difference in binding have been identified. Our findings differ from previous studies and support that mutant-specific mechanisms may contribute to the range of actin-mediated vascular disease. Our data also highlights that the yeast model continues to be an excellent system to investigate the role of actin in human disease.

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PREVALENCE OF GASTROINTESTINAL PERFORATIONS IN PRETERM NEONATES DIAGNOSED WITH PATENT DUCTUS ARTERIOSUS WHO WERE TREATED WITH IBUPROFEN IN COMPARISON TO INDOMETHACIN

S Bauer, T Thomson, P Chhangani, M Shareef, Stritch School of Medicine, Div. of Neonatology, Dept. of Pediatrics, Loyola University Medical Center, Maywood, IL.

Purpose of Study: Patent Ductus Arteriosus (PDA) is a common heart problem in premature neonates. Indomethacin has been used to treat PDA, but complications can arise such as decreased blood supply to the gastrointestinal (GI) tract and kidneys. GI perforations are detrimental because they can lead to surgery. Recently, treatment changed to ibuprofen, as it causes less of a decrease in blood supply. It is thought that with less blood supply interruption, fewer GI perforations would occur. By exploring ways to decrease the risk of perforations, neonates with PDA will hopefully have fewer treatment side effects. Our goal was to determine which treatment leads to fewer GI perforations and the effectiveness in closing the PDA. **Methods:** A retrospective chart review was performed for all preterm neonates admitted to the NICU between 2006-2010 who were diagnosed with PDA and treated with either ibuprofen or indomethacin. Demographic data such as birth weight, gestational age, and Apgar scores, with specifics about GI perforation and additional complications was collected. Chi-squared analysis was then performed. **Results:** A total of 200 preterm neonates diagnosed with PDA were identified, 101 treated with indomethacin and 99 with ibuprofen. There was inconclusive data as to PDA closure for 3 neonates (2 indomethacin and 1 ibuprofen); thus, 99 infants treated with indomethacin and 98 neonates treated with ibuprofen were included in the final analysis. There was no statistical difference between the two groups. Neonates treated with indomethacin had a significantly higher rate of PDA closure than those treated with ibuprofen. Of 99 neonates treated with indomethacin, 75 (76%) were treated successfully, compared to ibuprofen in which 59 out of 98 (60%) were successfully treated (p=0.019). There was no significant difference in GI perforations between neonates treated with indomethacin or ibuprofen. Perforation occurred in 9 neonates out of 99 (9%) treated with indomethacin, compared to 11 out of 98 (11%) treated with ibuprofen (p=0.62). **Conclusions:** Indomethacin appears to be more effective at closing PDA. Furthermore, we found no difference in GI perforations between the two treatments. However, due to the study limitations, we cannot conclusively state that indomethacin is a better treatment than ibuprofen. Future, larger studies may be necessary to determine if this difference in efficacy persists.

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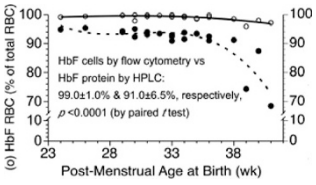
FETAL HEMOGLOBIN EXPRESSION IN INTACT CORD RED BLOOD CELLS MEASURED BY FLOW CYTOMETRY VERSUS BY HPLC DURING 24–41 WEEKS POST-MENSTRUAL AGE

P Bhandary[1], D Nalbant[2], R Schmidt[1], G Cress[1], N Matthews[3,4], D Mock[3,4], J Widness[1], Departments of [1]Pediatrics, [2]Pharmacology, U Iowa, Iowa City, IA; Departments of [3]Biochemistry & Molecular Biology, [4]Pediatrics, U Arkansas Med Sciences, Little Rock, AR.

Background and Objective: Red blood cells (RBCs) at birth have predominately fetal hemoglobin (HbF) and very little adult hemoglobin (HbA). The switch from HbF to HbA production increases rapidly after ~32 wks gestation and by 1 year HbA is the predominant Hb species. Our initial studies using FITC-tagged HbF antibody on intact RBCs in term infants showed that nearly 100% of the RBCs had detectable HbF and the proportion of HbF+ RBC observed was not consistent with prior reports for HbA and HbF protein content obtained from HPLC analysis of hemolyzed blood. Although the nature of this developmental switch from HbF protein to HbA protein has been studied using high pressure liquid chromatography (HPLC) of hemolyzed RBCs, there are no data on the switch of HbF cells in intact RBCs. We hypothesize that the HbF cell proportion obtained by flow cytometry will always be >%HbF obtained by HPLC and the developmental decline in HbF RBC proportion will lag behind that of HPLC %HbF protein. **Study Design and Methods:** Subjects enrolled included a convenience sample of 23 neonates from 24 to 41 wks post-menstrual age at birth. Analysis of the same cord blood samples was performed by: 1) flow cytometry of intact RBCs using FITC conjugated-antibody against HbF for HbF RBCs; and 2) quantitation after HPLC separation of HbF from other Hb species. Paired *t* test was used to examine the relationship of %HbF+ RBCs and %HbF protein. **Results:** As indicated (Fig), the %HbF+ RBCs were always greater than HbF protein (paired *t* test, $p < 0.0001$). The decline of %HbF+ RBCs lagged behind %HbF protein beginning at ~36 wk. **Conclusions:** The developmental decline of HbF+ RBCs lags behind that of HbF protein. Our results indicate that the switch from HbF to HbA production in fetal RBCs happens such that RBCs contain both HbA and HbF instead of separate clonal populations of HbA and HbF RBCs. Understanding of this process in neonatal RBCs can be applied in developing techniques for performing RBC kinetic, ie, RBC survival and RBC volume, studies using HbF+ and HbF- RBCs of transfused neonates in whom radioactive ^{51}Cr is not ethically justified.



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SCREENING FOR CONGENITAL HEART DISEASE IN NEWBORNS: DO BLOOD PRESSURE MEASURMENTS IMPROVE THE PROCESS?

KL Boelke, J Hokanson. University of Wisconsin Hospitals and Clinics, Madison, Wisconsin. United States.

Purpose: The purpose of this study was to evaluate the effectiveness of neonatal pulse oximetry (POx) and blood pressure (BP) screening in identifying infants with unrecognized critical congenital heart disease (CCHD). **Methods:** A retrospective chart review was performed at a single tertiary care hospital where both BP and POx screening for CCHD are performed. These screenings were performed after 24 hours of age or prior to discharge. The POx measurement was done in either leg and is considered abnormal if < 95%. The BP measurements were taken in the right arm and either leg. The BP was considered to be abnormal if the right arm was 15 mm Hg > the leg. Abnormal measurements were repeated within two hours. An echocardiogram was performed if the repeat measurement was also abnormal. All charts of infants born at greater than 34 6/7 weeks gestation between 2-12-08 and 12-31-10 were reviewed. To evaluate the possibility of a false negative screening test, the charts of all infants admitted with congenital heart disease under 30 days old to the city's three hospitals were reviewed. **Results:** 10,436 infants greater than 34 6/7 weeks gestation were born during the study period. 325 did not undergo the congenital heart disease screening, 99 had incomplete screening, and 10,012 infants completed the screening. 9,999 infants passed the screening (99.87%). Of these infants 151 required a repeat screening because of an abnormal initial result. The BP screening was responsible for 128 of these initial abnormal screenings. Ultimately, 12 infants failed the BP screening, 1 infant failed both screenings, and no infant failed only the POx screening. Only 6 of the 12 infants failing the BP screening had an echocardiogram performed and none had CCHD on echocardiography. The infant failing both screenings had a normal echocardiogram. No infants were identified who had been discharged home with a diagnosis of missed CCHD. There were no true positive or known false negative results. There were 13 false positives. The false positive rate was 0.13% for the BP and 0.01% for the Pox screening components. **Conclusion:**In this small sample in which all children with CCHD were detected by prenatal ultrasound or physical examination, only the adverse consequences of screening could be evaluated. In this population, the false positive rate of BP screening was greater than that of Pox screening.

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LUNG COMPUTED TOMOGRAPHY DURING LUNG RECRUITMENT MANEUVER IN PEDIATRIC PATIENTS WITH ACUTE LUNG INJURY

*J.P Boriosi**, R.A Cohen[#], E Summers[#], A Sapru[†], J.H Hanson[#], G Gildengorin[#], V Newman MD[#], and H Flori[#]. *University of Wisconsin, Madison, Wisconsin, [†]University of California San Francisco, San Francisco, California, [#]Children's Hospital and Research Center Oakland, Oakland, California.

Purpose: To describe CT-scan lung aeration changes after a recruitment maneuver (RM) in ventilated pediatric patients with acute lung injury (ALI). **Methods:** Prospective pilot study in Pediatric intensive care unit (PICU) at an urban, tertiary children's hospital. Patients: Six ventilated pediatric patients with ALI. Intervention: RM using incremental positive end-expiratory pressure (PEEP). **Results:** There was an overall increase in lung aeration after the RM (range 1-72 %, p = 0.03). All patients improved the PaO2/FIO2 ratio after the RM (range 7- 99 %, p = 0.03). In four of six subjects ventilation, as measured by PaCO2, improved after the RM (p = 0.5). The only subject with clinically significant yet transient hypercapnia (41% increase in PaCO2) during the RM had the smallest increase in lung aeration (1%). All patients tolerated the RM without hemodynamic compromise, barotrauma, hypoxemia, or dysrhythmia. There was one case of transient hypercapnia during the RM that resolved entirely by the end of the maneuver. **Conclusions:** The modified lung RM used in this study resulted in statistically significant increase in lung aeration and oxygenation. In 4 of our 6 subjects ventilation and oxygenation improved with increase in lung aeration. Assessing lung recruitability using CT-scan in early ALI seems safe and may have therapeutic and prognostic implications. Implementation on a wider scale is required to further validate our results.

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A DISEASE MUTATION OF BESTROPHIN CAUSES ALTERED CHLORIDE CHANNEL AND MAY PROVIDE INSIGHT INTO MACULAR DEGENERATION

S K Brar[A], P J Halbach[A], B R Pattnaik[B], S A Tokarz[A], D M Pillers[C]. [A]Pediatrics; [B]Pediatrics, Ophthal & Visual Sci, Eye Research Institute; [C]Pediatrics, Eye Research Institute; University of Wisconsin, Madison, WI, USA.

Background: Macular degeneration impairs the vision of countless adults and is commonly understood to be a consequence of aging. In reality, macular degeneration, specifically Best Vitelliform Macular Dystrophy (BVMD), is also a serious pediatric concern. BVMD is caused by dominantly-inherited mutations of bestrophin, a protein localized on the basolateral membrane of retinal pigment epithelial (RPE) cells. In functional RPE cells, bestrophin complexes form the Ca^{2+}-activated Cl^- channels responsible for the flux of Cl^- anions across the membrane. Since Cl^- regulates essential cellular events, mutations in bestrophin may be detrimental to RPE cell function. **Objective:** Using a simplified cell model, we sought to determine the influence of one BVMD-associated bestrophin mutation on cell function. We hypothesized that altered Cl^-transport, resulting from this bestrophin mutation, will cause cellular dysfunction similar to that seen in BVMD patients. **Methods:** Using site-directed mutagenesis, we introduced the N296H genetic mutation associated with BVMD into a vector containing PIRES, GFP, and human bestrophin 1 (hBest1). The vector was sequenced to verify the mutation was present. The hBest1 wild-type and hBest1 N296H mutant were introduced to cultured Chinese Hamster Ovary (CHO) cells via electroporation. The effectiveness of the electroporation was evaluated using live-cell fluorescence imaging. Ionic flux due to the two clones was measured as current-amplitude using the whole-cell mode of patch-clamp electrophysiology. **Results:** GFP-positive cells confirmed the ectopic expression of hBest1and hBest1 N296H in CHO cells. Under conditions of high intracellular Ca^{2+}, recorded Cl^-current due to hBest1 exhibited both inward and outward current. The corresponding current-amplitude plot was linear with resting membrane potential close to 0mV. Substitution of bath $Cl^-$with other anions matched previously published characteristic bestrophin Cl^- channel current. In comparison, mutant hBest1 N296H transfected cells had significantly altered Cl^-current magnitude and preference for other anions. **Conclusion:** Our results suggest that hBest1 is part of a complex Ca^{2+}-regulated Cl^- channel. We expect the current magnitude and anion preference of BVMD RPE cells to be similar to that of the hBest1 N296H mutant CHO cells, suggesting that this is an underlying etiology for BVMD.

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THE IMPACT OF A PEDIATRIC PALLIATIVE CARE CURRICULUM ON THE EMOTIONAL BURDEN OF CARING FOR DYING CHILDREN

K Catrine, M Moreno, University of Wisconsin School of Medicine and Public Health, Madison, WI.

Background: The long-standing need for improved palliative and end-of-life care in pediatrics has led academic training programs to develop educational curricula in this area. However, the effects of such curricula have not been well studied. Residents report high levels of emotional distress when caring for the pediatric palliative care population. The current study examined whether their feelings of guilt and anger could be assuaged by a palliative care curriculum. **Methods:** Pediatric interns were surveyed before and after a palliative care educational experience during the 2010-2011 academic year at a medium-sized academic institution. The educational programming consisted of four 3-hour interactive seminars based upon a progressive case example, a home visit, hospice experience, parent-written novel review and reflective journal. Seminar topics included; 1)overview of palliative care, 2)communication skills, 3)ethics, 4)pain management, and 5)self-care. Trainee attitudes towards palliative care and comfort in its provision were assessed before and after the educational intervention using the Professional End-of-Life Attitude Scale-Pediatric Version (PEAS-P). **Results:** A total of 14 residents completed the pre test, and 12 completed the post test. Among these residents, 8 had experience in palliative care training prior to residency. These experiences consisted of home hospice visits (2 residents), inpatient hospice visits (4) and palliative care service (1). Among participants who had no exposure to palliative care training prior to residency, residents mean score on the question "I feel guilty when a child patient of mine dies" was in the category of "strongly agree." However, after palliative care training this mean score dropped one category to "agree." Residents who had previous experience with palliative care training mean score began and remained in the "agree category." The mean score for the question "I feel angry when a child patient of mind dies" was in the category of "agree" regardless of prior training and encountering the residency palliative care curriculum. **Conclusions:** Results suggest that an intern palliative care curricula can help residents learn about palliative care and reduce the feelings of guilt that can accompany care of such patients. This may indicate that the educational programming contributed to a shift in perspective from viewing a patient death as a personal failure to a sometimes unavoidable situation. The consistency of angry feelings may be an attestation to the strength of a provider's existential struggle after the death of a child.

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INHIBITION OF NA^+/H^+ EXCHANGER ISOFORM 1 (NHE-1) ACTIVITY IN HIPPOCAMPAL REACTIVE ASTROCYTES REDUCES OGD-REOX MEDIATED GLUTAMATE RELEASE

Cengiz P[1], Kintner D[2,3], Kendigelen P[1], Akture E[1], Begum G[2], Ferrazzano P [1,2], Sun D [2,3] [1]Department of Pediatrics, [2]Waisman Center, [3]Department of Neurosurgery, University of Wisconsin, Madison, WI, USA.

Background: Selective hippocampal injury has been associated with hypoxia ischemia (HI) related brain injury in neonates. Mechanisms underlying ischemic hippocampal neuronal injury is unknown. Hippocampal astrocytes respond to HI by developing reactive astrogliosis. NHE-1 was dramatically increased in the hippocampal $GFAP^+$reactive astrocytes at 3 days after neonatal HI[1]. Inhibition of NHE-1 with its selective and potent inhibitor HOE 642 decreased CA1 pyramidal neurodegeneration and improved motor and spatial learning[1]. We hypothesized that up-regulation of NHE-1 in hippocampal reactive astrocytes contributes to hippocampal pyramidal neurodegeneration after HI via changing the intracellular pH (pH_i) and releasing of gliotransmitters. **Methods:** Hippocampal astrocyte cultures were prepared from P4 Black6/C57 mouse hippocampus (>99% astrocytes). OGD was induced by incubating cells with a bicarbonate-buffered OGD solution (pH_o 7.4) at 37°C in a hypoxic incubator for 2 hours. Live cell imaging is performed for measurement of pH_i. After 2 h of OGD and 1-5 h of REOX, hippocampal astrocytes are undergone immunohistochemical staining to identify GFAP and NHE-1 expressions and pH_i was measured. 1 μM HOE-642 was added to the media during OGD and REOX in order to establish the effect of NHE-1 inhibition. Glutamate was determined using an amplex red glutamic acid /glutamate oxidase assay kit. **Results:** OGD led to a significant increase in NHE-1 protein expression and H^+ efflux in response to acidification in hippocampal astrocytes. Stimulation of NHE-1 activity was sustained for 1-5 h REOX. Inhibition of NHE-1 activity with its potent inhibitor resulted in less intracellular alkalosis under normoxic and ischemic conditions. Astrocytes released significant glutamate by 5 and 24 h REOX that was reduced when NHE-1 was inhibited. **Conclusions:** HI causes overstimulation and up-regulation of NHE-1 in hippocampal reactive astrocytes after OGD resulting in intracellular alkalosis and disruption of ionic homeostasis contributing to the damage of pyramidal neurons in the hippocampus. Inhibition of NHE-1 reduces OGD/REOX mediated glutamate release from hippocampal astrocytes.

References:

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HASHIMOTO'S ENCEPHALOPATHY IN A 17 YEAR OLD FEMALE PRESENTING WITH NYSTAGMUS, ATAXIA AND SEIZURE

S Chandratre, M Clements, Children's Mercy Hospital, University of Missouri at Kansas City, Kansas City, Missouri, USA.

Background: Hashimoto's encephalopathy is a syndrome of acute or subacute encephalopathy associated with elevated anti-thyroid antibodies. Clinical Case: We report a case of a 17 year old female presenting with headache, nystagmus, ataxia, tremor, seizure and altered mental status with a Glasgow coma scale of 8, all consistent with encephalopathy. She also had frontal lobe dysfunction including impulsivity, disinhibition, poor insight, and weak abstraction skills. She had several month history of brittle hair and hair loss, as well as constipation. Thyroid peroxidase antibody titer was found to be elevated at 813 international units/ml. Thyroid globulin antibody was not elevated. Her Thyroid stimulating hormone was 4.37 mcIU/mL (0.35-5.50) and free T4 was 1.0 ng/dL (0.8-1.9) both within normal limits. Her cerebrospinal fluid (CSF) protein was elevated at 87 mg/dL (12-54) with normal glucose and cell count. Bacterial & viral CSF cultures, CSF- Polymerase chain reaction test for Enterovirus and Herpes simplex, analysis for paraneoplastic syndrome, N Methyl D Aspartic acid receptor antibodies and anti-neuronal antibody was negative. She had a normal electroencephalogram. Computed tomography head was reported normal. Her brain Magnetic Resonance Imaging showed small focus of increased T2 signal intensity in the left subcortical parietal white matter of doubtful clinical significance. She responded very well to empiric therapy with levothyroxine and glucocorticosteriod. Her symptoms improved within 48 hours and was symptom free by 5 days. She was on steroid taper for 10 weeks after which it was discontinued. She continues to be on levothyroxine supplementation and remains to be clinically euthyroid with regular follow up in the endocrine clinic. **Conclusion:** To the best of our knowledge, this is the first reported case of Hashimoto's encephalopathy with nystagmus and ataxia as presenting feature in pediatric population.

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BIRTHWEIGHT DISTRIBUTION OF SEVERE IVH/PVL AND DEATH IN PREMATURE INFANTS TREATED WITH INHALED NITRIC OXIDE

Sharie C Cooper[1], Jeremy D Marks[1], Rachel Meisel[1], Melissa K White[1], Dezheng Huo[2] and Michael D Schreiber[1] , [1]Dept of Pediatrics and [2]Dept of Health Studies University of Chicago, Chicago, IL U.S.A

Background: Inhaled nitric oxide (iNO) has been reported to decrease, increase or not affect the incidence of severe IVH/PVL in premature infants.We previously reported that inhaled nitric oxide decreased severe IVH/PVL in premature infants with moderate RDS (Schreiber, NEJM, 2003). Of note, because the study population was extremely high risk, the use of antenatal steroids (ANS) was only modest. Kinsella and colleagues also reported a decrease in severe IVH/PVL in somewhat smaller premature infants (NEJM, 2006). However, in premature infants with severe hypoxic respiratory failure, Van Meurs and her colleagues reported an increase in severe IVH in premature infants weighing <750g (NEJM, 2005).And most recently, Mercier and his colleagues reported no effect of iNO on the incidence of severe IVH or PVL (Lancet, 2010). Whether these differences in reported results are secondary to differences in birthweight, initial disease severity, study population or protocol differences, or a combination of these factors, is unknown. **Objective:** To evaluate the influence of birthweight, initial oxygenation index (OI) and ANS exposure on the iNO-associated decrease in severe IVH/PVL and death. **Methods:** A randomized, double-blind placebo-controlled study of iNO in 207 infants <34 weeks gestation who were undergoing mechanical ventilation for RDS. Infants were randomized to receive iNO (10 ppm on day 1, followed by 5 ppm for six additional days) or placebo for seven days. Data analysis of previous study was then performed using an unpaired t-test, chi-square, odds ratio, relative risk, associated confidence intervals, and standard deviation. **Results:** Compared to placebo group (n=102), the iNO group (n=106) demonstrated a significant decrease in severe IVH/PVL or death. Within the ≤ 750g group, a decrease was observed in both severe IVH/PVL (placebo: 35.0%; iNO: 15.6%) and death (placebo: 40.0%; iNO: 28.1%). The decrease in severe IVH/PVL and death was restricted to those infants with lower initial oxygenation indices (OI <6.94: RR 0.46 [0.23-0.92] vs OI ≥6.94: RR 0.84 [0.48-1.44]).The effect of iNO on severe IVH/PVL may be greatest in infants who do not receive antenatal steroids (placebo: 21%; iNO: 9%). **Conclusions:** Low birthweight is unlikely the dominant reason for the discrepancy of the reported effects of iNO on severe IVH/PVL and death. Premature infants not receiving ANS may receive the most benefit from iNO. Future studies of iNO therapy for premature infants should not restrict entry based on birthweight alone and must control for additional factors, such as pulmonary severity and ANS exposure.

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CAN COMMONLY AVAILABLE CORD BLOOD TESTS PREDICT HISTOLOGIC CHORIOAMNIONITIS AND OR FUNISITIS IN THE SETTING OF PRETERM BIRTH?

DT Costakos[1,2], JM Walden[1], LR Dahlen[1], JM Yuvienco[1]. Mayo Clinic Health System-La Crosse[1], La Crosse, WI USA. Mayo Clinic College of Medicine[2], Rochester, MN USA.

Histologic chorioamnionitis and funisitis are retrospective diagnosis of a clinically silent process. Cord blood can be collected quickly and easily after birth. Cord high-sensitive Cord Blood Reactive Protein (hs-CRP), Cord Blood immature to total granulocyte ratio (I:T) are likely abnormal (high) in the presence of histologic chorioamnionitis or funisitis. Informed consent, IRB approved prospective cohort study to calculate sensitivity and specificity of these tests to diagnose histologic chorioamnionitis or funisitis. There were 84 women delivered who delivered at Mayo Clinic Health System-La Crosse, Wisconsin, 91 babies (gestational 24 – 36 weeks), 89 cord and placenta, 10 sets of twins and 1 set of triplets. Eighty-eight percent of cord blood attempts were successful for hs-CRP, and 80% success for cord blood I:T. None of the babies had growth of bacteria (aerobic) blood cultures, and none of the babies died.

Blood Test	Prevalence	Sensitivity ± 95% C.I.	Specificity ± 95% C.I.
Cord hs-CRP	6%	0 (0 – 0.37)	0.97 (0.89 – 1.0)
Cord I:T	11%	0 (0 – 0.4)	0.9 (0.8 – 0.96)

In a subgroup of 49 of the 91 cord blood and infant pairs, there was not only cord blood I:T measured, but infant blood I:T. In this subgroup, range gestational age 27 to 36 weeks, the cord blood and infant I:T samples compared and there was a poor correlation. (Pearson correlation coefficient of 0.33). Three placentas were abnormal independent of changes associated with infection: 1 for villitis and 2 positive for iron staining consistent with subchorionic hemorrhage. Cord and placenta histology cannot be replaced by cord blood hs-CRP or cord I:T in the evaluation of histologic chorioamnionitis and/or funisitis. The sensitivity of either test is very poor. Inaddition,cord and placenta histology can reveal other clinically significant abnormalities such as villitis and subchorionic bleeding which are otherwise not detected unless histology is performed. Our study is important because some of our results contrast with a study of 313 women delivered in Korea (Yoon BH J of Mat Fet Med 2003;4: 85-90).

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HOW MUCH NICU RESOURCES DO MICROPREMIES REALLY CONSUME? COMPARED TO WHAT AND COMPARED TO WHOM?

Rajesh Dudani[1], William Meadow[1]. University of Chicago, Chicago, IL[1]

Background: NICU care is often criticized as being overly expensive and wasteful, suggesting that an inordinate amount of dollars and resources are directed toward sick infants, particularly those with very poor prognoses. **Objective:** We compared survival outcomes to NICU resources consumed by infants born at each gestational week from 23 to 42 weeks gestation for one year in our hospital. **Methods:** We determined gestational age (GA), birth weight (BW), length of NICU stay (LOS), and survival for every admission in 2009 to our NICU. We used NICU bed-days as a proxy for NICU resource consumption. **Results:** 724 infants were admitted to our NICU in 2009. 26 (4%) died, and 698 (96%) survived. Overall, survivors occupied 19,656 (94%) of 20,991 NICU bed-days. Even at the lowest gestations, bed-days were still disproportionately occupied by survivors – for 21 infants born at 23-24 weeks gestation, 2759/3090 NICU bed days (90%) were occupied by infants who survived to NICU discharge. 77 infants (10% of all admissions) ≤28 wks gestation accounted for 38% of all NICU bed days. Term infants accounted for 318 patients (44%) but consumed only 18% of NICU resources. **Conclusions:** 1) NICU bed-days are disproportionately occupied by infants with low BW/GA; nearly half of all NICU bed days are devoted to infants born <=30 wks gestation. 2) Nonetheless, the more than 90% of NICU bed-days are occupied by surviving infants, even at the lowest gestation. 3) NICU resources used at different gestational age from 23 weeks to term is fairly constant; babies at lower gestation are less in numbers but stay for longer time.

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OUTCOMES AFTER ADMISSION TO THE PEDIATRIC INTENSIVE CARE UNIT FOLLOWING PEDIATRIC HEMATOPOIETIC STEM CELL TRANSPLANT

A Dvorak, M Neumann, C Diamond, American Family Children's Hospital, University of Wisconsin, Madison, WI.

Purpose: Pediatric hematopoietic stem cell transplant (HSCT) has been used with increasing frequency for the treatment of an expanding number of diseases over the past 20 years. This has resulted in an overall improvement of outcomes for many children with otherwise difficult to cure disorders. Even so, the risk of death associated with pediatric HSCT and subsequent need for admission to the pediatric intensive care unit (PICU) has been recognized as substantial. Reviewing patient data collected in our PICU we attempted to define characteristics predictive of mortality. We were also interested in learning how and when palliative care was initiated. Finally, because we often do not know the exact etiology of death in this patient population, we sought to learn when or if autopsy was discussed with families, and if families agreed to autopsy. **Methods:** We performed a retrospective chart review of all pediatric patients who underwent HSCT between 1999 and 2009 and subsequently died in the PICU. From these records the information gathered included patient demographics, diagnosis, HSCT details, and reason for transfer to the PICU. In addition, data for the modified SOFA score calculations was obtained. Information regarding the end of life was collected, including whether palliative care was initiated, and documentation of discussions regarding withdrawal of support. Finally, discussion and occurrence of autopsy was recorded and whether new information was obtained from the autopsy. **Results:** In this 11 year period there were 94 pediatric HSCT completed including 88 children. Of these, 33 patients were treated in the PICU at some point post-transplant, for a total of 49 different visits to the PICU. There were 16 patients who died in the PICU, accounting for 48% of HSCT patients cared for in PICU. Of these 16 patients, 11 had palliative care interventions, but only three occurred more than 5 days prior to withdrawal of support. There were a total of 9 patients who had autopsy discussed, of whom only 5 consented. There were only 3 patients that autopsy was discussed prior to death, all of whom consented. **Conclusion:** Early after admission to the PICU it is difficult to identify a strong predictor of death, though a rapid decline in SOFA score or increased length of stay is consistent with increased mortality. Therefore, we believe early introduction of palliative care should be provided for all patients. As well, because we rarely know the exact etiology of death we believe autopsy should be discussed and discussed prior to withdrawal of support.

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HYPOCARBIA AND INTRAVENTRICULAR HEMORRHAGE IN MECHANICALLY-VENTILATED PRETERM INFANTS

IC Fields, E Culp MD, J Sinacore PhD, M Shareef MD Loyola University Chicago Stritch School of Medicine, Chicago, IL

Objective: To examine whether hypocarbia in mechanically-ventilated preterm infants during the first 24 hours of life is associated with increase incidence of severe intraventricular hemorrhage (IVH). **Background:** Brain injury continues to be a significant problem in the care of premature infants. Although the etiology of brain injury is multifactorial, disturbances of cerebral blood flow plays an important role. Increasing survival of preterm infants has focused attention on quality of life, particularly long-term developmental outcome. The presence of IVH and periventricular leukomalacia (PVL) can lead to profound neurodevelopmental problems, and the relationship between IVH and PVL with cerebral palsy has been well established. In the immediate postnatal period, hypocarbia is one variable that has been postulated as an influence on the occurrence of IVH. Mechanically-ventilated infants run a greater risk of developing hypocarbia as a result of inadvertant hyperventilation. If a correlation exists between hypocarbia and development of IVH, it will be important to avoid hyperventilation in mechanically-ventilated premature infants and decrease the risk for poor neurodevelopmental outcomes. **Methods:** We conducted a retrospective study of all infants born at less than 29 weeks gestation who were admitted to the Neonatal Intensive Care Unit at Loyola University Hospital from September 2006 through May 2010. A total of 226 charts were reviewed with gestational ages ranging from 23 to 28 weeks and birthweights from 250 g to 1430 g. Medical charts were reviewed and data was obtained from antenatal and perinatal records. Particular attention was paid to ventilatory details, including arterial and capillary carbon dioxide levels, ventilator settings, and radiologic data. Review of cranial ultrasounds was carried out routinely on days 7 and 28 of life, or more frequently as was clinically indicated. Statistical analysis was performed using a logistic regression model with severe IVH (defined as Grade III or Grade IV) as primary outcome, correcting for gestational age, birthweight, sex, use of antenatal steroids, and CRIB (neonatal illness) score. **Results and Conclusion:** Our initial analysis shows that hypocarbia has no apparent effect on the development of severe IVH. It does reveal a high incidence of hypocarbia in mechanically-ventilated preterm infants in the first 24 hours of life. This has important clinical significance, and attempts should be made to avoid hyperventilating infants early on in their clinical course.

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RELIGION AND SEX IN COLLEGE FRESHMEN: A LONGITUDINAL STUDY OF FACEBOOK

K Gannon, M Moreno, T Becker, University of Wisconsin, Madison, WI.

Purpose: The relationship between religion and sexual behavior has been well studied in adolescents. However, the role of religion in decreasing sexual activity is less clear in college students. Freshmen year is an ideal time to monitor this relationship. Facebook is a social networking site used by the majority of college students to create personal profiles; it provides a venue to evaluate beliefs and behaviors over time. The purpose of this study was to conduct a longitudinal evaluation of references to religion and sexual behavior on Facebook profiles during freshman year of college. **Methods:** Public Facebook profiles of freshmen undergraduates age 18 to 19 from a large state university were examined at four time periods during freshmen year. At Time 1, data was collected during high school until starting college. Time 2 occurred early in fall semester, Time 3 at the conclusion of fall semester and Time 4 at the end of freshmen year. Content analysis included self-displayed demographic information, religious affiliation, references to religiosity, and references to sexual behavior. A 20% subsample was evaluated for interrater reliability; Cohen's kappa for categorization of sex references was 0.71. Analyses included mixed-effects logistic regression, mixed-effects Poisson regression, and nonparametric Kruskal-Wallis test. **Results:** Of 330 screened Facebook profiles, 150 profiles met inclusion criteria. 97% were 18 years old; 55% were female. At Time 1 52% of profiles displayed a religious affiliation; by Time 4 13% of profiles displayed a religious affiliation. References to religiosity increased from 7% of profiles at Time 1 to 10% at Time 4. At Time 1 51% of students displayed references to sexual behavior; at Time 4 23% of students displayed sexual references. Women were more likely than men to decrease sexual references (OR=4.7). When compared across all time points, adolescents who displayed at least one reference to religiosity had 65% fewer sexual references than those who did not display references to religiosity (p=0.002). **Conclusion:** During freshmen year, students use Facebook to publicly display religion and sexual behavior. College freshmen removed both religious affiliation and references to sexual behavior from their profiles, demonstrating a decreased willingness to share this information with the public. Freshmen who displayed references to religiosity displayed fewer references to sexual behavior. These findings suggest that the relationship between religiosity and sexual behavior established by self-reported data in adolescents may persist into freshmen year of college.

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EXPRESSION OF SURFACTANT PROTEIN-A IN THE NEONATAL MURINE INTESTINAL TRACT

EM Theisen, CLS George, University of Minnesota – Twin Cities, Minneapolis, MN.

Surfactant protein-A (SP-A) plays a critical role in the innate immune system and has well characterized effects in the lung where it attenuates inflammatory responses and controls invasion of microorganisms. Extra-pulmonary sources of SP-A have also been identified: SP-A mRNA has been detected in adult and neonate gastrointestinal tracts, while significant levels of SP-A protein have been located in amniotic fluid. To date it is not clear if neonatal intestinal exposure to SP-A comes from ingested amniotic fluid or from internal production in the newborn intestinal tract. RNA *in situ* hybridization with a digoxigenin-labeled anti-sense SP-A probe was used to detect cells with SP-A mRNA in the intestinal tract of post-natal day (PND) 3 and PND 6 mice. Immunohistochemistry in PND 7 mice was used to determine intestinal SP-A protein expression. We report positive staining for SP-A protein in cells of the lamina propria of the intestinal tract. RNA *in situ* hybridization produced positively staining cells for SP-A mRNA in the muscularis externa and lamina propria of the intestinal tract, similar cells that stained positive for SP-A protein. These results indicate that SP-A is likely produced in the neonatal murine intestinal tract.

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USE OF SYNTENY CONVERSION IN IDENTIFICATION OF CANDIDATE GENES FOR CONGENITAL VERTEBRAL MALFORMATIONS IN HUMANS

Philip Giampietro, MD PhD[1], Denis Larkin, PhD[2], Robert Blank, MD PhD[1], Cathy Raggio, MD[3] and Harris Lewin, PhD[4]. [1]University of Wisconsin, Madison, Wisconsin, United States; [2]University of Aberystwyth, Wales, UK;[3]Hospital for Special Surgery, New York, New York; [4] University of California, Davis, California.

Background: Understanding the genetic contributions of vertebral malformations in humans has relied on the assumption that somitogenesis is conserved across species. Synteny block analysis, a methodology that compares the distribution of genes in breakpoint regions to synteny blocks, may be used to determine whether somitogenesis is conserved across species since evolutionary conserved genes tend to lie within synteny blocks and genes which are not conserved lie within evolutionary breakpoint regions (EBRs). **Purpose:** We hypothesized that vertebral patterning genes are conserved in amniotes and their location is within stable or "synteny" regions of chromosomes. **Methods:** 78 patterning genes involved in FGF, Wnt and Notch signaling pathways were analyzed in order to determine their location within synteny blocks or EBRs in the genomes of several amniotic species including (human, chimp, macaque, mouse, rat, dog, pig, opossum and chicken). The human genome was divided into 1 Mbp intervals and a comparison was made to determine whether these genes were preferentially localized within homologous synteny blocks or breakpoint regions which are associated with rapid evolution. **Results:** The results indicate that genes associated with vertebral development in humans are preferably located away from the evolutionary breakpoint intervals, 0.014 genes in breakpoint intervals on genome average vs. 0.030 on average in other parts of the genome (t test p-value = 0.01). Examine of large blocks of 7 homologous synteny >16.3 Mbp in human coordinates) demonstrated 0.04 genes from the 78 genes studied are positioned on synteny blocks on genome average while 0.03 genes on average are situated on the rest of the genome. **Conclusions:** The concentration of vertebral patterning genes in synteny blocks as opposed to evolutionary breakpoint intervals provides evidence that developmental pathways involved in vertebral morphogenesis are likely conserved across amniotes, which is consistent with their known function. These data support prior observations indicating that gene networks associated with major developmental processes such as neuronal, central nervous system, bone and blood vessel development, some of which were mediated by WNT and NOTCH signaling pathways were less likely to be localized at EBRs.

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A HOMOZYGOUS LETHAL MUTATION IN CONNEXIN43 LEADS TO ABNORMAL SKELETOGENESIS IN ZEBRAFISH

JI Lin, Y Matos, Jack Howe and *MI Goldsmith* Department of Pediatrics, Washington University School of Medicine, St Louis, MO 63110

Purpose: The underlying mechanisms regulating the size and shape of body structures remains largely unknown. In zebrafish, mutations in the gap junction gene, *connexin43* (*cx43*), lead to shortened fin ray segments and a short fin phenotype. In mice, Graziello et al demonstrated hypomineralization of the calvaria in heterozygous *cx43* $^{+/-}$ litter-mates. Finally, humans with oculodentodigital dysplasia (ODDD), a syndrome associated with craniofacial and limb malformations, have *cx43* mutations. In concert these data suggest that gap junctional communication plays a critical role in skeletogenesis. We have identified a new zebrafish mutant with short fins. We have now cloned this mutant and we have begun functional studies to characterize the mutant protein. **Objectives:** (1): To clone a newly identified zebrafish short fin mutant. (2) To characterize the axial and appendicular skeletal phenotype in this mutant. (3) To perform functional studies of the mutant protein. **Methods and Summary of Results:** A single exon containing the entire coding sequencing of *cx43* was amplified from both genomic DNA and cDNA. For skeletal histomorphometry, simple measurements using stereomicroscopy and μCT were employed. The constructs for connexin43 functional assays were generated from PCR fragments by using oligonucleotides with Hind III sites engineered at the 5' ends and EcoRI sites at the 3' ends. Amplified products were subcloned into pEGFP-N1 and pCS2+. Alleles expressed in pEGFP-N1 will be used for dye coupling assays in microinjection of HeLa cells. Sense RNA transcribed from the pCS2+ vector will be injected into Xenopus oocytes for ionic clumping assays. Gap junctional coupling between oocyte pairs will be measured using the dual whole-cell voltage clamp technique. We report here that our newly identified short fin mutant is a novel *cx43* allele, V96F. Interestingly, unlike previously described *sof* mutants, the V96F mutation results in homozygous lethality and thus is likely a null allele. Heterozygous alleles of V96F exhibit shorter fin ray segment length and shorter caudal fins, but paradoxically have hypermineralization of the axial skeleton. **Conclusions:** We report here the identification of a novel, homozygous lethal *cx43* mutant. Progress regarding histomorphometric analyses and functional studies of the mutated *cx43* protein will be presented.

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EXPLORING THE INFLUENCE OF MEDIA USE ON ADOLESCENT MENTAL HEALTH

LA Jelenchick, AM Grant, RN Koff, MA Moreno. University of Wisconsin, Madison, WI.

Purpose: The average adolescent reports spending almost eight hours a day consuming media. Some forms of media use may be associated with adverse mental health outcomes. The purpose of this study was to explore associations between electronic media use and both anxiety and depression within a sample of college students. **Methods:** Students enrolled in an introductory communications course at a large Midwestern university and between the ages of 18-21 were recruited to complete an online survey for class credit. Survey components included demographics, media use questions including: average hours per day spent listening to music, using the Internet, watching television, and gaming, as well as the Generalized Anxiety Disorder 7-item Scale (GAD) to assess symptoms of anxiety, and the Patient Health Questionnaire-9 (PHQ) to assess symptoms of depression. Scores can range between 0-21 on the GAD, and between 0-27 on the PHQ; scores over 4 on either measure correspond with increasing symptoms of anxiety/depression. While controlling for age and gender, a total of eight regression models were used to assess the relationships between media use variables and both GAD and PHQ overall score. All models were repeated with the inclusion of quadratic terms for all media use variables to test for potential curvilinear relationships. **Results:** A total of 263 students participated (71% response rate). The average age reported was 18.8±.8 years, 64% were female, and 91% were Caucasian. Music was the most intensely used media format, 88% of participants reporting listening for more than two hours a day, followed by Internet use (79%), television (64%), and gaming (23%). Mean scores on the GAD and PHQ were 5.7±4.3 and 5.3±4.1, respectively. No significant relationships were observed between any of the media use variables and overall GAD scores. There was a positive relationship between daily internet use and overall PHQ scores (β=.15, 95%CI .16-1.46, p=.01). A u-shaped curvilinear relationship was noted between amount of daily television watched and overall PHQ score (β=.78, 95%CI .28-1.02, p=.001). No other significant relationships were noted between either gaming or listening to music and overall PHQ score. **Conclusions:** Internet and television use may be related to depression symptoms. Although these relationships reached statistical significant, clinically significant changes in depression symptoms were not observed. Future work should investigate the relationship between adolescent electronic media use and mental health longitudinally to determine if effects will magnify over time.

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ASSESSING PROBLEMATIC INTERNET USE IN US COLLEGE STUDENTS.

LA Jelenchick, MA Moreno. University of Wisconsin, Madison, WI.

Purpose: Problematic Internet use is a growing concern among adolescents. However, both a clear understanding of the mechanisms driving problematic behaviors and a gold standard instrument for assessing symptoms are lacking. Symptoms are more commonly reported among older adolescents and those with concurrent symptoms of depression; prevalence may also vary by gender. The purpose of this study was to assess the construct validity of a widely used screening instrument for problematic Internet use, and relationships with both depression and gender using a sample of US college students. **Methods:** Students aged 18-20 were recruited from two universities to complete an online health risk behavior survey, which included demographic questions, the Young Internet Addiction Test (IAT) to measure problematic Internet use, and the Patient Health Questionnaire-9 (PHQ) to assess symptoms of depression. Participants scoring 40 or above on the IAT were classified as 'problematic users'. Those with PHQ scores over 10 were designated as 'depressed'. Exploratory factor analysis was used to assess the underlying structure of symptoms associated with problematic Internet use. Subscale scores were created by taking the mean of all items loading on each factor. Subscale scores were compared across depression category and gender, and correlated with overall PHQ score. **Results:** 215 participants completed the survey (RR=70%); average age was 18.8±.7 years, 53% were female, and 70% were Caucasian. Mean IAT score was 28.7±10.5; 12% of participants were classified as problematic users. Using exploratory factor analysis, we extracted two factors from the IAT, *dependent use* and *excessive use*, which together explained 91% of the total variance. Depressed participants scored higher on both the *dependent use* (1.4±.6 vs. 1.0±.5, p=.000) and *excessive use* (2.8±.8 vs. 2.0±.7, p=.000) subscales. Females score higher on the *excessive use* subscale (2.2±.8 vs. 1.9±.6, p=.000), no gender differences were found for the *dependent use* subscale. Significant correlations were also noted between total PHQ scores and both the *dependent use* (r=.28, p<.01) and *excessive use* (r=.41, p<.01) subscales. **Conclusions:** The IAT appears to be a valid screening tool for use in college students. Further, two underlying factors my account for problematic behaviors, *excessive use* and *dependent use*. Female adolescents and those with symptoms of depression may have more difficulty controlling their online behavior, leading to problematic use. Findings highlight the need to further explore the content validity of the IAT, and mechanisms underlying the relationship between depression and problematic Internet use.

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RESIDENT ADVISOR INVOLVEMENT AND FACEBOOK USE

LE Kacvinsky, MA Moreno, University of Wisconsin, Madison, WI

Introduction: College students who reside in residence halls are typically assigned a resident advisor (RA) who is a peer leader in charge of maintaining a positive environment and dealing with any problems that may arise on their floor. This study aimed to determine what types of RA involvement are most effective and how RAs can maximize their ability to assist and refer residents with alcohol or mental health problems. **Methods:** Freshmen students were asked to rate how involved their RA is in their life (1 being not involved and 10 being extremely involved) during an interview. Next, participants were asked to list the ways their RA is involved and whether they were Facebook friends with their RA. Four focus groups were conducted with RAs who were asked to discuss their views on using Facebook to identify residents that may be at risk. **Results:** A total of 72 freshmen (56% male) completed an interview. The average for how involved the RA was in the resident's life was 4.03. Twenty-nine of the participants (40%) reported that they were Facebook friends with their RA. The category of RA involvement with the highest involvement score given by residents was "the RA asks questions about how the resident is doing." RAs participated in focus groups, with group size varying from five to seven participants and consisting of both males and females. RAs reported that they have seen references to depression and problem drinking on the Facebook pages of their residents. They agreed that, although they may initially learn of an issue with a resident through Facebook, the follow up with the resident should be face-to-face. **Conclusion:** Certain types of RA involvement seem to be more effective in making residents feel their RA is involved. The RAs that spend time talking and getting to know their residents were rated higher than those who only planned meetings or sent emails. Facebook could possibly be used by RAs with their residents to help the residents feel that they are more connected with the RA and help the RAs identify residents that may need help, although maintaining an offline relationship with the residents is still extremely important.

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SOCIAL NETWORKING AND THE FEMALE COLLEGE STUDENT: PRIVACY SETTING ATTITUDES

ME Lederer, SA Rastogi, MA Moreno. University of Wisconsin Hospitals and Clinics, Madison, WI, United States.

Background: The use of Facebook, the social networking site, is extremely prevalent among college students. Concerns about privacy and safety surrounding this method of communication have increasingly been raised in the media, among law enforcement, healthcare personnel, educators and parents. These concerns may be particularly salient for women given their increased risk of unwanted attention resulting from online disclosures. **Objective:** We conducted focus groups with college women to discuss privacy and safety concerns on Facebook and to identify thoughts, behaviors and actions taken in response to these issues. **Design/Methods:** A convenience sample of university women between 18 and 22 years of age were recruited from several campus organizations between September and October of 2009. Two trained facilitators asked subjects for their views about privacy and safety on Facebook. All tape-recorded data was fully transcribed and then analyzed to identify common themes. **Results:** 39 female undergraduate students were divided into five focus groups. Each participant had an active Facebook account and nearly all reported accessing it multiple times a day. Several themes emerged from our data: 1. Students consistently identified certain types of Facebook information, such as addresses and phone numbers, as being too personal to share public; 2. Most students were interested in discussing, learning about, and enhancing their privacy settings. Some students stated that they planned to increase their privacy settings as a result of the focus group discussion; 3. Students who reported having privacy settings enforced by their student organizations described significant efforts to subvert these enforcements. **Conclusions:** In our study population, Facebook is a very common method of communication and consumes a substantial amount of female student's time. These students were largely aware of privacy settings and had some concern about the safety of the information available about them on Facebook , leading them to increase their privacy settings over time. While students were interested in learning about privacy settings from others, enforced settings were seen as punitive and a substantial amount of time was spent subverting these enforcements. This suggests that efforts towards education rather than enforcement may benefit this population.

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AAP CPTI LEGISLATIVE ADVOCACY MODULES: IMPLEMENTATION AND ANALYSIS

Cathy A Lee-Miller, MD[1] and Dipesh Navsaria, MD[1]. [1]Department of Pediatrics, University of Wisconsin, Madison, WI, United States.

Background: An essential part of residency education is advocacy training. The AAP created a series of Legislative Advocacy training modules, which are housed within the Community Pediatrics Training Initiative. These modules are designed to teach residents about advocacy in an interactive and efficient way. While these modules have existed since 2008, they have never been studied nor has their efficacy been evaluated. **Objective:** To study the efficacy of the AAP's Advocacy Teaching Modules in changing the knowledge, attitude and values of pediatric residents with respect to legislative advocacy. **Design/Methods:** A pre-module survey of pediatric residents' attitudes and opinions regarding legislative advocacy and experience was administered electronically. Four of the five modules were conducted. After each module was completed, residents were encouraged to give qualitative feedback anonymously. Following completion of all of the modules, the survey was again completed. **Results:** The pre-module survey response rate was 71% (27 of 38 eligible pediatric residents). Approximately 60% of residents who completed the survey were able to attend at least one module. The immediate post-module response rate was 66% (25 of 38). Pediatric residents showed change within the domains of understanding legislative advocacy and the pediatrician's role therein. Prior to the modules, residents rated their understanding of what is involved in legislative advocacy an average of 3.41 on a scale of 1 to 10; afterwards, this self-rating increased to 5.04. Similarly, they rated their understanding of the role of the pediatrician in legislative advocacy 3.7 prior to the modules and 5.58 afterwards. More residents were interested in being involved in advocacy both during and after the completion of residency. Following the modules, residents felt more comfortable contacting a governmental official to advocate on behalf of a patient as evidenced by a self-reported comfort level increasing from 4.33 to 5.46 on a scale of 1 to 9. **Conclusions:** The Legislative Advocacy Training modules were successful in bringing advocacy to the forefront of residents' minds and concerns. Having protected learning time to think about legislative advocacy and having modules designed specifically to teach this topic are instrumental in maintaining this interest amongst residents.

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ABNORMAL SWEAT CHLORIDE TESTS AND PFT'S IN A 17 YEAR OLD MALE WITH CROHN'S DISEASE

MG Arnold, JL Kantor. University of Missouri, Columbia, MO.

Summary: A 17 year old male presents with a 5-6 year history of failure to thrive, abdominal pain, intermittent diarrhea, and productive cough who was shown to have two abnormal sweat chloride tests in addition to abnormal pulmonary function tests. Two previous endoscopy's showed mild gastritis and duodenitis. The patient was initially diagnosed with Cystic Fibrosis but failed to improve or gain weight after starting pancreatic enzymes. Genetic testing for CF later came back negative. Repeat endoscopy again showed mild gastritis/duodenitis with a normal appearance of both the colon and terminal ileum. After several failed Remicade infusions in addition to increasing abdominal pain and diarrhea, bowel resection was performed with a pathological confirmed diagnosis of Crohn's Disease involving the small bowel only. We suggest that the patient's abnormal sweat chloride tests could be attributed to severe malnutrition. Furthermore, we suggest this patient's chronic cough and abnormal PFT's were extraintestinal manifestations of Crohn's Disease.

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ASSESSING THE EDUCATIONAL IMPACT OF A PEDIATRIC SLEEP MEDICINE ROTATION FOR FIRST YEAR PEDIATRIC RESIDENTS

C Matthews, University of Wisconsin School of Medicine and Public Health, Madison, WI

Purpose of study: Limited exposure is provided during pediatric residency training in sleep medicine. Because sleep is an important aspect of development and multiple sleep disorders arise in childhood and adolescence, there is a need to develop curricula that provide fundamental training in this field for pediatricians-in-training. The purpose of this study was to assess the impact of a pediatric sleep medicine rotation for first year pediatric residents. **Methods:** A pediatric sleep medicine curriculum was developed that included pre- and post self assessment questionnaires, a pre and post knowledge based exam, handouts on normal sleep in addition to pediatric sleep disorders in children and adolescents, pediatric sleep review articles, and an overall satisfaction survey. 23 residents over a 2 year period completed the curriculum. One preceptor was in charge of the curriculum. Time in clinic was devoted to reading nocturnal polysomnograms and evaluation of patients in a pediatric sleep medicine clinic. Specific sleep disorders that were addressed in the curriculum included obstructive sleep apnea, insomnia, restless legs syndrome, periodic limb movement disorder, circadian rhythm sleep disturbances, behavioral sleep disturbances and narcolepsy. **Results:** Time in clinic ranged from 2-6 half days over a 3 week period with average time in clinic at 4.1 half days. All of the scores increased to a significant degree. Mean scores on self-assessment skills in sleep medicine improved pre- to post-participation in all spheres measured. The exam consisted of approximately 30 items and scores were assessed on a percent correct of total score. A significant increase (58.8% +/- 16.9% to 64.7% +/- 17.2%, p=0.013) was observed for the sleep rotation exam from the initial to the final assessment. Participants preferred paper over web-based materials, and review articles and pediatric sleep handouts were rated as most helpful. **Conclusions:** This pediatric sleep medicine curriculum effectively improved self- and test-assessed knowledge of sleep and its disorders among first year residents. By providing this training early on in pediatric training, improved awareness and treatment of pediatric sleep disorders can occur.

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EFFECT OF MULTIPLE RISK FACTORS ON FETAL IRON STATUS AT BIRTH

[a]*HM McLimore*, [a]AK Phillips, [a]SE Blohowiak, [b]D Q-D Pham, [a]CL Coe, [a]PJ Kling. [a]University of Wisconsin-Madison, Madison, WI; [b]University of Wisconsin-Parkside, Kenosha, WI.

Introduction: Fifty percent of the iron needed for proper infant growth is obtained before birth. Poor fetal iron acquisition places infants at-risk for inadequate tissue iron levels required for normal organ and neurologic development. A recent AAP statement acknowledges that poor iron status at birth may increase the risk for abnormal iron screening in late infancy. Our goal was to determine if multiple risk factors for impaired fetal iron status placed infants at additive risk for poorer indices of iron status at birth. **Methods:** Newborns ≥35 weeks gestational age with one or more risk factors for iron deficiency (ID) were recruited. Risk factors included: maternal ID, maternal diabetes, newborns small or large for gestational age (SGA or LGA), mothers from ethnic minority groups, and/or mothers with lower socioeconomic status. In review of our dataset, we found that maternal obesity at delivery (BMI ≥30 kg/m^2) should also be included. Cord blood indices of storage iron (serum ferritin), transport iron (serum transferrin), steady state RBC iron (ZnPP/H), and recent RBC iron (reticulocyte-enriched or RE ZnPP/H) were measured and compared against cord blood of healthy controls. ZnPP/H is a sensitive index of early pre-anemic ID and increases with inadequate availability of iron for incorporation into the protoporphyrin ring of hemoglobin. RE ZnPP/H increases sensitivity of this index as it measures inadequate iron availability in the most recently made RBCs, the reticulocytes. The number of risk factors for each baby was determined and risk groups were demarcated as high-risk (≥3 risk factors) and low-risk (1-2 risk factors). **Results:** We identified 111 high-risk and 181 low-risk newborns. Mean values of all parameters in the high-risk and low-risk groups differed from controls, $p<0.05$. High-risk newborns exhibited higher ZnPP/H and higher RE ZnPP/H than the low-risk group, $p<0.04$, but had similar serum ferritin and serum transferrin levels. Three specific risk factors: LGA newborns born to obese, diabetic mothers were compared to newborns with 3 or more alternate risk factors. The LGA/obese/diabetes group had higher ZnPP/H, RE ZnPP/H, lower serum ferritin, $p<0.03$ and similar serum transferrin. **Conclusions:** Our results support the AAP recommendations to screen at birth for historical factors that place infants at-risk to develop infantile ID; additionally we found that multiple risk factors may confer greater risk. The combination of gestational obesity and diabetes compounded with a LGA newborn conferred greater risk than other combinations of ≥3 risk factors. Identification of these newborns is very important as these newborns are at greatest risk for ID.

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URBAN, SUBURBAN, AND RURAL: ADOLESCENTS' USE AND PREFERENCES FOR FITNESS PROMOTION TECHNOLOGIES ACROSS COMMUNITIES

EJ Mikulec, NF Goniu, LN Brockman, MA Moreno, University of Wisconsin, Madison, WI, USA.

Introduction: Adolescent obesity remains a significant public health concern, with the majority of adolescents expressing concern about their weight. Previous studies have demonstrated daily use of a variety of technologies by adolescents. However, little is known about how their use of technology varies across ages and different communities. This knowledge would allow for future age and community specific implementation of different technologies and resources to promote and support fitness. **Methods:** Focus groups consisting of adolescents were recruited from rural, suburban and urban cities in Wisconsin, Washington and Ohio between the months of March and May of 2011. Focus groups were led by a trained facilitator. Facilitators used both open-ended and prompting questions to explore how participants used technologies and how they applied them for fitness purposes. All focus groups were audio recorded and manually transcribed. Analysis was conducted by three investigators using an iterative process in which the investigators initially reviewed transcripts individually and then jointly discussed findings to determine the representative themes and quotations. **Results:** A total of 5 focus groups with 3 to 8 participants per group were conducted with adolescents between the ages of 12-18 (20 females and 8 males.) All participants reported using technology daily. Three themes were derived from our data. First, we found differences between younger and older adolescents' use of technology. Younger participants described technology as being a complement to fitness. In contrast, older participants designated technology as a motivator for fitness. Second, differences exist in fitness approaches between rural and urban adolescents. Adolescents in rural communities go outdoors for fitness, while urban adolescents relied on video games. Lastly, both urban and rural communities relate having a lack of fitness-focused resources. **Conclusions:** Our findings indicate that significant differences exist between adolescents' application of technology for fitness, with younger adolescents utilizing it as an accessory to their fitness and older adolescents requiring it in order to achieve their fitness goals. Additionally, despite adolescents' differing uses of resources across communities, a common need exists to expand upon their resources. New approaches for fitness promotion should address these differences as they will allow for targeted and effective technology interventions that satisfy the specific needs of different adolescents and communities.

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ALCOHOL DISPLAYS ON FACEBOOK: INTERVENTION CONSIDERATIONS

Moreno MA, Egan KG, Fleming MF. University of Wisconsin, Madison, WI; Northwestern University, Chicago, IL.

Purpose: College students frequently display references to alcohol on social networking sites (SNSs). Our previous work investigated displayed references to intoxication and problem drinking on Facebook. We found that these Facebook references were associated with self-reported risk for problem drinking measured via a validated clinical scale. Before next steps towards screening or intervention based on SNS content can take place, views of this population regarding displayed content and communication preferences must be understood. **Methods:** Freshmen college students from a large state university were interviewed. During the interview, we informed participants that we frequently noted references to alcohol on SNS profiles and that some of these references described problem alcohol behaviors. Participants were asked for their views regarding displayed alcohol content on college student profiles and communication preferences regarding feedback on content of their own profile. All interviews were audio recorded and fully transcribed. Analysis was conducted using an iterative approach. **Results:** A total of 132 participants completed the interview (70% response rate), the average age was 18.4 years (SD 0.49) and our sample included 64 males (48.5%). Three themes emerged from our data. First, the vast majority of participants stated that they viewed displayed alcohol content as indicative of alcohol use. Second, participants explained that they would prefer to be approached by someone they knew regarding content of their profile, and approached in a direct face-to-face manner. Most participants did not want to be approached by a stranger, but those who did preferred an indirect manner such as using Facebook or email. Finally, the style of approach was considered critical; participants wanted the approach to include asking questions about profile content rather than implying judgment without discussion. **Conclusion:** Findings support that alcohol references on Facebook are taken at face value by peers. When approaching college students regarding concerning alcohol messages on SNSs, both the relationship and the approach are key factors in whether the message is heard. In considering future directions for SNS interventions regarding college student alcohol use, messages sent by known or trusted individuals are likely to be better received since peers accept alcohol displays at face value, peer leaders may be possible intervention partners. In designing future programs, motivational interviewing approaches could be considered.

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BONE DENSITY CORRELATES WITH INSULIN LEVELS IN MIDDLE SCHOOL CHILDREN

Oluyomi Obafemi, Jens Eickhoff, Aaron Carrel University of Wisconsin School of Medicine and Public Health, Madison, WI.

Introduction: Recent studies in mice have demonstrated that insulin plays a role in osteoblast stimulation, and may also decrease bone resorption. While much research has focused upon the role of insulin and insulin resistance (IR) on body composition and obesity, especially lean mass and adipose tissue, little is known of the connection of insulin with bone density in children. **Methods:** Dual-energy x-ray absorptiometry (DXA) was performed in 98 middle school children to assess lean mass (muscle), adipose tissue (% body fat) and bone content (BMC). The children also had fasting blood work for insulin and cardiovascular fitness testing (maximal VO_2) calculation of body mass index (BMI) on the same day. **Results:** After adjusting for age, height, gender, lean mass and body fat, total BMC (kg/m) was a significant predictor of fasting insulin levels (beta=-0.47, p=0.0351) in the multivariate regression analysis. However, stepwise regression analysis showed that total BMC was not an independent predictor from other known predictors (body composition and cardiovascular fitness) for fasting insulin. When stratified by gender, and after adjusting for age, height, lean mass and body fat, BMC had a statistically significant negative correlation with fasting insulin levels in males (partial correlation=-0.36,p=0.0354) while the partial correlation for females was not significant. **Conclusions:** Previous studies have attempted to elucidate the connection between insulin levels and bone metabolism. Our study shows that insulin may play a role but this differs by gender. However, hormones during puberty also play a major role in bone maturity. Since males and females differ in the onset of puberty, the negative correlation in our study could be due to males lagging behind females in puberty. To correct this, future directions could use the subjects' bone age rather than chronological age.

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STRUCTURAL BASE ANALYSIS FOR ESTROGEN RECEPTORS ALPHA VS. BETA INTERACTION WITH CAV-1 OF UTERINE ENDOTHELIAL CELLS FROM LATE PREGNANT EWE

Pastore, M.B.[1], Hofeld, B.C.[1] Chen, D.B.[4], and Magness, R.R.[1, 2, 3] [1]Ob\Gyn Dept., Perinatal Research Labs, [2]Animal Sciences, [3]Pediatrics, UW-Madison, Madison WI, [4]Ob\Gyn Dept., UCI, Irvine, CA

Background: Estrogen receptors (ERs) localize to the plasma membrane of endothelial cells and are responsible for rapid vasodilatory responses. ER-α/β have been previously demonstrated to activate ERK signaling and nitric oxide (NO). NO production is partly responsible for the maintenance of vasodilatation during physiologic states of high circulating estrogen levels such as pregnancy. It has been reported that ER-α interacts directly with the main scaffolding protein in the caveolae, Cav-1, however, it is unknown if there exits a similar protein-protein interaction between Cav-1 and ER-β. **Hypothesis:** We hypothesize that ER-α and ER-β maintain similar protein-protein interaction with Cav-1 in an "unstimulated" state in uterine artery endothelial cells and that structural analysis of these ERs will provide evidence for ERs' association at the plasma membrane. **Method:** Bioinformatic database approaches using SDSC Biology WorkBench web-base tool and UCSF Chimera molecular 3D modeling system were employed to obtain structural information on transmembrane domain and crystal structure-based analyses. The structural differences and similarities between receptor subtypes were overlaid to draw conclusions regarding 3D orientation within the plasma membrane. Proof of principle was established using *in vitro* immunoisolation column assay using Cav-1 antibody and Western blotting analysis. **Results:** Database bioinformatic analyses on ER-α, ER-β and Cav-1 secondary structure showed that these proteins do not possess any transmembrane domains. UCSF Chimera software revealed that ER-α and ER-β have identical configurations suggesting that 3D structure alone cannot account for ER and Cav-1 interactions. The "pull-down" assay demonstrated that ER-α has tightly association with to Cav-1, which was not seen with ER-β. Finally, protein database sequence analysis revealed a candidate sequence that may be specific to caveolin binding motif in both ER-α and ER-β. **CONCLUSION:** These data collectively support the thesis that unlike ER-α, ER-β maybe at least loosely associated with the caveolar lipid raft, but not via a transmembrane loop. The direct/indirect association between ER-β and Cav-1 needs further study. NIH GM083252, HL49210, HD38843, HL87144, HL70562.

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AN EVALUATION OF SOURCES OF STRESS IN THE CURRENT EDUCATION OF PEDIATRIC RESIDENTS: A QUALITATIVE STUDY

M Peters, M Cercone, M Moreno, American Family Children's Hospital, University of Wisconsin, Madison, WI.

Introduction: In the past 10 years, rules regarding medical resident work restrictions have changed substantially. This shift in training necessitated a change in mentality, but most studies on this topic reported modest to no improvement in number of hours worked per week, number of hours of sleep per night, and the amount of exhaustion while on call. However, as new duty hour restrictions go into effect, how have existing rules affected sources of stress in resident life? The purpose of this study was to explore residents' thoughts on the stress associated with their training. **Methods:** Focus groups of pediatric residents were assembled by convenience sampling, and questions included sources of stress in residents' educational experiences. Discussions were audio recorded and transcribed. Transcriptions were read by 3 readers who discussed common themes that arose in each group. **Results:** Four focus groups of 15 pediatric residents including all years of residency training were conducted. Residents cited new responsibilities and uncertainty with their new roles as physicians as major stressors in their training. They expressed feelings of pressure to master their craft in a brief period of time. Residents stated they struggle with the paradoxical nature of taking care of sick children while not always tending to their own health. Balancing obligations between family, friends, and the workplace provided another described source of stress. **Discussion:** These themes of resident stress discussed here are recurrent in the literature despite changes in work hours. Despite measures taken by the Accreditation Council for Graduate Medical Education (ACGME) in part to improve physician well-being, residents still identify a high number of stressors as inherent in their training. Though hours spent on the job have decreased, medical training is by its nature mentally and physically demanding. Future policies and investigations should incorporate strategies to improving resident life and well-being rather than focusing solely on number of hours worked. Qualitative changes in residency training may have a broader impact on resident well-being than quantitative changes.

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USING NEWBORN SCREENING CARDS TO DETECT IRON DEFICIENCY IN NEWBORNS?

CE Leyns[1], AA Ovasapyan[1], SE Blohowiak[2], PJ Kling[2] and DQD Pham[1] (1) Dept Biological Sciences, University of Wisconsin-Parkside, Kenosha, WI, (2) Dept Pediatrics, University of Wisconsin, Madison, WI.

Background: Iron deficiency (ID) acquired in infancy can lead to a multitude of metabolic and neurological vulnerabilities later in life. Fifty percent of the iron needed for growth in infancy is acquired before birth. Earlier screening is advantageous, but an optimal diagnostic screening tool for ID must first be available; the iron storage protein, ferritin, emerges as a good candidate. Although cord plasma ferritin levels reflect iron stores, whole blood levels in newborns have not been studied. Our goal was to test the feasibility of measuring whole blood ferritin and using dried blood spots on Newborn Screening Program cards to examine newborn iron status *via* whole blood spot ferritin concentrations. **Methods:** Enzyme-Linked ImmunoSorbent Assays (ELISA) were performed on whole cord blood, cord erythrocytes, cord plasma and dried, spotted whole cord blood samples to determine if ferritin levels of these samples were strongly correlated. In addition, for an internal control, the ferritin levels of whole cord blood samples were also compared to those of the sum of cord plasma and erythrocytes samples. **Results:** Cord whole blood, erythrocyte, and plasma ferritin concentrations were positively correlated with dried blood spots from the screen cards ($r = 0.43$, $p = 0.01$, $n = 37$; $r = 0.54$, $p = 0.00$, $n = 35$; and $r = 0.53$, $p = 0.00$, $n = 33$, respectively). The sum of the cord plasma & erythrocyte ferritin shows a positive correlation to whole blood ferritin ($b = 0.70$, $p = 0.00$ and $n = 40$). **Conclusion:** Fetal iron allotment impacts iron status in later infancy, and newborn screening of iron status is possible should a diagnostic tool be developed. Although variability in the relationships was seen, measuring whole blood ferritin, on newborn screening card blood spots is feasible and should be further examined.

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FERRITIN, ERYTHROPOIETIN, AND TRANSFERRIN RECEPTOR RELATIONSHIPS IN INFANTS

AA Ovasapyan[1], SE Blohowiak[2] , PJ Kling[2] and, DQD Pham[1]. (1) Dept Biological Sciences, University of Wisconsin-Parkside, Kenosha, WI (2) Dept Pediatrics, University of Wisconsin, Madison, WI.

Purpose: Iron deficiency in infants can lead to neurologic problems later in life. The first step toward analyzing the effects of and diagnosing infantile iron deficiency is to develop an accurate and easily accessible diagnostic tool to screen for iron deficiency at birth and follow these cases through the first year of life. For this study, three iron-dependent factors, Ferritin (Fer), erythropoietin (Epo), and transferrin receptor (TfR), were examined. **Methods:** In a study of children at-risk for iron deficiency in infancy, intra- and inter-relationships between the three iron-dependent factors were investigated at birth and at 6 months. All studies were performing using commercially available Enyzme-Linked ImmunoSorbent Assays (ELISA). **Results:** *Intra-relationships:* At birth, cord plasma Fer levels show no correlation to either cord plasma Epo or TfR levels, whereas cord plasma Epo levels show a positive correlation with TfR levels ($r = 0.48$, $p = 0.00$ and $n = 135$). At six months, plasma Fer levels show a negative correlation with both Epo ($r = -0.298$, $p = 0.04$ and $n = 47$) and TfR ($r = -0.47$, $p = 0.00$ and $n = 74$) levels, while plasma Epo levels show a positive correlation with TfR levels ($r = 0.353$, $p = 0.02$ and $n = 45$), just as that seen at birth. Relationships between the factors at 6 months reflect those observed in adults. *Inter-relationships:* Cord plasma Fer levels at birth shows a significant positive correlation to plasma Fer levels at 6 months ($r = 0.49$, $p = 0.00$ and $n = 59$). No clearly significant relationship is shown between cord plasma Epo or TfR levels at birth and plasma Epo or TfR levels at 6 months, respectively. **Conclusion:** In newborns at-risk for infantile iron deficiency, the iron index TfR is indicative of erythropoietic stimulus and not iron storage, similar to that observed in a study for healthy newborns. At 6 months, TfR levels reflect storage iron and erythropoietic stimulus, as in older individuals. Intra-relationships between the three iron-dependent factors in at-risk children sometimes mirror those seen in adults, however this is not always the case. Because the plasma Fer levels tracked in early infancy and 50% of the iron needed for infant growth is obtained before birth, cord plasma Fer levels may be an important tool in the assessment of both fetal iron allotment and iron status in late infancy.

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PERCEPTION, INFLUENCE, AND SOCIAL ANXIETY AMONG ADOLESCENT FACEBOOK USERS

MA Pumper, MA Moreno, University of Wisconsin-Madison, Madison, WI, USA

Purpose: Adolescence is a life stage where one is easily influenced. Facebook is speculated to be a major source of influence as well as a popular social tool for adolescents all over the globe. There is uncertainty on how the use of this site is impacting adolescent's social interactions offline. This study examines the psychology of Facebook users through views of Facebook's perceived effects on social interactions offline, amount of time spent on Facebook, and social anxiety. **Methods:** College freshman (ages 18-19) were examined through an in person interview asking them their overall views on Facebook and its effects on their offline social interactions. They were also asked about the amount of Facebook they used when compared to their overall internet use. They then completed the Liebowitz Social Anxiety Scale Test (SAS). Descriptive statistics were calculated and qualitative analysis was used to evaluate responses. **Results:** Of 72 participants (ages 18-19) included in the study, 55.6% were male. Overall, 73.6% viewed Facebook positively, 12.5% viewed Facebook as neutral and 13.9% viewed Facebook negatively. On average, the participants logged onto Facebook 71.3% of the time they logged onto the internet and scored a 34 on the SAS. 22.6% of positive and neutral viewers met criteria for moderate or higher social anxiety disorder, and 65.3% reported high use of Facebook. Among the 13.9% who viewed Facebook negatively, none met criteria for social anxiety. **Conclusion:** Facebook, the popular social tool for adolescents, is seen to have a positive effect on offline social interactions in general. With the small number of participants that were in the moderate social anxious category, there was a relation with high use. Therefore, our results suggest that people who perceive Facebook as having positive social effects use Facebook more often, which may increase social anxiety and lead them to use Facebook even more often. The study in turn sheds light on the need to educate adolescents about these types of sites and ways to balance offline and online social interactions.

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SOCIAL NETWORK SITES HERE AND ABROAD: EXAMINATION OF FACEBOOK AND SOCIAL CONNECTEDNESS

MA Pumper, MA Moreno, University of Wisconsin-Madison, Madison, WI, USA

Purpose: A recent report by the American Academy for Pediatrics (AAP) suggested that Facebook causes depression offline. It is unclear whether this statement is supported by evidence, if so, this phenomenon should be observed across cultures. Depression has been shown to have a strong link to social connectedness, and Facebook is a key element of social connectedness among US adolescents. It is not known how adolescents outside the US see social networking sites playing a role in their social connectedness. The purpose of this study was to examine the perceived views regarding social connectedness of Facebook users in the United States compared to Ecuador. **Methods:** College freshman, ages 18-19 from two universities in the US and Ecuador completed an in person interview. The interview asked for their views on social network sites and perceived impact on their social interactions. They were also asked about the amount of time spent on social networking sites compared to overall internet use. Descriptive statistics were calculated. **Results:** Of 124 participants (ages 18-19) included in the study, 58.1% were from the United States and 55.6% were male. Of 31.9% participants from Ecuador, 30.8% were male. Overall, 86.1% of the United States participants and 63.5% of the Ecuadorian participants had positive and neutral views of Facebook. 36.5% of the Ecuadorians had a negative view on social networking sites compared to only 13.9% of the participants from the United States. Among females, 50.0% of Ecuadorian females had a negative view of Facebook compared to only 15.6% of the females from the United States. Among positive and neutral participants from the United States, 77.4% had high use, while of the positive and neutral participants from Ecuador, only 36.4% reported high use. **Conclusion:** Overall, participants from the United States demonstrated a more positive view towards social network sites and their influence on offline social interactions. Our next steps will be to apply qualitative analysis to this dataset to understand themes in participant responses.

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THE EFFECTS OF ERYTHROPOIETIN ON IRON AND TRANSFERRIN RECEPTOR CONCENTRATION IN NEWBORN RAT TISSUE

[a]*AT Quilling*, [a]C Pittner, [a]SE Blohowiak, [a]PJ Kling. [a]University of Wisconsin Department of Pediatrics School of Medicine and Public Health, Madison, WI.

Background: Transferrin, an iron transport protein found in the mammalian milk and small intestine, is critical to iron metabolism. Concentrations of transferrin and its receptor may be influenced by erythropoietin (Epo), a hormone found in milk that stimulates iron utilization in red blood cells. It is unclear if Epo also stimulates iron absorption to meet increased iron needs. To examine how enteral Epo affects iron metabolism in newborn rats, we hypothesized that transferrin, transferrin receptor and liver iron concentrations would be greater in both dam-fed (Dam) and iron-deficient (IDA) rats that were fed Epo in milk. If Epo can be shown to have these effects in newborn rats, it could be used as a treatment for neonatal iron deficiency. **Methods:** Both Dam and IDA Sprague-Dawley rats were fed either therapeutic enteral Epo or PBS/BSA for 12 days following birth. IDA was induced by artificial formula fed through gastrostomy (pup-in-cup method). Blood and tissues were harvested at day 12. Plasma transferrin and ferritin were measured by ELISA, and intestinal transferrin receptor expression was measured by Western blot. Body and liver iron content were measured by atomic absorption, plasma iron by ferrozine, and iron distribution in duodenal and liver tissue was analyzed using stains for both normal (Fe3+) and enhanced (Fe2+/3+) Prussian blue (PBR) stains. Photomicrographs were used for quantitative image analysis. **Results:** Dam rats had higher body iron than IDA, with intermediate levels in IDA+Epo. Liver iron content was higher in IDA groups, with enhanced PBR staining in a central vein distribution. Duodenal transferrin receptor staining was greater in IDA+Epo than other groups and was located on the apical surface of villus enterocytes. While enhanced PBR stain was more sensitive at detecting iron in liver than normal PBR, little iron was seen in duodenal tissue with either stain. Plasma levels of transferrin and iron were not different between treatments, but plasma ferritin levels were higher in both Dam groups, compared to IDA, with intermediate results in IDA+Epo. **Conclusions:** Rats with IDA have a blockade in iron trafficking from liver into the blood, with both liver tissue iron concentration and enhanced PBR staining showing similar patterns. In IDA rats, Epo raises transferrin receptor levels in duodenum and may increase plasma ferritin levels, which likely reflect the greater liver stores. Further analysis of the impact of milk-borne Epo on trafficking of iron into and out of liver tissue in suckling rats is necessary.

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PRO-APOPTOTIC BNIP3 REQUIRES POSH FOR C-JUN N- TERMINAL KINASE (JNK) DEPENDENT CELL DEATH

M Castronovo, A Grimes, T L Schmit, M Maes, J C Ralphe, M Wilhelm, Department of Pediatrics, Waisman Center, University of Wisconsin School of Medicine and Public Health, Madison, WI

Background: Myocardial hypoxia is a leading cause of cardiomyocyte apoptosis. However, the signaling pathways leading to cardiac cell death are not well understood. Previous data has shown that BNip3 interacts with and stabilizes POSH. Our data indicates that BNip3 requires POSH for JNK mediated cell death. **Hypothesis:** We hypothesized that BNip3 requires POSH for induced cell death in cardiomyocytes. **Methods:** We used wild type (WT) and POSH KO HEK-293T cells transfected with BNip3 to evaluate JNK activation and cell death. We also subjected WT and POSH-null cardiomyocytes to hypoxia/acidosis (H/A) and measured JNK activation and the requirement for POSH in BNip3-regulated cell death. TUNEL staining of cardiomyocytes was used to determine the levels of cell death and immunoblotting was performed to measure p-JNK and BNip3 expression levels. **Results:** Overexpressing BNip3 promotes JNK activation in WT but not in POSH KO cells (140 +/- 18 vs 89 +/- 23), resulting in a lack of BNip induced cell death in POSH KO cells. Further, in a H/A cardiomyocyte cell death model, we show that POSH KO cardiomyocytes have a decrease in BNip3 expression and JNK activation, and a decrease in cell death after exposure to H/A. **Conclusion:** These data indicate that BNip3 relies on POSH to activate JNK and promote cell death and thus indicate a potential therapeutic target to improve cell survival in myocardial infarction and ischemia.

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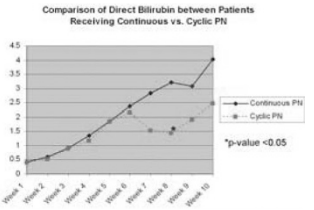
DECREASED INCIDENCE OF CHOLESTASIS IN INFANTS ≤ 1500 GRAMS WITH CYCLIC PARENTERAL NUTRITION (PN)

MJ Shareef, B Petro, P Nicoski, J Hrejsa, J Corrales, Loyola University Medical Center, Maywood, IL.

Purpose of Study: To determine the incidence of cholestasis in continuous vs. cyclic parenteral nutrition (PN) for infants ≤ 1500 grams. Parenteral nutrition-associated cholestasis is a major complication of PN and can develop as early as two weeks after birth, as indicated by an elevated direct bilirubin (DB) level > 2 mg/dL. Cyclic PN has been effective in reducing cholestasis in children and adults, but minimal research has been done in very low birthweight infants. **Methods:** A prospective, randomized controlled study from July 2007 to June 2010 compared continuous PN ($n = 58$) vs. cyclic PN ($n = 56$) beginning at 1 week of age. Cyclic PN was infused over 20 hours, with only dextrose and electrolytes for the remaining 4 hours. Weekly serum liver profiles and the incidence of cholestasis were compared between the groups. Statistical analysis included t-test, chi-square analysis, and Fisher's exact test. **Results:** The incidence of cholestasis was 14 (24%) in the continuous PN group vs. 7 (12.5%) in the cyclic PN group ($p=0.109$). There was a downward trend in DB beginning in week 7 favoring cyclic PN which was statistically significant by week 8: 3.2 mg/dL vs. 1.4 mg/dL ($p=0.042$) for continuous vs. cyclic PN groups, respectively. **Conclusions:** Very low birthweight infants anticipated to be on PN for an extended period may benefit from cyclic PN. Further research is needed to determine when cyclic PN should be initiated.



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INCIDENCE OF IBD IN ICELANDIC CHILDREN 1950–2010: NATIONWIDE POPULATION BASED STUDY

Agnarsson, Ulfur [1]; Björnsson, Sigurdur [1]; *Sigurdsson, Luther* [1, 2] 1. Childrens Hospital, LSH University Hospital of Iceland, Reykjavik, Iceland. 2. Pediatrics, University of Wisconsin School of Medicine and Public Health, Madison, WI, United States.

Aim: Describing the changes in incidence of IBD in Iceland. Material and methods: All IBD patients < 16 years of age diagnosed in Iceland 1950-2010 included. From 1950-1989 patients were identified retrospectively reviewing charts and pathological specimens. Since 1990 we prospectively obtained data on IBD patients. Only patients fulfilling Lennard-Jones1 criteria for Ulcerative colitis (UC) and Crohn's disease (CD) were included. Colitis not clearly UC or CD was called indeterminate colitis (IC). **Results:** 110 children were diagnosed to have IBD; 61 with UC, 44 CD and 5 were indeterminate. The median age of children was 13.7+/- 2.6 years; there were 70 boys and the sex distribution varied decade to decade (M:F 1.6:1 to 5:1). Twenty percent of both patients with UC and Crohn's disease had a family history of IBD. From 1951 until 2000 there was a dramatic increase in the incidence of IBD from 1.2 per 100,000 children < 16 years of age to 5.6 per 100,000; however, in the last decade (2000-2010) the incidence stabilized at 5 per 100,000. Right sided disease became more prominent in last 3 decades. **Conclusion:** In this population-based pediatric cohort that included all children in one country over 6 decades, a dramatic increase in the incidence of IBD was observed during the last 30 years. These findings are similar to the few other studies available in children however the stabilization of incidence in the last decade is a new finding.

1 Lennard-Jones JE Scand J Gastroenterol 1989, 24(suppl 170), 2-6 Incidence of IBD per 100.000 children< 16 years in Iceland

	IBD	UC	CD	IC
1951–1960	1.2	1.1	0.2	
1961–1970	0.9	0.7	0.1	
1971–1980	0.9	0.7	0.1	
1981–1990	2.5	1.2	1.2	0.1
1991–2000	5.6	2.9	2.5	0.3
2000–2010	5.0	2.4	2.3	0.3
p-value (trend)	0.022	0.065	0.014	

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BREAST FEEDING INITIATION (BFI) RATE AND BREAST FEEDING (BF) RATE AT 3 WEEKS POSTPARTUM IN AN URBAN INNER CITY POPULATION

V. Geraldo, C. Asiedu-Osei, M. Newman, N. Dulao, G. Srinivasan and H. Srinivasan. Mount Sinai Hospital Chicago, IL.

Background: The objective of Healthy People 2010 was to achieve BFI rates of 75% and 50% continuing to BF at 6 months post partum. Wide discrepancies exist in BFI and duration of BF amongst various racial and ethnic groups. **Objectives:** To determine the BFI rate amongst African American and Hispanic population in an urban inner city population. To determine BF rate at 3 weeks post partum in a select cohort. **Material and Method:** The study cohort included all mother infant pair admitted to the mother- baby unit of Mount Sinai Hospital from Jan 2009 to May 2011. The BF rate at 3 weeks post partum was determined in a subset group of 107 consecutive mothers admitted to the mother -baby unit. A lactation consultant met with all mothers postpartum and helped them with BF. At 3 weeks post partum, a bilingual clinician conducted a phone interview with the mother. **Results:** During the study period, 7580 infants delivered at Mount Sinai Hospital. Of these, the 6582 admitted to the nursery formed the study group. 54% were Hispanic, 41% African American and 5% Caucasian. The BFI rate was 78% and exclusive breast feeding (EBF) rate was 18%. During the study period a subset of 107 consecutive mothers admitted over a 2 month period were followed up prospectively at 3 weeks with a telephonic interview to assess the BF rate. In this cohort, 75% were Hispanic, 22% African American and 3 % Caucasian. 44 mothers could not be contacted and the data of the remaining 63 is presented. During the hospital stay the BF intention rate was 95.2%, BFI rate was 85.7% and EBF rate was 28%. At 3 weeks postpartum, BF rate was 62.5% and the EBF rate remained the same at 28%. The two most common reasons for this decline in breast feeding rate were insufficient milk production and maternal perception of an unsatisfied infant. **Conclusion:** The BFI rate of 85.7% in this cohort of inner city population is well above the national goal. However there is a steep decline in the BF rate from 85.7% to 62.5% at 3 weeks, with the EBF rate remaining the same. Hence this decline in BF rate is seen only in the group of mothers who initiate mixed feeding during hospital stay. Continued lactation support either over telephone or during well child visit would help to greatly reduce this decline.

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POSTNATAL GROWTH FAILURE (GF) IN VERY LOW BIRTH WEIGHT (VLBW) INFANTS

L. Grindeanu, G. Srinivasan and H Srinivasan, Dept of Pediatrics, Mount Sinai Hospital, Chicago, IL

Background: Postnatal GF of Very Low Birth Weight (VLBW) infants may result from a complex interaction of genetic and environmental factors. **Objective:** To document the incidence of postnatal GF, (weight< 10^{th} percentile) at discharge from NICU and at 1 year corrected gestational age in a cohort of VLBW infants from an urban inner city population. **Methods:** The study was a retrospective review of medical records of all infants admitted to Mount Sinai Hospital NICU from 2007 to 2009. Exclusion criteria included infants who were small for gestational age at birth, had congenital malformations, who were transferred out or expired prior to discharge and those who had incomplete records. During the study period, standardized nutritional protocol was in place, which included starting hyper alimentation with amino acids on the first day of life. The follow-up at 1 year corrected gestational age is presented only for a subset of neonates born in 2009 who were discharged and followed up in the high risk clinic. **Results:** During the study period, 181 infants were admitted to the NICU. 68 infants were excluded and the data for the 113 infants is presented in the table below.

Birth weight (g)	N	GF at discharge (%)
500–750	11	45.4
750–1000	31	32.3
1000–1500	71	35.2
Total	113	35.4

The table below shows the data for the 51 VLBW infants seen in the high risk clinic.

Birth weight (g)	N	GF at 3 m (%)	GF at 6 m (%)	GF at 9 m (%)	GF at 12 m (%)
500–750	6	3/4 (75)	3/4 (75)	3/4 (75)	3/4 (75)
750–1000	15	5/8 (62.5)	6/8 (75)	5/7 (71.4)	4/6 (66.6)
1000–1500	30	7/19 (36.8)	6/15 (40)	4/11 (36.4)	5/10 (50)
Total	51	15/31 (48.4)	15/27 (55.5)	12/22 (54.5)	12/20 (60)

Conclusion: Despite aggressive nutritional practices, 35% of VLBW infants show GF at discharge. At 1 year corrected gestational age, 60% of infants show GF. We speculate that this increase in GF may be due to the low follow-up rate as only VLBW with complex medical conditions follow up in the high risk clinic.

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MIGRATION PATTERNS OF PERIPHERALLY INSERTED CENTRAL VENOUS CATHETERS (PICC) AT 24 HOURS POST INSERTION IN NEONATES

A. Tjin-A-Tam[1], R. Galang[1], A. Hecht[2], G. Srinivasan[1] and H. Srinivasan[1]. Department of Pediatrics[1] and Department of Radiology[2], Mount Sinai Hospital, Chicago, IL

Background: PICC are commonly used in the Neonatal Intensive Care Unit (NICU) for long term intravenous access. Migration of the catheter post insertion is a serious complication which can cause neonatal death due to cardiac tamponade. **Objective:** To document the migration patterns of PICC at 24 hours post insertion in neonates. **Material and methods:** Retrospective chart review of 100 consecutively placed PICC lines in the NICU at Mount Sinai Hospital (from January 1, 2010 to March 31, 2011), All PICC were placed by 2 certified NICU nurses. X-ray was obtained immediately after insertion and 24 hours later. The position of the arm was abducted and externally rotated at shoulder and extended at the elbow on all the X-rays. All X-rays were reviewed by a single board certified radiologist. **Results:** The mean birth weight of the study group was 1266 grams (range 420 – 3795g). The mean dwell time of the PICC lines was 29.8 days (range 1 – 45 days). Of the 100 PICC lines 76 were placed in the basilic vein, 19 in the cephalic vein, and 5 in the saphenous vein. Of the PICC lines placed in basilic veins, 35.5% migrated inferiorly and medially (1.48 cm average, range 0.5 - 3.4cm), 14.5% migrated laterally (1.74cm average, range 0.5 – 3.6cm) and 50% did not change in position. Of the PICC lines placed in cephalic veins, 21% migrated inferiorly and medially (1.36cm average, range 0.6 – 2.5cm), 15.7% migrated laterally (1.75 cm average, range 1 – 2.5cm), and 63.3% did not change in position. None of the PICC lines placed in the saphenous veins migrated. **Conclusion:** 45% of all PICC placed in either the basilic or cephalic vein migrated at 24 hours post insertion. Hence all neonates after PICC insertion should have x-rays done 24 hours post insertion and PICC lines adjusted accordingly.

Insertion Site	Number	Inf. Med migration	Lateral migration	No migration
Basilic Vein	76	27 (35.5%)	11 (14.5%)	38 (50%)
Cephalic	19	4 (21%)	3 (15.7%)	12 (63.3%)
Saphenous	5	0	0	5 (100%)
Total	100	31 (31%)	14 (14%)	55 (55%)

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THE HELA CELL LINE CONTAINS A GENETIC VARIATION IN TOLL-LIKE RECEPTOR 2

Sara A Tokarz, PhD[1], Jessica A DeValk[1], Bikash Pattnaik, PhD[1] and De-Ann M Pillers, MD, PhD[1]. [1]Pediatrics, University of Wisconsin, Madison, WI, United States.

Background: HeLa cells are commonly used in studies to investigate inflammation pathways, including the up-regulation inflammatory cytokines in response to pathogen exposure. Toll-like receptors (TLRs) are a family of trans-membrane receptor proteins present in many cell types and serve as the first point of defense in the innate immune system. Infectious microorganisms interact with TLRs to initiate the inflammatory cascade in response to infectious microorganisms. Single nucleotide polymorphisms (SNPs) in genes for TLRs occur in up to 20% of the population. **Objective:** We sought to determine the presence of a subset of common TLR SNPs associated with inflammation in a cohort of cell lines that are used extensively for inflammation studies. **Design/Methods:** DNA was isolated from the several commonly used cell lines including HeLa cells. TLR genotype was established using ABI TaqMan® SNP Genotyping Assays. The Step OnePlus™ software was used to analyze the intensity of each fluorescent probe assigned to either the wild-type (WT) or natural variant (NV) allele and to produce an amplification plot of the data along with the C_t value. **Results:** HeLa cells were heterozygous for WT and NV alleles at TLR4 299, TLR4 399 and TLR2 631 while all other cell lines were homozygous for the WT alleles. **Conclusions:** TLR 2 functions as a heterodimer with TLR1 and 6. TLR2 P631H has been shown to be associated with a decrease in the innate immune response and acts as a dominant-negative allele. The presence of the TLR2 P631H allele impacts the activity of each of these complexes, affecting the cell"s ability to respond to a wide variety of pathogens have been studied using the HeLa cell line, including Ureaplasma, fungi, and bacteria. HeLa cells are commonly used as a cell model for many inflammation studies, which may not be appropriate considering the presence of TLR SNPs in this cell line.

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TRACKING PATIENTS THAT LEAVE WITHOUT BEING SEEN FROM AN URBAN PEDIATRIC EMERGENCY ROOM

AS Tothy, E Kaufman, SD Johnson, D Johnson* *The University of Chicago, Chicago Illinois, United States

Background: For the past 20 years, overcrowding in the Emergency Room (ER) has worsened. From 2000 to the present, the number of ER patient visits has increased to more than 15 million. The number of patients that leave an ER prior to receiving medical care, or the Left Without Being Seen rate (LWBS), correlates to the growing number of ER visits. Pediatric Emergency Departments are seeing increases in patient volumes and increases in LWBS rates. Elevated LWBS rates and overcrowding can have drastic consequences with regards to patient care and patient satisfaction. Few studies have evaluated the LWBS rates in children and even fewer have looked at what happens to the pediatric LWBS patient when they leave the Pediatric ER. In collaboration with The Urban Health Initiative, we undertook this study to determine what was happening to the large LWBS volume after leaving our Pediatric ER. Methods: This project took place in the Pediatric Emergency Department at the University of Chicago Comer Children's Hospital. A Patient Care Advocate identified all LWBS patients from April 2009 until March 2011. The LWBS data was gathered from our Pediatric ER electronic medical record system. Once patients were identified, they were called on the first day after their visit and if they were not reached, 1 more attempt to contact them occurred either on the same day or the following day. All phone numbers available in the patient's chart were attempted. Results: From the time period of April 2009 and April 2011, 3,476 patients LWBS in the Pediatric Emergency Room at the University of Chicago Comer Children's Hospital. Sixty-three percent of these children's families were reached by phone by a Patient Care Advocate. Thirty-four percent of the families contacted sought medical care elsewhere or had scheduled an appointment for follow up medical care. Eleven percent of children received care at an outside hospital patient care site on the same day they left the Pediatric ER. When contacted, 13% of the families felt that no further medical appointment/care was needed at that time and 14% refused assistance from the patient care advocates. **Conclusion:** This study of LWBS data was a very good start in adequately determining what happens to our patient population that leaves prior to receiving medical care. The responses we received from family members could help us reeducate families in the need for medical homes and what is appropriate medical use. Furthermore, it could begin to help us as a medical community, begin to understand and subsequently determine needs of patients and their families that visit the pediatric ER.

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FACTORS INFLUENCING SUCCESSFUL DISCONTINUANCE OF CAFFEINE AT 34 WEEKS CORRECTED GESTATIONAL AGE FOR PREMATURE INFANTS TREATED FOR APNEA OF PREMATURITY

P Hummel, C Vega-Barrera, I King, C Sajous. Loyola University Medical Center, Maywood, IL.

Purpose of Study: The objective of this investigation is to determine, the factors that predict successful cessation of caffeine at 34 weeks gestational age (GA) in premature infants treated for apnea of prematurity, potentially shortening hospital stay. **Methods:** Retrospective chart review of all infants born at < 34 weeks GA admitted to Loyola University Medical Center NICU from August 15, 2006 to August 15, 2010, with diagnosis of apnea of prematurity who were started on caffeine therapy. Data collected include GA at birth, birth weight (BW), mode of delivery, race, gender, singleton or multiple pregnancy, presence of intracranial hemorrhage or other brain pathology, bronchopulmonary dysplasia, patent ductus arteriosus, necrotizing enterocolitis, and GA when successfully weaned off caffeine. **Results:** A total of 663 infants < 34 weeks GA were admitted during the study period. 75% of these infants were treated with caffeine, 48% were female, 52% male. The GA ranged from 22-33 weeks. The BW ranged from 390 to 3070 grams. Of the infants treated with caffeine, 78% weaned successfully at 34 weeks. 22% percent of the infants failed discontinuance at 33-35 weeks gestation; 65% of these were male, 35% female. The mean GA at birth of those infants who were successfully weaned was 29 weeks versus 25 weeks. The mean BW of those infants who were successfully weaned was 1316 grams versus 906 grams. **Conclusions:** The infants who failed were more likely to be male, and to have lower gestational age and lower BW than those in whom caffeine was successfully weaned. Infants who weaned successfully were female, had a higher BW and GA.

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SHOULD AN EXTREMELY LOW BIRTHWEIGHT INFANT WITH A PROVEN PATENT DUCTUS ARTERIOSUS BE EXTUBATED BEFORE PHARMOCOLOGICAL CLOSURE OF THEIR PATENT DUCTUS ARTERIOSUS?

C Vega-Barrera, YJ Khan, J Muraskas. Loyola University Medical Center Maywood, IL.

Purpose of study: To evaluate the rate of reintubation in extremely low birthweight (ELBW) infants weighing <1000g, with patent ductus arteriosus (PDA) and determine if it is clinically reasonable to extubate before their PDA has functionally closed. **Methods:** This retrospective study analyzed data from the NICU at Loyola University Health System from 2006 to 2009, with a limitation on ELBW infants with ECHO proven PDA. The collected data included day of extubation, day of PDA closure and day of reintubation during pharmacological treatment of PDA. **Results:** 79 infants with birth weight (BW) <1000g and PDA were identified, 35 females and 44 males. Of these 79, 11 infants were excluded due to death before extubation; thus 68 infants were evaluated. All 68 infants underwent initial pharmacological treatment of their PDA resulting in ECHO proven closure. Of the 68 infants, 62 (91%) were extubated prior to their PDA treatment and 6 (9%) were not extubated. 24 (39%) of the 62 infants remained extubated, and 38 (61%) were reintubated. Of the 38 infants who were reintubated, 13 infants were reintubated (34%) before PDA closure, and 25 infants (66%) were reintubated after PDA closure. **Conclusions:** This study demonstrated that of the ELBW infants who presented with hemodynamic significant PDA, 13/62 (21%) infants extubated required reintubation prior to medical treatment of PDA. 25/62(40%) infants were reintubated after PDA closure due to other causes. 24/62(39%) infants remained extubated before and after their PDA treatment. Approximately 80% of ELBW infants will remain extubated during the medical treatment and closure of PDA. A PDA is not an indication for maintaining ELBW infants on mechanical ventilatory support.

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AN ANALYSIS OF FITNESS BEHAVIORS PROMOTED ON FACEBOOK FOR ADOLESCENTS

H Villiard, M Moreno, Department of Pediatrics, University of Wisconsin, Madison, WI.

Background: When students transition from high school to college, they gain independence and establish their own fitness behaviors. Due to an alarming increase in adolescent obesity, this group is an important target for an intervention promoting improved fitness behaviors. The purpose of this project was to evaluate fitness-related content on Facebook. **Methods:** Three key words (fitness, nutrition, and diet) were used as search terms for identifying Groups, Fan Pages, and Applications on Facebook. A Group allows for communication and sharing of common interests among members. Fan Pages are promotional pages created by businesses. Applications are a way to more actively engage with Facebook members via interactive programs. Among search results for the three searched terms, a random subsample of 10 Groups, Fan Pages and Applications was evaluated to determine the purpose of the page and content of comments on the page. **Results:** Among Groups, the majority (94.8%) of content was user generated and included postings of fitness articles and product promotion. Among Fan pages, three categories of content included: product promotion, offering advice and postings of news articles. Among Applications, the majority of displayed content was advertisements or suggestions for workouts. Most of the reviewed Applications were not directly accessible via Facebook, but instead re-directed users to an outside website. **Conclusions:** Facebook Groups seem to function as a user-generated forum and Fan pages tend to show many advertisements while Applications do not clearly define their purpose. Therefore, when adolescents are seeking out information on Facebook on how to improve their fitness, the majority of the material displayed may not be helpful or relevant. Implications may include the development of an age-appropriate forum or interactive page on Facebook for adolescents where they can learn how to eat healthy and stay physically active.

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FITNESS ON FACEBOOK: ADVERTISEMENTS GENERATED IN RESPONSE TO PROFILE CONTENT

H Villiard, M Moreno, Department of Pediatrics, University of Wisconsin, Madison, WI.

Background: Obesity is a challenging problem affecting almost half of college students. In order to solve this complex health problem, innovative approaches must be utilized. Over 94% of college students maintain a Facebook profile, providing them a venue to publicly disclose current fitness behaviors. Displayed advertisements on Facebook are tailored to profile content and may influence college students' fitness efforts. Facebook may be an innovative venue for improving college students' fitness behaviors. The purpose of this project was to determine 1) how and to what extent college students are discussing fitness on Facebook and 2) how user-generated fitness information is linked to advertisements for fitness products and advice. **Methods:** First, public Facebook profiles of individual college students were evaluated for displayed fitness references based on ten fitness behavior categories. Interrator reliability between two coders was 91.18%. Second, 10 fitness "status updates" were generated and posted by a researcher on a Facebook profile; the first 40 linked advertisements to these statements were examined. Advertisements were categorized and then examined for relevance to the college population. **Results:** A total of 57 individual profiles were examined, owners had an average age of 18.3 years (SD=0.51) and 36.8% were female. 71.9% of profiles referenced one or more fitness behavior; 97.6% referenced exercise; 4.9% dieting and 4.9% unhealthy eating. Among the first 40 ads linked to generated "status updates", 40.3% were fitness related. Most advertisements were for charity runs (30.4%), fitness apparel (24.2%), or fad diets (9.9%). **Conclusions:** Students reference both healthy and unhealthy fitness behaviors on their Facebook profiles, and these trigger the display of fitness-related advertisements of which few appear applicable. A community or university based intervention could be designed and implemented to provide relevant and tailored information to students on Facebook.

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INVESTIGATING THE RELATIONSHIP BETWEEN NEWBORN IRON DEFICIENCY AND EOSINOPHILIA AT 6 MONTHS-12 MONTHS

[a]*RM Weigert,* [ab]TW Guilbert, [ab]AK Phillips, [ab]SE Blohowiak, [ab]PJ Kling. [a]University of Wisconsin School of Medicine and Public Health, [b]Department of Pediatrics, Madison, WI.

Introduction: Many environmental, genetic and immunological factors have been associated with wheezing and asthma in young children. Newborns with iron deficiency (ID) also have an enhanced risk of recurrent wheezing in infancy and subsequent asthma diagnosis. These two illnesses may be related because previous work found lower umbilical cord iron was related to wheezing in infancy. Since eosinophilia (eosinophils ≥4% of WBC or absolute number ≥470/mm^3) at 6-12 months of age is a biomarker for recurrent wheezing in infancy, we hypothesized that eosinophilia will be correlated with lower fetal iron stores at birth. **Methods:** Newborns ≥35 wks gestation with one or more risk factors for ID were recruited. Risk factors included: maternal ID, maternal diabetes, fetal overgrowth or undergrowth, mothers from ethnic minority groups, and/or mothers with low socioeconomic status. Cord blood indices of storage iron (plasma ferritin), steady state erythrocyte (RBC) iron (zinc protoporphyrin/heme, ZnPP/H), and recent RBC iron (reticulocyte-enriched ZnPP/H) were measured. At 6-12 months, eosinophil % and absolute eosinophils were determined. Linear regression and unpaired t tests examined the relationship between infant eosinophilia and iron status at birth. **Results:** No significant linear relationship was found between either 6-12 month eosinophil % or absolute eosinophil count and any cord blood iron status index. Mean plasma ferritin did not differ based on % eosinophilia, but was lower in those with absolute number eosinophilia, $p=0.02$. Cord RE ZnPP/H trended higher with ≥4% eosinophils, $p=0.053$ and was higher with absolute number eosinophilia, $p=0.02$. Delta ZnPP/H (RE ZnPP/H minus ZnPP/H) was higher in those with percent and absolute eosinophilia, $p=0.02$, $p=0.007$, respectively. Few ferritins in children with either percentage or absolute number eosinophilia were above the 75 percentile of normal cord ferritins. **Conclusions:** An inverse relationship was seen between eosinophilia at 6-12 months and both erythrocyte and storage iron indices in cord blood. Poor fetal iron status in late gestation can affect crucial development processes, including immune cell differentiation. Studies show that a predominance of T helper type 2 (Th2) cells is seen in asthmatic children. Immediately after birth, neonatal immune cells experience a 100-fold amplification, which may exacerbate an existing Th2 prevalence. *In vitro* work shows that Th2 clones are relatively resistant to cellular iron depletion. Further investigation is necessary.

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QUANTITATIVE MRI ASSESSMENT OF IMPENDING HEPATIC FAILURE DUE TO STEATOHEPATITIS IN A 10 YEAR OLD NATIVE AMERICAN CHILD WITH ACANTHOSIS NIGRICANS

Peter M Wolfgram, MD[1], Ellen L Connor, MD[1], M Tracy Bekx, MD[1] and David B Allen, MD[1]. [1]Pediatrics, University of Wisconsin-Madison, Madison, WI, United States.

Background: Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in children. Obesity and insulin resistance (IR) increase the risk for NAFLD and are more common in Native American youth. Current screening for NALFD in obese children includes measurement of ALT, with confirmatory hepatic biopsy. However, since elevated ALT and clinical symptoms lag behind steatosis, hepatic injury, and fibrosis, a better test for NAFLD diagnosis, grading of severity, and therapeutic monitoring is needed. **Objective:** To report use of QMRI to diagnose and grade NAFLD in an obese Native American child presenting with imminent hepatic failure and evidence for prior insulin resistance. **Design/Methods:** An obese (BMI 24.9; >97th%tile) 10yo Native American male had a 3 week history of abdominal pain, epistaxis, and jaundice following a gradual 15kg weight loss during the previous 17 months. Marked acanthosis nigricans and hepatomegaly were noted. Initial fasting labs: AST 410 U/L, ALT 106 U/L, INR 1.7, TG 346mg/dL, HDL-C 7mg/dL, and LDL-C 137mg/dL, insulin 15uIU/mL, glucose 95mg/dL, HgbA1c 4.5%; Viral hepatitis studies were negative. Postprandial insulin was 62uIU/mL and glucose 90mg/dL, consistent with insulin resistence. **Results:** Hepatic MRI revealed increased fat fraction, 20-22% (nl < 5%), and decreased elasticity, 10kPa (nl < 2.9). Liver biopsy revealed severe steatohepatitis, with fat fraction and fibrosis correlating closely with MRI findings. Treatment with metformin and vitamin E was begun. Follow-up after 9 months showed a BMI of 20.0, resolution of hepatic steatosis, reduced acanthosis nigricans, AST 46U/L, ALT 44U/L, INR 1.2, TG 108mg/dL, HDL-C 15mg/dL, and LDL-C 89mg/dL. **Conclusions:** Heightened susceptibility to obesity and IR in Native American children increase the risk for early and severe NAFLD. NAFLD is reversible if identified and treated before fibrosis develops; in this case elevation of AST greater than ALT already indicated severe fibrosis. QMRI provides NAFLD diagnostic information equal to biopsy, but with reduced discomfort and risk, and potentially less cost.

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AVAILABILITY OF WEB-BASED GLOBAL HEALTH EDUCATION INFORMATION FOR APPLICANTS TO PEDIATRIC RESIDENCY PROGRAMS

*J Yaeger,** D Allen, M Moreno. University of Wisconsin School of Medicine and Public Health, Madison, WI, USA.

Many pediatric residency programs are developing training opportunities in global health (GH) to meet rising interest among prospective applicants. Studies reveal wide variation in the content and extent of this education, but have not specifically assessed the content and presentation of information that is available on programs' websites. Electronically accessible information is becoming increasingly important since it is frequently the only way that applicants have to evaluate residency programs. Our objective was to quantify the number of programs that present opportunities for GH education on their websites and to describe the content of this information. The Fellowship and Residency Electronic Interactive Database (FREIDA) was used to identify all pediatric residency programs in the United States as well as their websites. Each website was searched systematically for any information about GH education and specifically for indicators of level of structure and curricular components for each program. Evaluation took place between April 2011 and June 2011. Of 194 total programs, 177 had operational websites listed and were included in the study and 93 of the 177 training programs (53%) mentioned a GH educational opportunity. Of these 93 programs, 98% offered an elective experience, and 28% described a structured curricular program at the home institution. Other evaluated content included information regarding: existence of established global elective site (53%), payment of malpractice insurance for GH experiences (1%), cultural sensitivity training (8%), presence of curriculum for elective experience (24%), and whether a GH experience is a scheduled rotation or the resident is required to take vacation (28%). Our findings suggest a wide variety of programmatic approaches to GH described on pediatric residency program websites. This diversity likely stems from the evolving nature of GH education as program directors and administrators continue to work toward feasible and cohesive GH experiences during residency training. At the same time, this diversity presents challenges to applicants trying to evaluate and compare the role of GH in their prospective residency programs; e.g. whether GH is included in scheduled, structured curricula or restricted to elective opportunities. One helpful approach would be to ascertain key criteria for GH education, construct a template to facilitate presentation and comparison of these criteria, and investigate its utility in on-line pediatric residency websites.