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DEVELOPMENTAL INFLUENCES ON THE INNATE IMMUNE AND CARDIOVASCULAR RESPONSES TO SEPSIS

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Background: Sepsis is a major cause of morbidity and mortality in neonates, and the mortality rate doubles in patients who develop cardiovascular compromise and septic shock. Despite sepsis being a leading cause of death of preterm infants, mechanisms underlying sepsis-related cardiac dysfunction are not well established and therapies for the treatment in this uniquely susceptible population are very limited. **Objectives:** To establish a murine model and test the hypothesis that neonates have a heightened innate immune response to LPS compared to adults, which is linked to inflammation-related cardiac dysfunction. **Design/Methods:** 4 day old (neonate) and 5 week old (young adult) C57B/L6 WT mice were treated with a single injection of low-dose LPS (1.5 mg/kg). Noninvasive measures of cardiac function were obtained at baseline and after LPS injection. Animals were then sacrificed and serum and cardiac tissues collected for analysis of TNF-α, SAA and IL-6 via ELISA, RT-PCR and immunohistochemistry. Data were analyzed by t-tests, two-way ANOVA and Bonferroni post-tests. **Results:** We were able to reliably classify the cardiac response to LPS in 4 day old mice (ave. body weight 2.5gm) using new echocardiographic technology (Vevo770, VisualSonics) and demonstrate increased cardiac dysfunction relative to adult animals. Within one hour of LPS exposure, neonatal mice demonstrated a 30% decrease in left ventricular fractional shortening that was not seen in the adult mice ($p=0.006$). The neonatal mice also showed increases in plasma TNF-α ($p=0.02$), IL-6 ($p=0.0002$) and SAA ($p=0.0003$) 3 hrs after LPS injection; post-LPS cytokine levels in neonates were ~10-fold (for TNF-α) and~3-fold (for IL-6) higher than changes in adult mice. Myocardial tissue TNF-α was also increased to a greater extent in neonatal vs. adult mice (438.6 ± 206 vs. 86.3 ± 17 pg/mg protein, $p=0.0026$). **Conclusion:** We have established a murine model of neonatal sepsis that can be used to evaluate the innate immune and cardiovascular responses to endotoxin. Neonatal mice have a significant decrease in cardiac function in response to low-dose LPS that is not seen in adult WT mice and is associated with an increase in both baseline and post-LPS levels of pro-inflammatory cytokines. Further investigation of the developmental influences on the systemic vs. cardiac innate immune responses to sepsis is warranted, as a better understanding of these processes could lead to the development of successful therapies for sepsis in premature neonates.

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DIFFERENTIAL EFFECTS OF EXENATIDE AND CAPTOPRIL ON GLUCOSE HOMEOSTASIS AND SURVIVAL IN A MURINE MODEL OF DILATED CARDIOMYOPATHY

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Background: While a close bidirectional relationship exists between impaired glucose homeostasis and congestive heart failure, the mechanisms contributing to this association remain incompletely understood. We have previously demonstrated that acute blockade of glucose transport by the GLUT4 antagonist ritonavir accelerates the progression to decompensated heart failure in a murine model (TG9) of dilated cardiomyopathy. TG9 mice, which express high levels of the cre recombinase under theα-myosin heavy chain promoter, develop insulin resistance together with dilated cardiomyopathy and predictably die of heart failure by 12 weeks of life. We hypothesize that cardiac function and survival in the setting of heart failure is directly affected by myocardial glucose uptake. **Experimental Design and Results:** To investigate the relationship between altered insulin sensitivity and survival in the setting of heart failure, the incretin mimetic exenatide (40 mcg/kg/d, n=9) was administered to TG9 mice from 8 weeks of age until the time of death. Blood glucose levels were improved under both fasting and post-glucose conditions. Myocardial 2-deoxyglucose uptake was also significantly improved (37.7 versus 14.5 umol/100 g/min in exenatide and vehicle-treated mice, respectively, n = 6/group $p<0.001$). This was correlated with improved survival (89.6 $\pm$ 2 for exenatide-treated mice versus 80.8 $\pm$ 2.68 days for vehicle-treated mice). Cardiac GLUT4 and Cre levels were not different between groups. The ACE inhibitor captopril (15 mg/kg/d) also improved survival in TG9 mice (86 ± 1.2 days, n=5) but did not improve systemic glucose homeostasis. Ritonavir abrogated the beneficial effects of exenatide and captopril on survival (83 $\pm$ 0.8 and 80.2 $\pm$ 2.68 days, respectively). **Conclusions:** Improved survival of exenatide-treated TG9 mice is correlated with improved systemic and myocardial specific glucose uptake, whereas the beneficial effect of captopril is independent of changes in systemic glucose uptake. Acute ritonavir-induced blockade of GLUT4 partially abrogates the beneficial effects of these drugs. These data provide insight into the importance cardiac specific glucose uptake on survival and function in the setting of advanced dilated cardiomyopathy.

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HYPOXIA PROMOTES PROLIFERATION OF PULMONARY ARTERY SMOOTH MUSCLE CELLS THROUGH INDUCTION OF FOXM1 BY HYPOXIA-INDUCIBLE FACTOR

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Purpose of the Study: Pulmonary arterial hypertension (PAH) is a devastating microarterial disease characterized by pulmonary artery remodeling due to smooth muscle cell proliferation and de-differentiation. Hypoxia is a well established stimulus for PAH as seen in several animal models. However, the mechanisms underlying hypoxia-mediated PAH remain unclear. The forkhead box M1 (FoxM1) is a transcription factor that regulates cell cycle and controls cell proliferation. We sought to determine whether FoxM1 participates in hypoxia-induced proliferation of human pulmonary artery smooth muscle cells (HPASMC). **Methods Used:** HPASMC were obtained from Lonza (Basel, Switzerland) and cultured in SmGM-2 medium. Hypoxic conditions (1.5% or 3% O_2) were achieved in a HEPA Class 100 CO_2 incubator (Thermo Scientific, Waltham, MA) and the oxygen tension was continuously monitored. The protein levels of FoxM1 and hypoxia-inducible factor (HIF) were determined with Western blot analysis. To measure FoxM1 gene transcription, we transfected HPASMC with a luciferase reporter construct containing the FoxM1 promoter and then exposed cells to hypoxia. In addition, we determined FoxM1 activity by transfecting HPASMC with a luciferase reporter construct containing 6 copies of the FoxM1 binding site. The proliferation rate of HPASMC was measured by bromodeoxyuridine incorporation assay. **Summary of Results:** We found that HPASMC exposed to hypoxia have increased FoxM1 protein levels and hypoxia increased FoxM1 promoter and transcriptional activity. During hypoxia, both HIF-1α and HIF-2α were stabilized in HPASMC and hypoxia induced HPASMC proliferation. Moreover, chemicals such as $CoCl_2$ and DMOG as well as overexpression of a peptide containing oxygen-dependent degradation domain (ODDD) of HIF-1α to stabilize HIF-α in normoxia also increased HPASMC proliferation, suggesting that hypoxia induces HPASMC proliferation via HIF. Furthermore, overexpression of the ODDD peptide increases FoxM1 promoter activity, suggesting that HIF induces FoxM1 gene transcription. **Conclusions:** Our results suggest that during hypoxia, stabilization of HIF induces FoxM1 expression, leading to HPASMC proliferation. Thus FoxM1 may represent a potential therapeutic target in PAH.

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BRONCHOPULMONARY DYSPLASIA AND POLYMORPHISMS OF PULMONARY VASCULAR GENES

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Bronchopulmonary Dysplasia (BPD) is the chronic lung disease that develops after preterm birth. BPD is characterized by a lack of alveolar development and concomitant decreased pulmonary vascular development. Pulmonary vascular development requires intact signaling from several genes including *Vascular Endothelial Growth Factor (VEGF)*, *Endothelial Nitric Oxide Synthase (eNOS)*, and *Extracellular Superoxide Dismutase (EC-SOD)*. Signaling from these genes can be affected by single nucleotide polymorphisms (SNPs). We performed a case control study to determine whether SNPs in *VEGF*, *eNOS,* or *EC-SOD* are associated with the development of BPD. **Methods:** We used previously collected DNA samples from the University of Iowa that were linked to clinical outcome data to perform a case control study. All infants included in the study were European American and born at$\leq$ 32 weeks EGA. Cases were defined as infants who either required supplemental oxygen for the first 28 days of life, or died at less than 28 days of age. Samples were genotyped for several SNPs in each gene. The SNPs which were analyzed were chosen by review of the literature and Hapmap data to ensure good coverage of each gene. Samples were genotyped using mass spectroscopy with matrix assisted laser desorption/ionization with time of flight detection. We used a chi-square test of association to determine significance. We then used a family based association test analyzing the same index cases along with corresponding parental data to confirm the association. **Results:** We included 219 samples in the analysis, 128 cases and 91 controls. None of the SNPs in *VEGF* or *EC-SOD* were associated with BPD. Two of the SNPs in *eNOS*, rs2373961 ($p=0.006$) and rs3918188 ($p=0.035$) were associated with BPD when cases and controls were compared. These same SNPs were also significant when analyzed with the family based association test (rs2373961- $p=0.031$, and rs3918188- $p=0.022$). **Conclusion:** Our data support an association between SNPs in *eNOS* and the development of BPD.

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DOES MORBIDITY PARALLEL MORTALITY FOR EXTREMELY PREMATURE INFANTS? AND WHAT WOULD IT MEAN IF IT DIDN'T?

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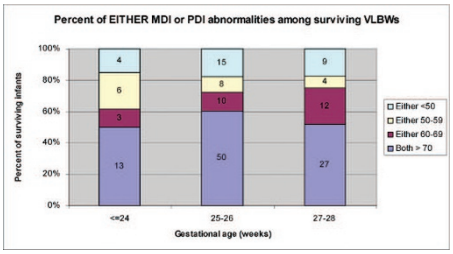
Background: For the first 30 years of neonatology, morbidity paralleled mortality. If an infant population had a 10% risk of dying then the survivors had 10% risk of permanent morbidity; 50% mortality meant 50% morbidity in survivors. **Objective:** We wondered whether this phenomenon held true for infants born at the extremes of prematurity (23–28 weeks). **Design/Methods:** We studied the outcomes for 199 infants born between 23 and 28 weeks gestational age(GA) whose excellent 2 year follow-up (Bayley MDI/PDI) was assured by participation in clinical trials in our NICU. **Results:** 52/199 infants (26%) were born at less than or equal to 24wks, 93/199 (47%) were born between 25–26 wks, 54/199 (27%) were born between 27–28wks. 38/199 (20%) infants died before hospital discharge. 90/199 (45% of births; 56% of survivors) had a normal neurologic outcome at 2 years corrected age (both MDI and PDI $\geq$70 71 infants (35% of births; 44% of survivors) were neurologically impaired at 2yrs of age (MDI or PDI $<$70). Survival to 2 years increased significantly as a function of GA subgroup – 50%, 89%, and 96% respectively. However the impairment rate among survivors was similar comparing gestational age subgroups [Fig 1]. 50%, 40% and 48% of survivors had either MDI or PDI $<$70 in gestational age groups $\leq$24, 25–26 and 27–28 respectively. More stringent cut-offs for neurologic impairment (MDI/PDI $<$ 60, $<$50) also did not differ comparing survivors among the GA subgroups. Similarly, the frequency of normal neurologic exam was not different comparing survivors among GA subgroups. **Conclusions:** 1) It is the rate of mortality, not morbidity, which differentiates outcomes among infants born between 23 - 28 wks GA. The rate of impairment in NICU survivors is similar across the 23–28 wk spectrum. 2) To the extent that permanent morbidity, and not the prospect of NICU death, drives parental decision-making, parents of infants born at the margin of viability should be informed of these outcome data.



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NEONATAL VISUAL ACTIVATION RESPONSE USING DIFFUSE OPTICAL IMAGING

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Background: Neurodevelopmental outcomes of infants in NICU is a major clinical concern. Traditional imaging modalities such as MRI and ultrasound of the head offer valuable structural information but may be limited in providing equally critical information on brain function. Diffuse optical imaging (DOI) based on near infrared spectroscopy (NIRS) technology is portable and relatively inexpensive and can be utilized at the bedside to study and monitor brain function. Previous DOI/NIRS devices suffer from stringent technical limitations and significant noise contamination, resulting in few functional imaging studies in neonates available in literature. Here we present images of functional/neurovascular responses to visual stimulation in term-born infants using our novel diffuse optical imaging system. **Methods:** we recruited healthy, term-born infants from well baby nursery within the first 3 days of life. Informed consent was obtained from parent(s) prior to each scan. Infants were scanned in a dim room and the imaging cap was placed over the occipital cortex. Each infant was presented with visual stimulus with alternating contrast in luminance. Functional or neurovascular response was recorded over a period of 15 minutes. **Results:** All infants tolerated our study well without adverse events. We were able to successfully measure functional response over the occipital cortex bilaterally in 5 term-born infants correlating with our visual stimulus. We are able to show at least a 3-fold improvement in contrast to noise ratio using our novel DOI system compared to traditional DOI/NIRS methods. **Conclusion:** Neonatal neurovascular/functional response can be safely measured using our novel DOI system at the bedside. More validation data are needed in terms of reliability as well as utility and safety in preterm neonates. Overall, development of DOI technology remains a worthwhile undertaking.

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EFFECTS OF A NOVEL MATERNAL UTERINE SPACE RESTRICTION MODEL ON FETAL KIDNEY DEVELOPMENT IN SHEEP

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Background: Fetal growth restriction during pregnancy has been shown to reduce fetal renal development and nephron allotment due to *in utero* nutrient restriction. Decreases in fetal kidney weight are also associated with altered renal function after delivery (e.g. hyperfiltration). Purpose: In a novel model of uterine space restriction, we evaluated the effects of reduced uterine space on fetal growth and renal development in late ovine gestation. **Methods:** Ewes and fetuses were grouped as control non-uterine space restricted group, defined as single fetus in uterine horn (non-USR) and unilateral or uterine space restricted group, defined as multiple fetuses in the uterine horne (USR). USR was promoted by surgical ligation and disconnection of one uterine horn at least 2 months prior to pregnancy. For this study, fetuses were evaluated at either 120 or 130 days gestation (term 147). Kidney tissues were fixed, stained for collagen by Gomori Trichrome, and examined for glomerular generation number (as measured by branching patterns). Fetal umbilical arterial blood was analyzed for plasma creatinine. **Results:** In the USR group at 120 days gestation, fetal wts were not appreciably altered. At 130 days gestation, fetal wts were 18% lighter (3324$\pm$152 g) compared to non-USR (4073$\pm$297; P$<$0.03). At 120 days gestation, kidney wts were 13% lighter (P$<$0.03) in USR compared to non-USR. At 130 days gestation, kidney wts were 24% lighter (P$<$0.01) in USR compared to non-USR. USR had 12% less glomerular generations compared to non-USR (P$<$0.01) at 120 days, while at 130 days, USR had 14% less glomerular generations compared to non-USR (P$<$0.01). Fetal plasma creatinine levels were not appreciably altered at 120 days gestation, while at 130 days, creatinine levels were elevated by 44% (p$<$0.01) in USR (2.2$\pm$0.2 mg/dl) compared to non-USR (1.5$\pm$0.1 mg/dl). **Conclusion:** Ovine glomerulogenesis is complete between 120 and 130 days gestation. IUGR was seen at 130 days gestation. Decreased kidney wt and glomerular generations in the USR support that IUGR impairs renal development. Additionally, increased plasma creatinine levels at 130 days gestation may support decreased placental function. Space restriction did not alter placental efficiency, as based on fetal wt/individual placental wt at either time point, but the ratio was greater at the later gestation. Further research is needed for the adaptation of the ovine placenta during rapid fetal growth.

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HYDROCORTISONE NORMALIZES HYPEROXIA-INDUCED PDE5 ACTIVITY IN LAMBS WITH PERSISTENT PULMONARY HYPERTENSION OF THE NEWBORN

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Background: In the pulmonary vasculature, cGMP produced by soluble guanylate cyclase leads to vasorelaxation. This pathway is regulated by a cGMP-dependent phosphodiesterase, PDE5. After birth, lambs with PPHN demonstrate increased PDE5 activity, decreased cGMP, and increased reactive oxygen species (ROS). **Objective:** To evaluate the effects of stress dose hydrocortisone (HC) on PDE5 in an ovine PPHN model and in fetal pulmonary artery smooth muscle cells (FPASMC). **Methods:** The ductus arteriosus was ligated in fetal lambs at 126 days gestation. PPHN lambs were delivered at 135 days and ventilated with 100% O2 for 24 hours. Four lambs received 3mg/kg of hydrocortisone followed by 1 mg/kg/dose $\times$ 2 doses (PPHN-HC), and six lambs were ventilated with O2 alone (PPHN-O2). FPASMC from normal lambs were exposed to 21% or 95% O2 and treated for 24 hours $\pm$ HC (100 nM). We measured PDE5 activity by a colorimetric assay, PDE5 protein by Western blot, and ROS by dihydroethidium fluorescence. Controls included fetal PPHN lambs and spontaneously breathing 1-day lambs. We measured cell proliferation using a colorimetric assay system and cell death using an LDH cytotoxicity kit. Significance was defined as p$<$0.05. **Results:** O_2 ventilation increased PDE5 activity in PPHN lambs by 13.1$\pm$1.5 fold compared to fetal PPHN controls. HC significantly decreased PDE5 activity by 70$\pm$3% in PA, and 66$\pm$12% in lung relative to PPHN-O2 lambs, but did not affect PDE5 expression. ROS were decreased by 54$\pm$18% in PPHN-HC lambs vs PPHN-O2 lambs. In isolated FPASMC, hyperoxia alone increased PDE5 activity by 201$\pm$72%. Incubation with HC decreased PDE5 activity by 87$\pm$13% relative to hyperoxia-treated cells without increasing cytotoxicity or affecting cell proliferation. **Conclusions:** Ventilation with 100% O_2 increases PDE5 activity and expression in PPHN lambs, and HC treatment decreases PDE5 activity and ROS generation. HC appears to have direct effects on the pulmonary vascular smooth muscle, as it is sufficient to decrease hyperoxia-induced PDE5 activity in isolated FPASMC. We speculate that HC may decrease PDE5 activity by attenuation of oxidant stress, thus improving pulmonary vascular reactivity.

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THE GENETIC ARCHITECTURE OF CONGENITAL HEART DISEASE

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Nkx2–5 mutations cause pleiotropic heart defects with incomplete penetrance. We hypothesized that cryptic genetic polymorphisms contribute to phenotypic variability as well as the sporadic nature of congenital heart disease. *Nkx2–5*$^{\pm}$ mice in the C57Bl/6 strain background frequently have heart defects, whereas Nkx2–5$^{\pm}$ F1 hybrids to FVB/N and A/J generally have normal hearts. An analysis of $>$3000 Nkx2–5$^{\pm}$ F2 progeny from F1 back- or intercrosses reveals that inbred strains polymorphisms can buffer or enhance the effect of *Nkx2–5* mutation on cardiac developmental pathways. All three strains carry ASD and VSD susceptibility alleles. C57Bl/6 carries polymorphisms that confer even greater susceptibility to muscular VSD, and A/J for ASD and atrioventricular septal defects. Genome-wide linkage analyses identify multiple chromosomal loci (LOD-scores 3–6) that influence the susceptibility of *Nkx2–5*$^{\pm}$ animals to ASD, VSD, or both, most of which contain no known cardiac developmental gene. Interestingly, the distribution of defects in the F2 intercrosses resembles human epidemiologic patterns, suggesting that modifier genes may have as great a role as causative mutations in the presentations of congenital heart disease. Alleles of modifier genes either buffer perturbations on cardiac development or steer the heart toward a particular type of defect in response to an insult such as Nkx2–5 mutation. We propose that during evolution stabilizing selection produced a diverse set of modifier genes to counter the effect of unpredictable insults on the developing heart. This genetic architecture intertwines the robustness of cardiac development for the majority with the manifestation of disease in a few.

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GENETIC AND ENVIRONMENTAL FACTORS CONTRIBUTE TOWARDS VERTEBRAL MALFORMATIONS

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Purpose: The purpose of this study was to identify genetic factors which contribute towards congenital vertebral malformation (CVM) development. **Methods:** DNA sequence analysis was performed in 6 spinal patterning genes including *PAX1*, *DLL3*, *WNT3A*, *SLC35A3*, *TBX6*, *T* (brachyury) in a cohort of 50 patients with CVM. **Results:** Mutations were identified in *PAX1*, *DLL3*, *WNT3A*, and T (brachyury). Of the sequence variants observed, the c.1013 C>T allele (A338V) of *T* displays the most compelling evidence of pathogenicity, as it is significantly associated with CVM ($P = 9.87\times 10^{-4}$, Fisher's exact test). A broad spectrum of phenotypic features in T were observed among the 3 patients with the c.1013 C>T mutation including sacral agenesis, Klippel Feil syndrome and multiple cervical and thoracic vertebral malformations. One of the three patients in our cohort with the c.1013 C>T mutation had prenatal exposure to maternal diabetes and valproic acid, both known to be associated with birth defects including CVM. **Conclusion:** The *T* gene is a transcription factor whose function is essential to mesodermal development. The presence of the c.1013 C>T allele in each affected subjects' phenotypically normal parent demonstrates this alteration is not sufficient alone to cause CVM. Moreover, the variety of CVM exhibited in our sample suggests other factors, unrelated to *T* genotype, determine the precise manner in which CVM susceptibility is realized. This represents supporting evidence for a"two hit" mechanism for vertebral malformations. We postulate that some forms of CVM may have"multi-hit" kinetics, as seen for various neoplastic disorders. The first event would be genetic in nature and the second event could be genetic or environmentally mediated. This hypothesis accounts for the lack of phenotypic expression in the parents harboring the c.1013 C>T mutation and explain why most CVM represent a sporadic occurrence. Knowledge of genetic susceptibility to environmental agents associated with CVM has the potential to positively impact population prevention efforts and in the long term contribute to reduce their associated morbidity and economic burden.

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BNIP3 BINDS TO POSH TO PROMOTE PRO-APOPTOTIC JNK SIGNALING IN CRDIOMYOCYTES

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Background: BNip3 is a pro-apoptotic BH3-only protein that does not require its BH3 domain to promote apoptosis. Apoptosis of cardiomyocytes (CMs) following hypoxia and acidosis (HA) requires BNip3. We previously determined the BNip3 homologue, Nix promotes JNK-depdendent apoptosis by binding to and stabilizing the JNK scaffold protein POSH. **Hypothesis:** We hypothesize that BNip3, like Nix, binds to POSH to promote its stabilization and subsequent JNK phosphorylation (activation) and apoptosis. **Methods**: BNip3 was flag-tagged and cloned into pcDNA and pCMS-EGFP vectors. The transmembrane domain was deleted by site-directed mutagenesis to produce the dominant-negative form (ΔTM). POSH and c-Jun vectors have been previously described. Cell survival was determined by counting GFP+ cells on successive days. Nuclear morphology was determined by Hoechst 33342 staining. Immuno-precipitation (IP) was performed using beads conjugated to specific monoclonal antibodies. siPOSH cells, which constitutively express an shRNA targeting POSH and express reduced levels of POSH protein have been previously described. **Results:** When co-expressed in HEK293 cells, BNip3 was IP'ed in the presence of myc-tagged POSH, but not with empty vector. BNip3, but not (ΔTM), increased p-JNK when expressed in HEK293 cells. Co-expression of dominant-negative c-Jun blocked apoptosis induced by BNip3 in neuronal PC12 cells. JNK activation and apoptosis were inhibited in siPOSH cells. Co-expression of BNip3 increased POSH protein levels, and this was not inhibited by the JNK inhibitor SP600125 (20μM). Finally, JNK inhibitor, though slightly toxic to untreated CMs, promoted increased survival of CMs following HA. **Conclusions:** BNip3, like its homologue Nix, directly promotes increased POSH levels, and JNK-dependent apoptosis. BNip3 requires both POSH and c-Jun to promote apoptosis. Blockade of the JNK pathway, downstream of BNip3, protects cardiomyocytes from HA, which strongly suggests that BNip3 promotes POSH-dependent apoptosis in this paradigm.

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MINOCYCLINE ATTENUATES CEREBELLAR MICROGLIAL ACTIVATION *IN VIVO* DURING BILIRUBIN ENCEPHALOPATHY IN HYPERBILIRUBINEMIC GUNN RAT PUPS

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Background: *In vitro* studies demonstrate that unconjugated bilirubin can induce astroglial pro-inflammatory cytokine release and suggest that microglial activation and neuroinflammation may be proximal events in the cascade leading to bilirubin induced neurotoxicity. Microglial activation may be a target for inhibition by minocycline, a 2nd generation tetracycline that has recently been shown to provide neuroprotection against bilirubin-induced CNS injury in the Gunn rat model of hyperbilirubinemia and kernicterus. **Objective:** Characterize *in vivo* the status of cerebellar microglia in both quiescent and activated forms in hyperbilirubinemic j/j Gunn rat pups during sulfadimethoxine-induced bilirubin encephalopathy and the effect of minocycline pre-treatment on same. **Design/Methods:** Homozygous j/j Gunn rat pups were studied during bilirubin encephalopathy induced by sulfadimethoxine (200 mg/kg ip) at peak postnatal hyperbilirubinemia (15–18 days old) in the presence or absence of minocycline pretreatment (50 mg/kg or saline ip, 15-min prior to sulfa). 24 hr post sulfa dosing cerebellar tissue was studied for microglial activation using Iba1 (Wako, USA) and CD11b (OX-42; Serotec) immunohistochemistry. **Results:** 24 hr post sulfa cerebellar microglia evidenced moderate to intense puntate immunostaining with both Iba1 and OX-42 and an amoeboid phenotype characteristic of activated microglia. Minocycline pretreatment was associated with decreased to absent immunostaining; when staining was observed it was more diffuse with ramified processes and stellate morphology characteristic of resting, quiescent microglia. **Conclusions:** We conclude that i) sulfa-induced bilirubin encephalopathy in hyperbilirubinemic Gunn rat pups is accompanied by immunohistochemical evidence of microglial activation and ii) microglia number and activation is attenuated by minocyline pretreatment. We speculate that microglial activation may be a critical target for minocycline neuroprotection against bilirubin neurotoxicity. [Supported by CHP Scientific Program, MWRI CTRA, and Mario Lemieux Centers for Patient Care and Research]

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IMPACT OF EARLY VERSUS LATE HYPOXIA ON THE IMMATURE FERRET BRAIN

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Background: Animal models are valuable for the investigation of preterm brain injury. Previous studies in immature animals have shown that chronic hypoxia leads to patterns of hypomyelination, decreased cortical volumes, ventriculomegaly, and periventricular white matter injury. The immature ferret brain displays cortical folding and myelination after postnatal day 10 (P10). The impact of chronic hypoxia on cerebral injury and development in this model is yet to be determined. **Methods:** We exposed immature ferrets to 10% hypoxia for a period of 10 days at 2 different times of initiation (P10 and P20), with 2 different times of sacrifice (P20 and P30). Animals were divided into 3 groups: hypoxia P10-P20, sacrificed at P20 (n=5), hypoxia P10-P20, sacrificed at P30 (n=7), and hypoxia P20-P30, sacrificed at P30 (n=6). We then compared all groups to normoxic control animals of the same ages. Brains were imaged ex vivo and analyzed using conventional and diffusion MRI techniques with high resolution acquisition (voxel size 250–300 μm^2). Histological analysis is currently being completed. **Results:** Differences in diffusion measures were found between control and experimental animals at both P20 and P30. At P20, relative anisotropy (RA) was increased in the anterior ($p=0.05$) and central ($p=0.001$) gray matter for hypoxic animals, and decreased in the caudate ($p=0.02$). At P30, for the early hypoxia model (P10–20), there was a persistent increase in anterior gray matter RA only ($p=0.12$). In contrast, the late hypoxia model (P20-P30) showed increases in RA within the anterior ($p=0.02$) and posterior ($p<0.001$) white matter. **Conclusions:** A variation in the pattern of diffusion MR measures within white and gray matter was seen from hypoxia at differing developmental ages in the ferret. This suggested greater impact on the immature gray matter from early hypoxia, which persisted. In contrast, late hypoxia appeared to accelerate white matter microstructural measures.

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DETERMINATION OF OXIDATIVE METABOLISM IN THE DEVELOPING RAT BRAIN USING *IN VIVO* ^{13}C NMR SPECTROSCOPY

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Background: Neuronal glucose oxidation via TCA cycle is the main energy source for the brain. *In vivo* ^{13}C NMR spectroscopy is a unique tool for understanding cerebral glucose metabolism non-invasively in the mature brain. Unfortunately, the demanding technical requirements of the method have precluded its application to the developing brain *in vivo*. **Objective:** To determine cerebral TCA cycle activity in the developing rat brain using *in vivo* ^{13}C NMR spectroscopy at 9.4T. **Methods:** 30-day-old rats (n=4; average wt 95 gm) were intubated and mechanically ventilated. One femoral artery and both femoral veins were cannulated. Rats were placed in a custom-built cradle that allowed precise placement inside the 9.4T magnet. Plasma glucose was rapidly increased using an intravenous bolus of 99%-enriched [1,6-$^{13}C_2$] glucose, followed by continuous infusion of 70%-enriched $^{13}C_2$ glucose. Blood samples were collected at 0, 5, 15, 30, 45, 60, 90 and 120 min by removing the cradle from the magnet to determine isotope enrichment in plasma. Incorporation of ^{13}C label into metabolites was determined in a 360 μl volume of the cerebral cortex using continuous *in vivo* ^{13}C NMR spectroscopy for 120 min underα-chloralose sedation, followed by *ex vivo* ^{13}C NMR spectroscopy of the brain extract. The incorporation of ^{13}C in the C4 and C3 positions of glutamate (Glu) over time was quantified using LCModel analysis. Neuronal TCA cycle activity (V_{TCA}) was determined using one-compartment mathematical modeling. **Results:** The step-wise infusion protocol rapidly achieved hyperglycemia, which was maintained during the study period [plasma glucose (mean±SEM): 17.8±1.7 mmol/l (320±31 mg/dl)]. The fractional enrichment of ^{13}C glucose in the plasma was 56±4%. There was no loss of shims with repeated removal and repositioning of the rats from the magnet. The incorporation of ^{13}C into GluC3 and GluC4 could be easily determined in the *in vivo* ^{13}C NMR spectra. The V_{TCA} determined using metabolic modeling was 0.67±0.13μmol/g/min. **Conclusions:** Determination of cerebral oxidative metabolism *in vivo* is feasible in developing rats using ^{13}C NMR spectroscopy. The V_{TCA} values are comparable to previous *ex vivo* reports. The method may be useful for the non-invasive assessment of altered energy metabolism in the developing brain (Funded by NICHD).

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LOWER COMPLEMENT C4 GENE COPY NUMBER IS A GENETIC RISK FACTOR FOR TYPE 1 DIABETES MELLITUS IN PEDIATRIC PATIENTS

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In recent years, significant progress has been made in understanding the genetic susceptibility to type 1 diabetes (T1D). Complement component *C4* gene, which plays a role in autoimmunity and adaptive defense, maps to the Major Histocompatibility Complex class III region of chromosome 6, which is one of the major contributory determinants to genetic susceptibility of T1D. The aim of this study was to investigate differences in *C4* gene copy number variation (CNV) between T1D pediatric subjects and controls (CON). Subjects were recruited from Nationwide Children's Hospital in Columbus, Ohio. We investigated 96 pediatric T1D patients of European ancestry, their first-degree relatives, and 195 race-matched CON. T1D subjects ranged from 14 months to 16 years (6.80 ± 3.67 years, mean ± SD). Southern blot analyses were used to determine *C4A* and *C4B* CNV and the RCCX modular variations. *C4* protein allotypes were determined using EDTA-plasma by immunofixation. Total *C4* CNV varied from 2 to 6, and both *C4A* and *C4B* CNV varied from 0 to 4. Total *C4* CNV was significantly lower in T1D patients than CON (T1D 3.46 ± 0.09, mean ± SE; CON 3.82 ± 0.04, $p=0.00005$). Low *C4A* CNV was also a genetic risk for T1D susceptibility (T1D 1.81 ± 0.09; CON 2.1 ± 0.05, $p=0.0041$). T1D subjects were more likely to have a monomodular RCCX structure with a single short *C4B* gene (mono-S) which is in strong linkage disequilibrium with HLA DRB1*0301 (T1D 40%; CON 19%, $p<0.00009$). Among subjects with the presence of a mono-S gene, total *C4* CNV also notably lower in T1D patients (T1D 2.72 ± 0.07; CON 3.08 ± 0.07, $p=0.0006$). Our findings suggest that decreased total *C4* CNV and decreased *C4A* CNV are genetic risk factors for T1D susceptibility.

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ELECTRICAL PACING OF RAT ENGINEERED CARDIAC TISSUE (ECT) IMPROVES CARDIAC FUNCTION BY INCREASING CARDIOMYOCYTE DIFFERENTIATION AND SURVIVAL

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Rat ventricular cardiomyocytes can be grown in a three-dimensional matrix to form contracting cylindrical units called engineered cardiac tissues (ECTs). ECTs hold great promise for providing platform in which to study cardiac development, cardiomyocyte survival and regeneration, the functional effect of mutations and applications in gene therapy. To optimize culturing conditions, we found that electrical pacing, marginally above the intrinsic automaticity of the cardiomyocytes, result in a two-fold increase in twitch force, when compared to non-paced ECTs. We hypothesized that electrical pacing improves contractile function of ECTs by promoting cardiomyocyte survival and differentiation without altering Ca^{2+} handling. In the present study we concurrently measured force generation and Ca^{2+} transients in both paced and non-paced ECTs. While pacing increased twitch force by about two-fold. Ca^{2+} transients were not significantly different between the two groups. Histological analysis revealed that mature cardiomyocytes were predominantly found close to the surface of ECTs, and that mature cardiomyocytes were more prevalent in paced ECTs. Furthermore, quantitative RT-PCR and western blot analysis showed that paced ECTs have elevated mRNA and protein levels of cardiomyocyte-specific markers (cMyBP-C and Cx43), while expression levels of several housekeeping genes were unchanged. Taken together, these findings indicate that electrical pacing improves cardiomyocyte differentiation and survival, leading to increased force production without affecting Ca^{2+} handling.*$p<0.05$ *vs.* non-paced, Arbitrary Units (AU)

Mean ± SE	Non-Paced	Paced
Twitch Force (mN)	0.42 ± 0.13	1.00 ± 0.15*
Ca^{2+}Transient (nM)	90.5 ± 14.5	63.3 ± 13.0
GADPH RNA (AU)	60.4 ± 5.9	67,2 ± 2,4
Cx43 RNA (AU)	19.4 ± 1.8	35.1 ± 1.4*
cMyBP-C RNA (AU)	39.2 ± 2.6	63.5 ± 6.6*
Cx43 protein (AU)	69.3 ± 7.0	122.3 ± 8.1*
cMyBP-C protein (AU)	67.8 ± 9.2	116.5 ± 4.3*

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UREAPLASMA, A COMMON PATHOGEN ASSOCIATED WITH PRE-TERM LABOR, CAUSES A DISTINCT CYTOKINE EXPRESSION PATTERN IN HUMAN EPITHELIAL CELLS

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Background: Pre-term labor (PTL), and its associated infant mortality, is on the rise and it is important to understand its etiology. One major cause of PTL is infection and inflammation. *Ureaplasma* sp . is a common pathogen found in the female urogenital tract and has been associated with PTL. Single nucleotide polymorphisms (SNPs) in genes for Toll-like Receptors (TLRs) 2 and 4 have been shown to occur at a higher frequency in individuals with chronic inflammation disorders as well as in women with a history of PTL. It has recently been shown that *Ureaplasma* sp. participates in inflammation through the TLR1/TLR2 and TLR6/TLR2 heterodimers. This process may contribute to inflammation induced PTL. **Objective:** We sought to determine the cytokine expression pattern in human epithelial cells in response to *Ureaplasma* sp. exposure and to develop a cell culture based assay to study *Ureaplasma* sp . inflammation mechanisms that may affect the outcome of PTL. **Methods:** Human epithelial A549 cells (ATCC) were exposed to either *Ureaplasma* sp. or *E coli* endotoxin, lipopolysacharide (LPS) in a dose- and time-dependent manner. RNA was isolated and used as a probe of cytokine expression using the Oligo GEArray Human Common Cytokine Microarray (SuperArray). Targets were considered significant if they had a >1.5 fold difference and a P-value <.05. **Results:** In cells treated with either *Ureaplasma* sp. or LPS, significant up-regulation was observed in interleukin 8, which is a strong pro-inflammatory cytokine. In cells treated with *Ureaplasma* sp., 20 different cytokines were down-regulated including Interleukins, Tumor Necrosis Factor family members, and TGFβ. However, there was no down-regulation of cytokines in cells exposed to LPS. **Conclusions:** In a cultured cell model for inflammation, exposure to *Ureaplasma* sp., which activates the TLR1/2, TLR2/6, and TLR4 pathways, induced a cytokine expression pattern distinctly different from the pattern observed upon exposure to LPS, which acts through TLR1/2 and TLR4. Understanding the mechanisms of *Ureaplasma* sp. inflammation will yield a better of how TLRs contribute to PTL. This will lead to advances in preventative health care and improvement in clinical outcome in cases PTL.

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GENETIC ADMIXTURE AND PARENTAL AFFECTS ON PRETERM BIRTH IN ARGENTINA

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Background: Preterm birth (PTB) and its complications are a significant cause of worldwide infant mortality and a global public health challenge. Racial differences exist in rates of PTB even when controlling for socioeconomic status and education. The maternal and paternal genetic contribution to PTB has yet to be fully elucidated. **Objective:** Determination of genetic racial admixture and parental specific affects on PTB in Argentina through genotype analysis and sequencing of premature infants and their families. **Design/Methods:** Three maternal-fetal centers in Argentina collected study samples from November 2005 to July 2008, including saliva and whole, cord, and placental blood. Cases consisted of infants < 37 weeks of gestation. Candidate genes were selected through review of previous studies of PTB. Genotyping was carried out using TaqMan assay. Analysis was completed for parenteral transmissions using the Transmission Disequalibrium Test (TDT). Mitochondrial DNA (mtDNA) was sequenced in Hypervariable Regions I and II. Y-chromosome sequencing was performed for DYS 19 and DYS 199 ancestry markers. **Results:** Genotyping performed on 1473 individuals comprising 314 case families. 36 genes and 62 SNPs evaluated. Statistically significant associations in paternal transmission for PTB seen in defensin 6 (DEFA6: $p = 0.004$) and tumor necrosis factor receptor-associated factor 2 (TRAF2: $p = 0.005$). Significant associations for maternal transmission seen in the progesterone receptor (PGR: $p = 0.03$). MtDNA sequencing of 50 males and 50 females from a birthing center in northwest Argentina (where >90% of study samples were obtained) showed 89 individuals haplotyped into Amerindian mtDNA haplogroups A, B, C, D, or X. Y-chromosome sequencing of those same 50 males then revealed only 2 individuals were carriers of the Amerindian haplotypes DYS19*13/DYS199 T. **Conclusions:** An extremely asymmetric pattern of genetic racial admixture exists in our study population that is higher then previously reported studies from Argentina and South America. This result highlights possible genetic consequences for divergent genome admixture between Amerindian and European ancestral populations and a role for further investigation of parental specific effects on PTB.

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HEMOGLOBIN GENE EXPRESSION IN THE LUNG IS REDUCED IN IDIOPATHIC PNEUMONIA SYNDROME (IPS)

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IPS is a significant cause of morbidity and mortality post-hematopoietic stem cell transplant. We have shown that hemoglobin (Hb) genes are expressed in type 2 alveolar epithelial cells (JBC 281:5668) but expression in the context of lung injury has not been studied, nor is the role of lung-expressed hemoglobin known. To begin to understand the role of lung-Hb in the context of lung injury we used our established mouse allogeneic bone marrow transplant (BMT) model of acute IPS (JCI, 100:1015) characterized by host macrophage and donor T cell infiltrates, inflammatory mediators, decreased glutathione, diffuse alveolar damage, decreased compliance and total lung capacity. B6 mice were lethally conditioned (Cy/TBI) and transplanted with allogeneic B10.BR BM and 15 million T cells to cause IPS. We analyzed day 7 post-BMT lungs by in situ hybridization (ISH) and real-time RT-qPCR for Hb chains alpha and beta. Indeed, both alpha and beta Hb -expressing cells were seen by ISH in a pattern consistent with ATII cells (corners of alveolar septae). However, mice with IPS had fewer and less intensely stained cells in the lungs than BMT control mice. Compared to BMT controls, mice with IPS had a 14.4-fold decrease in lung mRNA for Hb-alpha and 15.3-fold decrease for Hb-beta by qPCR analysis (n=6/grp). Given the ability of the heme-globins to reduce nitrite in hypoxic conditions to generate bioavailable NO and increase vasodilation, our novel findings have wide implications in explaining the pathophysiology of the lung during post-BMT lung injury.

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BLOOD OUTGROWTH ENDOTHELIAL CELL BASED ENOS GENE THERAPY ATTENUATES MONOCROTALINE INDUCED PULMONARY HYPERTENSION IN RATS

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Introduction: Blood Outgrowth Endothelial Cells(BOECs) are a robust endothelial cell lineage cultured from peripheral blood and may serve as an effective autologous vehicle for gene delivery. BOEC-eNOS(endothelial nitric oxide synthase) gene therapy may modulate the pulmonary vascular bed and attenuate pulmonary hypertension. **Methods and Results:** We developed rat BOECs from F344 rats and genetically altered them to over express human eNOS with green fluorescent protein(r-BOEC/eNOS-GFP). A control cell population (rBOEC/GFP) was also constructed. Nitric Oxide (NO) production by rBOEC/eNOS-GFP was 1.8 fold above normal rat BOECs. rBOEC/eNOS-GFP also demonstrated greater tube formation in matrigel compared to the rBOEC/GFP; suggesting enhanced angiogenic potential. In vivo testing was performed in a monocrotaline (MCT 75mg/kg, intraperitoneal) induced pulmonary hypertension rat model. Three days after MCT injection test group 1 rats received rBOEC/eNOS-GFP cells ($1x10^6$) intravenously, control group 2 received rBOEC/GFP cells and control group 3 received no cells. Twenty-one days post cell delivery, Right Ventricular Systolic Pressure (RVSP), RV: LV+Septum ratio and total Serum Nitrate+Nitrite(NOx) of each animal were measured. The RVSP of group 1 rats (35.6mm Hg, $p<0.005$) was significantly lower than group 2(RVSP 48.3mm Hg) and group 3 (RVSP 49.8mmHg). The RV: LV+Septum ratio of the group 1(0.38, $p<0.05$) was lower compared to group 2(0.45) and group 3(0.46). Serum NOx of group 1(0.29nM/ul of serum, $p< 0.05$) was elevated compared to group 2(0.25nM/ul of serum) and group 3(0.19nM/ul of serum). The rBOEC/eNOS-GFP recipient animals demonstrated diminished arteriolar muscularization and preservation of alveolar sacs and lung architecture compared to MCT animals. Similar findings were obtained in a reversal model of established pulmonary hypertension. **Conclusion:** These findings suggest that rBOEC/eNOS-GFP significantly prevents MCT induced pulmonary hypertension in rats, and that BOECs may prove an effective vehicle for gene therapy.

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THE IMPACT OF SECOND HAND SMOKE EXPOSURE ON CARDIOVASCULAR FUNCTION IN CHILDREN IS AGE DEPENDENT

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Background: Links between second hand smoke (SHS) exposure, cardiovascular disease (CVD) and death in adults are well established, but little is known about the impact of SHS exposure on cardiovascular status in children. Many forms of chronic CVD originate in childhood and young adulthood, and at least one quarter of children in the United States are exposed to SHS. **Objectives:** A) To investigate the effect of SHS exposure on inflammation, endothelial stress/function, and prevalence of endothelial progenitor cells (EPCs) in toddlers (2–5yrs) and adolescents (9–18yrs); and B) To further define the impact of childhood obesity on these relationships. **Methods:** We recruited healthy subjects through public advertising and a primary care clinic affiliated with Nationwide Children's Hospital (NCH). Obese youth were recruited at the NCH Center for Healthy Weight and Nutrition. All ages had serum drawn for markers of inflammation (high sensitivity C-reactive protein [hs-CRP] and adiponectin, an anti-inflammatory adipokine), endothelial stress (soluble intercellular adhesion molecule [s-ICAM]), and endothelial repair (endothelial progenitor cells, determined by AC133+/CD34+/CD45- cells [FACS Calibur flow cytometer]). Endothelial function was measured via venous occlusion plethysmography (VOP) on the older age group. SHS exposure was determined by both a questionnaire and hair sampling for nicotine content. All relationships reported are significant at $p<0.05$. **Results:** We studied 52 toddlers ages 2–5, 55 youth ages 9–14, and 52 obese youth & adolescents ages 9–18. Toddlers had higher hair nicotine compared to adolescents, despite having similar reported home exposure to SHS (12.89 vs. 2.47 ng/mg, $p = .04$). In toddlers, there was a positive relationship between SHS exposure and endothelial stress (sICAM), which was magnified among obese toddlers. A negative relationship between SHS exposure and EPC prevalence was also found in toddlers. In obese adolescents, SHS exposed subjects had higher sICAM levels than non-exposed subjects. This interaction was present even after controlling for age, race, income, inflammation, and blood pressure. **Conclusions:** These data suggest that special populations of children may suffer greater consequences of SHS exposure. Toddlers appear to receive a 'higher dose' of SHS exposure with similar exposure histories relative to adolescents. Toddlers also have a greater degree of SHS-related endothelial stress and decreased EPCs in comparison to adolescents. In both age groups obesity enhances the cardiovascular toxicities of SHS exposure.

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EFFECTS OF PRENATAL INFLAMMATION ON CARDIAC FUNCTION AND REDOX STATUS IN ADULTHOOD

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Background: Cardiovascular disease in adulthood has been strongly associated with prenatal environment and preterm birth, but mechanisms underlying this fetal imprinting of adult disease are not defined. Recent studies suggest that the maternal innate immune response and attendant inflammation are important contributors to preterm birth, but the roles of inflammation and oxidative stress in prenatally programmed adult cardiac dysfunction are not known. **Objective:** To test the hypothesis that prenatal (maternal) exposure of low-dose lipopolysaccharide (LPS) impairs cardiac function and exacerbates cardiac stress responses in offspring during adulthood. **Design/Methods:** Pregnant C57BL/6J mice were treated with LPS (1.25 mcg/mouse/daily) i.p. or vehicle for 4 days starting at gestation E15. Male offspring were weaned and housed normally to adulthood (8wks). Basal cardiac performance and responses to inflammatory stress (LPS 1.0 mg/kg, ip) were evaluated by non-invasive echocardiography. Offspring were then sacrificed and cardiac tissues collected for immunohistochemistry and digital image analysis. **Results:** Compared to controls, offspring of LPS-treated dams had both lower birth weight (1.144 vs. 1.247g, $p<0.05$) and a lower postnatal growth rate (body weight at 8w: 22.1 vs. 24.6g, $p<0.05$). Prenatal LPS-exposed offspring also had a marked reduction of cardiac output (CO, 22.5 vs. 24.3 ml/min, $p<0.05$) and stroke volume (SV, 44.8 vs. 52.4 mcl, $p<0.05$) at 8wks of age, suggesting impaired cardiac contractility at baseline. This impairment was exacerbated after an acute LPS challenge, as offspring with prenatal LPS exposure showed more substantial reductions in CO, SV and left ventricular fractional shortening (LVFS%) than control animals ($p<0.05$), suggesting decreased cardiac contractile reserves. Immunohistochemistry and digital image analysis demonstrated no significant differences in toll-like receptor 4 (TLR-4), but a greater prevalence of protein 3-nitrotyrosine (biomarker of reactive nitrogen species) and nitric oxide synthase-2 (NOS-2) in the myocardium of prenatal LPS-exposed animals at adulthood. **Conclusions:** These data parallel known human phenomena and demonstrate that murine prenatal exposure to LPS and attendant maternal inflammation significantly impacts baseline cardiac function, cardiac contractile reserves, and myocardial nitration and oxidation status in offspring during adulthood. Further mechanistic studies of cardiac innate immune responses in relation to preterm birth-related adult cardiovascular disease risks, using this model and human studies, are warranted.

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CYCLIC STRETCH INCREASES CYTOSOLIC OXIDATIVE STRESS AND SOLUBLE GUANYLATE CYLCASE EXPRESSION IN FETAL PULMONARY ARTERY SMOOTH MUSCLE CELLS

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Background: In the pulmonary microvasculature, mechanical forces such as shear stress from circulating blood and stretch from respiratory cycles change intracellular signaling leading to changes in vascular tone, production of vasoactive molecules and vascular remodeling. Nitric oxide, a well studied vasoactive molecule in the pulmonary vasculature, is released by endothelial cells to activate soluble guanylate cyclase (sGC) to form cGMP, ultimately causing vasorelaxation. Reactive oxygen species (ROS) has been shown to trigger vasoconstriction, but little is known about whether mechanical forces such as cyclic stretch play a role in this process. **Objective:** To determine the effects of cyclic stretch on ROS and sGC expression and activity in the neonatal pulmonary vasculature. **Methods:** Fetal pulmonary artery smooth muscle cells (FPASMC) were isolated from fifth generation resistance pulmonary arteries of fetal lambs and were transduced with a recombinant adenovirus to express RoGFP, at ratiometric fluorescent protein thiol redox sensor targeted to the cytosol (RoGFP) or the mitochondrial matrix (mito-roGFP). FPASMC were then subjected to cyclic stretch for 24 hrs. Multilaser flow cytometry was used to measure the fluorescence, and thus oxidative stress, of these cells. Additional FPASMC were subjected to cyclic stretch for 24 hr, sGCβ expression was measured by Western blot, normalized toβ-actin. **Results:** FPASMC transduced with RoGFP and subjected to stretch for 24 hr showed a significant increase in cytosolic oxidative stress (stretch vs. static: 69.2% vs. 29.8% oxidized, $p<0.05$). There was no significant difference in mitochondrial oxidative stress with stretch. When exposed to stretch, sGCβ protein expression is increased relative to static FPASMC (2.47-fold, $p<0.05$). **Conclusion:** In FPASMC, mechanical forces such as cyclic stretch increase oxidative stress in the cytosol, but not the mitochondria, and increase sGCβ expression. This is in contrast to our prior work showing that hyperoxia increases mitochondrial but not cytosolic ROS, and decreases sGCβ expression. In contrast to our findings, ROS has been shown to downregulate sGCβ expression and activity in the adult systemic vasculature. Our findings suggest that the source of ROS may dictate differential downstream effects. Accordingly, sGC activators could provide another future avenue to treat infants with pulmonary hypertension.

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cMYBP-C NULL MOUSE HEARTS DEMONSTRATE UPREGULATION IN METABOLIC GENES ON POST-NATAL DAY 10 PRIOR TO DEVELOPMENT OF HYPERTROPHY

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Background: Cardiac myosin binding protein c (cMYBP-C) is a key sarcomeric regulatory protein. Mutations in *MYBPC* cause hypertrophic cardiomyopathy. cMYBP-C$^{-/-}$ mice demonstrate accelerated contractile kinetics implying increased basal energy demand. Hypertrophy develops early in this model raising the question of whether metabolic remodeling occurs prior to or after hypertrophic compensation. **Objective:** We evaluated the hypothesis that cMYBP-C$^{-/-}$ mice demonstrate upregulation of genes involved in energy metabolism earlier than genes involved in the hypertrophic response, indicating an underlying increased energy demand in the cMYBP-C$^{-/-}$ myocardium. **Design/Methods:** Total RNA was isolated from left ventricles from 10 day and 5 week old cMYBP-C$^{-/-}$ and matched wild-type (WT) mice. qRT-PCR was performed using TaqMan assay and primers to 48 genes involved in regulation of energy metabolism, sarcomeric function, and hypertrophic signaling. Expression was expressed as a percentage of house-keeping gene expression. **Results:** 10-day hearts from WT and cMYBPC$^{-/-}$ had similar weights and total RNA content, while 5 week cMYBPC$^{-/-}$ hearts had increased weight and RNA content ($n=3$ per group, $P<0.05$). At post-natal day 3, cMYBP-C$^{-/-}$ hearts demonstrated significantly increased transcription of genes involved in metabolism (GLUT1, creatine kinase, aldolase C), while sarcomeric and hypertrophic signaling transcripts were not elevated. At 5 weeks, metabolic gene transcripts were not different between groups, however sarcomeric genes (beta myosin heavy chain, alpha actinin, myosin) were increased ($P<0.005$). Also increased were hypertrophic signaling transcripts (ANP, BNP, c-myc). Interestingly, none of the metabolic genes remained increased at the 5 week timepoint. **Conclusions:** Early cMYBP-C$^{-/-}$ myocardium demonstrates activation of metabolic genes independent of the hypertrophic response. By the time the myocardium has developed hypertrophy, metabolic gene transcription has returned to levels found in WT myocardium. Metabolic remodeling may be an early compensatory mechanism in cMYBP-C$^{-/-}$ hearts. How this might relate to the development of the hypertrophic phenotype remains to be explored.

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ROLE OF PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR BINDING PROTEIN (PPARBP) IN THE PROLIFERATION OF HUMAN PULMONARY ARTERY SMOOTH MUSCLE CELLS IN HYPOXIA

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Nuclear Receptors such as Peroxisome Proliferator Activated Receptors (PPARs) constitute a large family of ligand activated transcription factors which work in concert with other proteins (coactivators) to regulate the expression of specific genes thereby controlling cellular proliferation, development, homeostasis and metabolism. Disruption of the gene encoding for the transcription coactivator peroxisome proliferator-activated receptor (PPAR)-binding protein (PPARBP/PBP/TRAP220/DRIP205/Med1) in the mouse results in embryonic lethality indicating that PPARBP is an essential and non-redundant coactivator. Recent studies have established that Pulmonary Hypertension (PH) in humans is associated with reduced PPAR gamma(PPARg) and PPAR alpha(PPARa) expression and that their specific ligands can attenuate the development of PH. Chronic hypoxia is an important contributing factor for the development of pulmonary hypertension. Hypoxia triggers pulmonary artery smooth muscle cell (PASMC) remodeling from a differentiated state to a proliferative state. However the role of PPARBP in human pulmonary artery smooth muscle cells (HPASMCs) proliferation in hypoxia is unknown. The objective of the study was to characterize PPARBP regulation in HPASMC response to hypoxia, affecting cell proliferation. This is important in the understanding the molecular mechanisms triggered by cell exposure to low oxygen tension in PH. HPASMCs were exposed to hypoxia (3% oxygen) for 24hrs with/without PPARa and PPARg agonists. Cells were processed for mRNA and protein studies. In the presence of PPARa agonist Wy-14,643 and PPARg agonist, rosiglitazone PPARBP mRNA and protein levels were decreased in the cells exposed to 24h hypoxia. Cell proliferation marker PCNA (Proliferating Cell Nuclear Antigen) showed increased levels after 24h hypoxia. Our studies suggest that PPARBP plays a role in hypoxia induced smooth muscle cell proliferation. This work was supported by RO1 HL075187–05A1.

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HYPEROXIA INDUCES ENDOPLASMIC RETICULUM (ER) STRESS IN NEONATAL RAT LUNGS AND CULTURED LUNG EPITHELIAL CELLS

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Purpose of Study: Conditions interfering with homeostasis of the ER are collectively called ER stress. Cells containing stressed ER demonstrate activation of serial signal transduction cascades that are collectively named the unfolded protein response (UPR). Prolonged oxygen therapy induces cell death and causes lung injury in premature infants, which play critical roles in the development of chronic lung disease in prematurity. Accumulating evidence has suggested that ER stress can induce programmed cell death (apoptosis). The aim of this study was to investigate if hyperoxia could induce ER stress and activate UPR pathway in neonatal rat lungs and cultured lung epithelial cells. **Methods:** Rats at 4 days of age were treated with room air and 95% oxygen for 1, 3, and 10 days. Cultured mouse lung epithelial cells were exposed to room air and 95% oxygen. Gene and protein expression was measured by real-time PCR, immunohistochemistry and Western blot. **Results:** BiP/GRP78 (immunoglobulin binding protein/glucose regulated protein 78 KD) is a chaperone protein in the ER lumen. It binds to UPR sensor proteins such as PKR-like ER kinase (PERK) and regulates UPR. Immunohistochemistry showed that the specific BiP/GPR78 staining was present in the cytoplasm of bronchial and alveolar epithelial cells in neonatal rat lungs. The staining was significantly increased in neonatal rat lungs treated with hyperoxia for 10 days. Activating transcription factor 4 (ATF4) is a downstream mediator of PERK and regulates C/EBP homologue protein (CHOP). ATF4 mRNA in cultured mouse lung epithelial cells was elevated after oxygen exposure for 16 and 24 hours. CHOP is a key mediator in ER stress-induced apoptosis. CHOP protein in neonatal rat lungs was expressed in a minimal level under the normoxic condition; however, its expression was increased by 2.8 fold and 2.5 fold after hyperoxic exposure for 3 and 10 days. Immunohistochemistry showed that specific CHOP staining in neonatal rat lungs was mainly localized in bronchial epithelial cells under the normoxic condition and was significantly augmented in both bronchial and alveolar epithelial cells after hyperoxic exposure for 10 days. **Conclusions:** These results demonstrate that prolonged hyperoxia can induce ER stress and activate UPR in the neonatal rat lungs and cultured lung epithelial cells. We speculate that ER stress-induced apoptosis might play an important role in the hyperoxia-induced lung injury and development of chronic lung disease in prematurity.

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MOUSE ENGINEERED CARDIAC TISSUE: A NOVEL PLATFORM FOR STUDYING THE PHYSIOLOGICAL EFFECTS OF HCM-CAUSING MUTATIONS ON CARDIAC FUNCTION

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Background: Hypertrophic cardiomyopathy (HCM) is an autosomal dominantly inherited cardiac disease that affects about 1 in 500 people characterized by left ventricular hypertrophy (LVH), and an increased risk of sudden cardiac death and heart failure. Mutations in cardiac myosin binding protein C (cMyBPC), a thick filament protein known to regulated cardiac contraction, is amongst the most prevalent causes of HCM. Certain mutations in cMyBPC cause severe form of HCM presenting in childhood. Ablation of cMyBPC in mouse models (cMyBPC-KO) cause LVH, diastolic dysfunction, as well as accelerated rates of force production and relaxation. Objective: To establish the usefulness of novel neonatal mouse cardiomyocyte 3D engineered cardiac tissue (ECTs) as model system to study the physiological effects of cMyBPC ablation. **Methods:** We generated ECTs from both one day old wild type (WT) and cMyBPC-KO pups. These ECTs were subjected to physiological analysis to assess contractile performance. **Results:** Similar to intact papillary muscle from cMyBPC-KO mice, maximal force production was marginally higher in cMyBPC-KO ECTs than in WT ECTs (0.760 ± 0.18 vs 0.413 ± 0.120mN when paced at 1Hz), rate of force production was accelerated (0.78 ± 0.003 vs 0.91 ± 0.005s when paced at 1Hz), and the rate of relaxation accelerated in the (0.063 ± 0.004 vs 0.082 ± 0.011ms). **Conclusions**: The kinetics of force production and relaxation in cMyBPC-ECTs are in agreement with that obtained from papillary muscles of cMyBPC-KO mice, indicating that ECTs mimic the physiological properties of intact tissue. These data support the feasibility of using the ECT model to study important contractile function and physiology in cardiomyocytes derived from neonatal mouse models of important cardiac disease before significant compensatory mechanisms have occurred.

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NEONATAL PMN EXHIBIT A DI2FFERENTIAL RESPONSE TO TNF-RELATED APOPTOSIS INDUCING LIGAND (TRAIL)-MEDIATED INFLAMMATORY AND APOPTOTIC STIMULI

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Background: We previously reported delayed apoptosis in neonatal (CB) neutrophils (PMN) compared with adult (AD) PMN; however, the contributory mechanisms remain enigmatic. The membrane-bound form of TNF-related apoptosis inducing ligand (TRAIL/Apo-2 ligand) can induce apoptosis of immune cells under selective conditions through interaction with specific membrane bound TRAIL receptors, although its biologic role in PMN is unclear. Our recent observations of greater surface TRAIL expression on neonatal vs. AD PMN lead us to hypothesize that age-specific expression of TRAIL in CB and AD PMN might contribute to differential longevity during inflammation. **Objective & Methods:** PMN were isolated from the umbilical venous cord blood of term placentas and for controls, from healthy AD volunteers. Basal and stimulated (TNF-α, 10 ng/mL; IFN-γ, 100 U) expression of TRAIL and TRAIL receptors (-R1, -R2, -R3, and -R4) was determined in paired CB and AD PMN by flow cytometry. SuperKiller TRAIL (SKT; 0–1 ng/mL) was added to PMN cultures to induce apoptosis. **Results:** We determined that CB and AD PMN express all TRAIL receptors (TR), and expression of decoy receptors (TR-3, TR-4) was markedly increased compared to death receptors (TR-1, TR-2). To begin to dissect TRAIL-mediated apoptotic mechanisms, PMN were incubated with inflammatory cytokines. TNF-α consistently increased surface TRAIL expression in CB but not AD PMN, while IFN-γ increased surface TRAIL and decreased intracellular TRAIL on AD but not CB PMN. In a preliminary study, SKT, a TRAIL agonist, induced PMN apoptosis in a dose-dependent manner, with a greater effect on AD PMN ($\sim$50% survival decrease) *vs.* CB PMN ($\sim$20%). **Conclusion:** We observed robust expression of TRAIL decoy receptors on both CB and AD PMN. We also determined differential TRAIL expression on CB and AD PMN in response to stimulation with TNF-α and IFN-γ. Furthermore, preliminary data suggest a greater apoptotic TRAIL-mediated response in AD vs. CB PMN. These data suggest that TRAIL contributes to disparate apoptotic mechanisms in CB and AD PMN during inflammation.

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ROLE OF RED CELL GLUCOSE METABOLISM IN SICKLE CELL DISEASE

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Background: There is broad phenotype variation in populations of patients with sickle cell disease (SCD), which may be due to energy metabolism in sickle red blood cells (RBCs). Inefficient energy metabolism in sickle RBCs disables endogenous antioxidant systems and enables hemolysis. RBC thiol based antioxidant defense is maintained by regenerating glutathione (GSH) from glutathione disulfide (GSSG) via glucose metabolism. Glucose is metabolized via the Embden-Meyerhof Pathway (EMP) or the Hexose Monophosphate Pathway (HMP), the latter of which generates the reducing equivalents for GSH recycling. Alternation between these two pathways is linked to oxygen gradients through competitive binding (between deoxy hemoglobin (Hb) and key EMP enzymes) to the RBC membrane protein Band 3. Since sickle Hb (HbS) has increased affinity for Band 3, we speculate that the HMP is constrained in individuals with SCD, creating a 'functional' G6PD deficiency thereby increasing their vulnerability to oxidative stress and ultimately causing hemolysis. **Objective:** To determine the coupling between O_2 gradients, the mode of glycolysis, and the ability of sickle RBCs to recycle GSH recycling during oxidative loading conditions. **Methods:** Sickle RBCs were collected from pediatric hematology patients at St. Louis Children's Hospital in St. Louis, MO. Sickle RBCs were oxygenated in a rotating tonometer at 21% O2 and 7% CO2 for 25 minutes and subsequently exposed to oxidative stress using hypoxanthine (HX)/xanthine oxidase (XO). NADPH and GSH redox ratios were measured spectophotometrically. The extent of RBC membrane oxidative modification was gauged by quantification of reduced thiol levels. **Results:** At baseline, sickle RBCs had significantly lower levels of total glutathione (730.4 ± 124.3 *vs.* 1082.4 ± 84.9), higher levels of oxidized glutathione (4.1 ± 5.0 *vs.* 0.6 ± 0.4) and higher reduction potential (-216.5 ± 25.4 *vs.* -286.4 ± 14.6) compared to normal healthy individuals (SCD individuals *vs* healthy individuals, respectively; $P<0.05$). Following exposure to HX/XO, SICKLE RBCs were not as able to recycle reduced glutathione when compared to controls. **Discussion:** We demonstrated that sickle RBCs have a higher oxidized redox state at baseline, suffer from total glutathione depletion, and have constrained ability to recycle reducing equivalents when subjected to oxidative stress. These data are consistent with our hypothesis that dysregulated glucose metabolism in sickle RBCs results from dysfunctional binding between Band 3 and HbS. The mechanism of abnormal glucose metabolism potentially leading to hemolysis is being explored further.

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EXUBERANT HYPEROSSIFICATION AND SKELETAL OVERGROWTH CAUSED BY A GAIN OF FUNCTION MUTATION IN THE NOVEL *RPZ* GENE

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Purpose: Mechanisms that regulate vertebrate skeletal development and homeostasis are protean and incompletely understood. The zebrafish mutant *rapunzel* has heterozygous defects in bone development, resulting in a exuberant skeletal hyperossification, thus identification of the genetic lesion underlying *rapunzel* might provide insight into the molecular basis of skeletogenesis. **Methods and Summary of Results:** *rapunzel* was identified through an ENU-based forward genetic screen. Genetic mapping was initially performed using centromere linkage analysis, and positional cloning using simple sequence polymorphisms identified the mutation. *In situ* hybridization (ISH) and qPCR were used to characterize gene expression in wild type and mutant embryos. Histomorphometry, including Alcian blue, Alizarin red, Sirius red and calcein staining, along with bone densitometry and microCT were used to characterize the *rapunzel* skeletal phenotype. The zebrafish mutant *rapunzel* has heterozygous defects in skeletogenesis, resulting in overgrowth of both the fin ray skeleton and hyperossification of the axial skeleton. We mapped *rapunzel* to a 46 kb critical region on chromosome 16. Then using a combination of genomic and cDNA sequencing, we identified a missense mutation (T269A) in the novel *rpz* gene, resulting a non-conserved amino acid substitution (V90E). Using the *rapunzel* homozygous embryonic phenotype as a surrogate, we showed that morpholino knockdown of *rpz* suppresses the homozygous lethal embryonic phenotype, demonstrating that rpz^{T269A} is a gain of function allele. ISH and qPCR demonstrate that *rpz* expression precedes the formation of definitive skeletal elements, suggesting a non-cell autonomous role for *rpz* in skeletogenesis. Differences in skeletal morphology (e.g. decreased scalloping of the vertebral bodies) between *rapunzel* mutants and wild type siblings manifest during larval development (2–3 weeks of development) and a marked hyperossification phenotype becomes apparent by several months of age, ultimately resulting in an almost 2-fold increase in bone mineral density. **Conclusions:** We have cloned *rapunzel* and identified a gain of function mutation in a novel gene (*rpz*) of unknown function. Although the function of *rpz* awaits further experimentation, the identification of the genetic basis for skeletal overgrowth in *rapunzel* provides new insight into the mechanisms underpinning vertebrate skeletal biology.

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EFFECT OF PRENATAL CHOLINE SUPPLEMENTATION ON KIDNEY DEVELOPMENT IN IRON DEFICIENCY

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Background: Iron deficiency (ID), a common nutrient deficiency, impairs both brain and kidney. Choline, is a key nutrient for brain development, especially regions impacted by ID, and renal development. We hypothesized that prenatal choline supplementation ameliorates the ID-induced disturbance in kidneys. **Methods:** We studied 4 groups of Sprague-Dawley rats at postnatal day (P)15; Iron sufficient (IS), IS+choline (IS+Chol), ID and ID+choline (ID+Chol). From gestational day 2, IS diet (198 mg Fe/kg diet) or ID diet (<6 mg Fe/kg) was fed. Water with 50mM saccharin was provided to all groups and+Chol groups received water with 25mM choline chloride. Choline was stopped at delivery and ID dams received IS diet at P7. At P15, rats were sacrificed. Trichrome-stained renal sections of the left kidney were blindly examined for glomerular size, number per cortical surface area to estimate nephron density and total glomerular planar surface area, relative to kidney size, as a measure of filtering capability. Radial glomerular counts (RGCs), an index of glomerular generations, were measured. **Results:** At P15, pup body weights in both ID groups were 15% smaller than in IS groups. The ratio of kidney/rat pup weight was similar in all groups. ID disrupted glomerulogenesis. Glomerular densities were similar in all groups, but mean glomerular size in the ID pup was 8–14% smaller ($P<0.02$) than other groups, with glomerular size in ID+Chol being similar to the IS or IS+Chol groups. Total glomerular planar surface area in ID was 14% lower than IS ($P<0.01$) and 20% lower than IS+Chol ($P<0.01$). ID+Chol was intermediate, being similar to both ID and IS, but 18% lower than IS+Chol ($P<0.04$). RGCs were lower in ID vs. IS ($P<0.0001$) or IS+Chol ($P<0.002$) and intermediate in ID+Chol ($P<0.05$). **Conclusion:** ID during rat pregnancy decreased glomerular surface area by decreasing glomerular size and generations, but maintained glomerular density. This contrasts with published work reporting lower glomerular density in adult rats with prenatal ID. It is necessary to study aging ID rats to see if smaller glomeruli have ongoing loss, or if choline protects against this loss. In ID rats, prenatal choline resulted in mildly improved glomerular size, generations and total glomerular planar surface area, but higher doses should be evaluated.

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ORTHOSTATIC PROTEINURIA AND BODY MASS

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In search for the prevalence of proteinuria in the adolescent overweight population we previously reported (PAS 2006) the surprising finding that mid-day proteinuria was significantly more common in the non-obese population, and by post-hoc analysis the phenomenon could be explained by higher rate of orthostatic proteinuria (namely no proteinuria in 1st morning urine specimen) in the non-obese population. The objective of the current study was to test the above finding by studying the anthropometric characters of patients diagnosed in the Renal Clinic with orthostatic proteinuria (OP). **Methods:** A computer search identified patients diagnosed in the Renal Clinic with OP between May1, 1999 and April 30, 2009. Data extracted from their medical records included; ethnicity, gender, age, weight, height, body mass index (BMI), the latter 3 expressed as centiles (%). **Results:** (when applicable presented as median; mean$\pm$SD): 89 patients (49 females) were identified; Caucasians 74, African-Americans 10, others 5. Females' age 13.0; 12.6 ± 3.1, weight 60.0; 59.6 ± 23.8, height 70.0; 60.4 ± 30.4, BMI 50.0; 52.9 ± 25.8. Males' age 14.0; 13.9 ± 2.2, weight 51.5; 60.6 ± 24.8, height 71.5; 66.6 ± 25.8, BMI 57.5; 53.3 ± 27.8. In only 1 child did BMI exceed the 95%. **Conclusion:** The above survey supports our earlier finding the OP is found more commonly in the non-obese population. The finding of proteinuria in a mid-day specimen in the overweight child has a high chance of indicating genuine proteinuria (non OP).

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LONG CHAIN POLYUNSATURATED FATTY ACID LEVELS IN U.S. DONOR BREASTMILK

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Current research shows that long chain polyunsaturated fatty acids (LCPUFA), like docosahexaenoic acid (DHA) and arachidonic acid (ARA), are critical for normal growth, vision and brain development. These essential fatty acids accumulate transplacentally in the last trimester. Infants born before this process is complete have less tissue accretion making a postnatal dietary source even more important. Assuring adequate DHA and ARA during early brain development improves neurodevelopment in premature infants. **Purpose of the Study:** Breastmilk is the optimal nutrition for all infants and contains all essential LCPUFAs. Many mothers may not be able to provide enough breastmilk for their infant in the neonatal intensive care unit. In this case, alternatives include formula or pasteurized donor breast milk. DHA and ARA levels in donor milk are not well established. The objective of this study is to determine the effect of pasteurization on LCPUFA levels in donor breastmilk and the average consistency of DHA and ARA content in the US donor milk supply. **Methods:** Pooled donor breastmilk from the Mother's Milk Bank of Iowa (MMBI) and several other Human Milk Banking Association of North America (HMBANA) members will be analyzed for LCPUFA content via gas chromatography. LCPUFAs levels, including DHA and ARA, will be calculated on a wt:wt%. Comparisons will than be made between pre and post pasteurization samples from the MMBI, to determine the affects of processing on these nutrients. We will also determine levels from multiple batches of various HMBANA banks to determine the consistency of these nutrients in the US donor milk supply. **Summary of Results:** Although still small in number (n=6), preliminary data from the MMBI shows that pre and post pasteurization levels of LCPUFAs remain fairly stable through processing with less than a 1.08% change in wt:wt%. (Linoleic acid 1.08%, a-linolenic acid 0.08%, ARA 0.013%, DHA 0.06%). Consistency and average LCPUFA levels from multiple batches with variable pools, including samples from other donor milk banks will be further studied in the months ahead. **Conclusions:** Knowledge of the LCPUFA content of donor breastmilk will ensure appropriate provision of these nutrients when donor milk is used as a dietary source in the NICU. Pasteurization does not appear to affect LCPUFA levels. This study will provide a foundation for further clinical studies of LCPUFAs in donor breastmilk fed preterm infants, aimed at improved growth and cognitive outcomes in preterm infants.

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EARLY HYDROCORTISONE THERAPY AND SHORT TERM OUTCOMES IN PERSISTENT PULMONARY HYPERTENSION OF THE NEWBORN

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Objective: To determine if the early administration of hydrocortisone (<24 hours of life) in newborns with persistent pulmonary hypertension of the newborn (PPHN) improves short term outcomes. **Design:** Over a 26 month period (2006–2008) we identified 72 newborn charts with the discharge diagnosis of PPHN. All newborns ≥34 weeks GA with PPHN (ECHO confirmed suprasystemic pulmonary artery pressure with a right to left shunt) who received ≥2 doses of hydrocortisone were included (N=35). Hydrocortisone (2 mg/kg IV) was initiated if despite volume resuscitation and maximal inotropic support (dopamine and dobutamine at 20 mcg/kg/min), systolic blood pressure remained <55 mm Hg and capillary refill was >3 seconds. The etiology of PPHN in these newborns included respiratory distress syndrome (12), meconium aspiration syndrome (8), pneumothorax (7), sepsis (5), and asphyxia (3). Weaning was based on hemodynamic stability. We identified 7/35 (20%) newborns that received the first dose of hydrocortisone before 24 hours of life (Group 1), and 28/35 (80%) after 24 hours of life (Group 2). **Results:** In group 1 (N=7) hydrocortisone was given at a mean of 16 hours of life. Group 2 (N=28) received hydrocortisone at a mean of 42 hours of life. The total number of doses received was similar for both groups. The etiology of PPHN for 6/7 (87%) newborns in group 1 was meconium aspiration syndrome. Table 1 identifies the short term outcomes between the two groups. **Conclusion:** Early hydrocortisone therapy (< 24 hours) did not shorten the length of iNO treatment, mechanical ventilation, or the duration of oxygen therapy for newborns with PPHN. Newborns with PPHN secondary to meconium aspiration syndrome more commonly developed inotrope resistant hypotension within the first 24 hours of life which warrants further investigation.

Table 1. *Short term outcomes in early versus late hydrocortisone treatment in PPHN*

Short term outcome	Group 1 (N=7)	Group 2 (N=28)	*P* value
Duration of iNO therapy (days)	7.6	7.4	0.8
Time to extubation (days)	7.4	8.1	0.5
Oxygen requirement (days)	19.6	15.6	0.3

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IMPACT OF BACK-UP VIRAL ISOLATION OR PCR TESTING ON CLINICAL MANAGEMENT OF HOSPITALIZED CHILDREN

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Background/Aims: Respiratory viral illness is common in childhood and rapid viral identification may enhance patient care by reducing unnecessary antibiotics and length of hospital stay (LOS). Although antigen tests for influenza and RSV provide rapid results they lack sensitivity often necessitating back-up confirmatory testing for appropriate patient management. The Luminex Diagnostics Respiratory Viral Panel (RVP) is a multiplex PCR that identifies 12 respiratory viruses. R-mix shell vial culture allows isolation and detection of 7 respiratory viruses. The aim of the current study was to describe the findings from back-up test results and determine the impact of back-up testing on clinical management of hospitalized patients. **Methods:** Respiratory specimens from hospitalized patients with RSV/Flu rapid antigen-negative test results were reflexed to viral culture (2007–08) or RVP (2008–09). An initial 100 charts from each season was evaluated to record viral culture isolation, differences in LOS and antibiotic usage. Patients with a LOS >10 days were excluded from analysis of LOS and antibiotic usage. **Results:** 34/100 (34%) patients from the viral culture group and 63/100 (63%) patients from the RVP group had positive test results. Viral coinfections (more than one virus detected) occurred in 11 patients from the RVP group and 4 patients from the viral culture group. RSV was the most commonly isolated virus in culture (26/38) while rhinovirus (33/74) followed by RSV (31/74) were the most commonly detected viruses by RVP. Other viruses detected in the culture group included adenovirus (7), parainfluenza 1 (4), and influenza B (1). Other viruses in the RVP group were human metapneumovirus (5), and adenovirus (4). 79/100 patients from the viral culture group and 87/100 patients from the RVP group stayed 10 or fewer days in the hospital. There was no significant difference between the two groups in age, sex, or race. The median LOS was 2 days for both groups. 53 in the culture group and 40 patients in the RVP group received antibiotics: the median length of total antibiotic therapy was 5 days and 3.5 days for the culture and RVP groups respectively ($p = 0.691$). **Conclusions:** Compared to viral culture, PCR testing produced nearly twice the detection rate for a respiratory virus. The LOH and antibiotic therapy did not differ between the two groups of patients. The higher detection rates of RVP should theoretically reduce antibiotic duration and promote improved infection control practice. Future analyses will evaluate the change in patient management practices following result availability from the confirmatory viral testing.

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CEREBRAL OXYGENATION, PHYSIOLOGIC STABILITY AND DEVELOPMENTAL OUTCOME IN PRETERM INFANTS

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Premature infants are at risk for perinatal brain injury and adverse neurodevelopmental outcomes. It is postulated that brain injury in some preterm infants is acquired during periods of physiologic instability due to effects on cerebral blood flow and oxygenation. Cerebral blood flow and oxygenation can be studied using a technique called near-infrared spectroscopy (NIRS). Cerebral oxygenation trends in healthy infants and the effects of physiologic instability and developmental outcomes in preterm infants have not been described. We will correlate regional cerebral oxygenation saturation index (rSO_2) with a score for neonatal acute physiology (SNAP), an index of physiologic stability and with a Test for Infant Motor Performance (TIMPSI) in 31–33 week infants. **Methods:** In 31–33 week infants from Prentice Women's Hospital at 12–24 hours of age, frontal NIRS monitoring was applied for 24 hours with rSO_2 data collected every 5 seconds. The minimum, average and maximum rSO_2 were calculated for each infant as well as a SNAP score. At discharge, the test of infant motor performance (TIMPSI) was performed. SNAP scores, rSO_2 values, TIMPSI scores were correlated using simple regression. **Results:** 22 infants (of a planned enrollment of 60 infants) ranged in age from 31 to 33 weeks with a mean GA of 31.9 wks (SE=0.21) and BW of 1874 grams (SE=91). SNAP scores calculated during the NIRS monitoring ranged from 0–10. The average rSO_2 was 78.9% (range 67–89, SD=6.66). An inverse correlation between SNAP and average rSO_2 was significant (r^2=0.524, p=0.001). Minimum rSO_2 ranged from 15–68% and maximum rSO_2 ranged from 82–95%. There was an inverse correlation between individual minimum rSO_2 and SNAP score (r^2=0.288, p=0.01) as well as individual maximum rSO_2 and SNAP score (r^2 = 0.227, p=0.025). GA was not significantly associated with average rSO_2 or SNAP scores. TIMPSI testing for 16 infants are available from our cohort of 22 infants. There is not a significant correlation between average, minimum or maximum rSO_2 values and TIMPSI scores. Also GA and SNAP scores were not related to TIMPSI scores. **Conclusions:** Physiologic instability was associated with decreased cerebral oxygenation. As more patients are assessed, we will evaluate for emerging trends in cerebral oxygenation and early neuromotor outcomes, but thus far, cerebral oxygenation is not associated with neuromotor outcomes at discharge.

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PREVELANCE OF STRESS REFERENCES ON COLLEGE FRESHMEN'S FACEBOOK PROFILES

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Purpose: Psychological stress is common among college students and is associated with adverse health outcomes including weight problems, depression and alcohol use. Although stress is common in college students, a method of identifying students who experience stress remains elusive. This study used the social networking website Facebook to explore stress and associated factors among college students. **Methods:** Facebook profiles of undergraduate freshman at a large Midwestern university were identified through a random search of publically available profiles. Profiles were included if they identified high school graduation year as 2008, college graduation year as 2012, and reported age as 18 or 19 years. Content analysis of Facebook profiles included demographic information as well as references to stress, weight concerns, depressive symptoms and alcohol. **Results:** Of 333 evaluated profiles, 300 profiles met inclusion criteria. The majority of profiles identified as female (62%) and reported age of 18 years old (61%). Stress references were present on 110 profiles (37%). Weight concerns were present on 6.3%, depressive symptom on 24.4% and alcohol references on 72.9% of profiles. Displaying stress references was positively associated with female gender (OR 2.8 [CI 1.7–4.7]), displaying weight concerns (OR 5.4 [1.9–15.34]), and displaying depressive symptoms (OR 2.7 [1.6–4.6]). No significant associations were found between referencing stress and referencing alcohol. **Conclusions:** College freshmen frequently display references to stress on Facebook profiles with prevalence similar to self-reported national survey data. Consistent with existing literature, our findings suggest a positive association between referencing stress and referencing either depressive symptoms or weight concerns. Contrary to our expectations, no relationship was found between the display of stress references and alcohol references. The exceptionally high prevalence of alcohol references may explain this lack of association. It is possible that Facebook may allow for early identification of a clinical disorder without the need for a person to first seek help. Future research will evaluate Facebook as a tool to identify mental and physical health conditions and potential interventions.

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REDUCING MEDICAL-LEGAL LITIGATION IN NEONATAL-PERINATAL MEDICINE

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Background: During the past 30 years, medical malpractice litigation involving practitioners of Neonatal-Perinatal Medicine has increased steadily. Technological advances that facilitate the survival of smaller, sicker newborns as well as parental and societal expectations for healthy outcomes have contributed to this. **Objective:** To identify preventable events in the care of sick newborns to reduce litigation. **Design/Method:** Over a 24 year period (1986–2009), 84 cases of alleged negligence in the care of the newborn were reviewed as an expert (JM). 59/84 (70%) were reviewed for the defense and 25/84 (30%) were reviewed for the plaintiff with 61/84 (73%) in state. A confidential file was kept for all cases. **Results:** For the defense, 42/59 (71%) were deemed defensible and 15/25 (60%) for the plaintiff were meritorious. 66/84 (78%) were settled or dismissed before trial. 8/84 (10%) went to trial with 6/8 (75%) resulting in a favorable verdict for the defense. 10/84 (12%) cases remain active. Table 1 identifies the 10 most common allegations brought against Pediatricians/Neonatologists. **Conclusion:** Airway and air leak management continue to be a major source of litigation. In the surfactant and steroid era, clinical experiences with procedures involving intubation and chest tube placement have dramatically declined. Regionalization has a critical role in the best interest of the mother and newborn. Desaturations with subtle gross motor abnormalities often represent seizures. Protocols for serial x-rays for potential line migration as well as ROP screening need to be followed rigorously. The evolution of computerized order entry should reduce medication dosing errors. The standard of care continues to evolve in our field. The importance of good communication and documentation cannot be overemphasized. Addressing the issues described can potentially have a favorable impact on the medical malpractice crisis.

Table 1. *Common allegations in 84 cases resulting in litigation*

Inadequate airway/intubation	29 (35%)	Cardiac tamponade (central line)	9 (11%)
Failure to recognize air leak (pneumothorax)	26 (31%)	Failure to do eye exam (blindness)	9 (11%)
Delayed transfer to Level III	20 (24%)	Medication error (overdose)	9 (11%)
Inadequate treatment of seizures	16 (19%)	Midgut volvulus	7 (8%)
Delayed attendance (delivery room/NICU)	14 (17%)	Hyperbilirubinemia (kernicterus)	4 (5%)

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NON-COMPACTION OF VENTRICULAR MYOCARDIUM MIMICKING OTHER CARDIOMYOPATHIES: STILL A MISSED DIAGNOSIS

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Introduction: Non-compaction of ventricular myocardium (NCVM) is a rare and unique cardiomyopathy (CMP) with distinct features that can be identified by echocardiography. The morphologic features are characterized by numerous trabeculations and deep intertrabecular recesses. A ratio of non-compacted to a compacted layer> 2 is diagnostic for NCVM. Arrhythmias such as complete heart block, left bundle branch block (LBBB), Wolff-Parkinson-White syndrome (WPW) and ventricular tachycardia may be associated with NCVM. Case studies: A 5-year-old boy has been followed with diagnosis of hypertrophic CMP and WPW. During evaluation of supraventricular tachycardia, echocardiogram (echo) showed marked thickening of the left ventricular (LV) wall with numerous trabeculations and deep intertrabecular recesses. A 2-year-old boy has been previously treated for congestive heart failure due to dilated CMP. EKG showed complete LBBB. Echo revealed enlarged LV with reduced systolic function (FS 21%) and a prominent trabecular meshwork was visualized in the LV wall. Another case was referred because of dilated CMP and complete heart block. A trans-venous pacemaker was implanted without improvement in the LV systolic function. A case of NCVM diagnosed with multiple muscular ventricular septal defects at 1-month, presenting as restrictive CMP at 11-month and diagnosed as NCVM at age of 11 year. Echo demonstrated left atrial dilatation, biventricular apical coarsely trabeculated, spongy myocardium, E-wave deceleration time was prolonged in Doppler profile at trans-mitral level. This patient showed restrictive hemodynamic consistent of high end-diastolic pressures and high pulmonary artery pressure at age of 11-month. **Conclusion:** The initial diagnosis of NCVM was missed in all the cases, the delay in diagnosis reflecting the similarities between this disorder and other cardiomyopathies. Echocardiographers should recognize the pattern of NCVM to enable early diagnosis, treatment and follow-up.

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RELEASE OF VOLATILE ORGANIC COMPOUNDS IN INCUBATOR AIR WITH PHOTOTHERAPY

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The results of a recently published study by our group concluded that the emission of two volatile organic compounds (VOCs): 2-heptanone and n-butyl acetate originated from inside of the incubator and that concentrations of these two VOCs were further elevated by increase in temperature and humidity. There is evidence that exposure to some VOCs may adversely impact the developing infant. Up to 80% of premature infants have hyperbilirubinemia and are treated with phototherapy during initial few days of their life. Most of them receive phototherapy while inside the incubator. The purpose of this study was to evaluate the effect of phototherapy on VOC levels inside of the incubator. This observational, descriptive, pilot study was performed in a level III Neonatal Intensive Care Unit and involved a series of air samples taken from 2 unoccupied incubators which were cleaned and prepared for use. Air sample collection was initiated after incubators were turned on for twelve hours to establish a steady state of compounds. Samples were collected in laboratory-evacuated electro polished stainless steel air sampling canisters using a grab air method at 0, 1, 2, 3, 6, 9, 12, 24, and 48 hours. Ambient air samples were collected simultaneously. One incubator was running at temperature of 28° C, no phototherapy, no humidity; and the second incubator was set at temperature of 37° C, with phototherapy, and humidity at 70%. Samples were analyzed following EPA TO-15 using a Tekmar AutoCan Interface Agilent 6890 Gas Chromatograph with a 5973 Mass Spectrometer calibrated for 30 EPA TO-15 method target compounds. Levels of 2-heptanone, n-butyl acetate, 2-butanone, toluene, and propene in the samples from incubator 2 were significantly elevated compared with incubator 1 samples. This study indicates that phototherapy causes significant release of several VOCs. The findings from this pilot study were useful in the design of a larger follow-up study that is currently underway.

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DEVELOPMENT OF GROWTH MODELS FOR CHILDREN WITH UNIVENTRICULAR PALLIATION

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Purpose: Delayed growth patterns plotted on standard clinical growth charts have been noted for patients with univentricular congenital heart disease. The disparity of growth models for children with univentricular palliation led to the development of length and weight growth charts for this specific patient population. **Methods:** A descriptive, retrospective chart review was performed of a cohort of surviving children and adolescents (n=75) who underwent single ventricle palliation between 1994 and 2009. Cardiac diagnoses included Hypoplastic Left Heart Syndrome n=24, Tricuspid atresia n=10, Pulmonary valve atresia n=14, Mitral stenosis n=2, Complete Atrioventricular canal n=6, and complex single ventricle n=19. Length and weight were also compared to an age matched normal cohort of 2,386 children with normal echocardiographic examinations. Length and weight growth charts were developed using regression splines for this population using 478data points from two years to seventeen years of age. **Results:** Children were divided into age groups to facilitate analysis (2–4.99, 5–7.99, 8–10.99,11–13.99, and 14–16.99 years). Significant differences ($p<0.01$) in height and weight are noted between normals and single ventricle children in all groups except for the 8–10.99 ($p>0.05$) year olds. **Conclusions:** Understanding growth patterns in children with univentricular palliation will assist primary care providers and pediatric cardiologists in caring for these children and adolescents. Further study is needed to determine length and weight risk threshold for morbidity and mortality in this group.

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ACUTE PHYSIOLOGICAL EFFECTS OF RED BLOOD CELL TRANSFUSION IN PRETERM INFANTS TRANSFUSED USING LIBERAL OR RESTRICTIVE GUIDELINES

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Preterm infants frequently require packed red blood cell (RBC) transfusions as treatment for anemia. Despite several recent randomized clinical trials, the safe lower hemoglobin or hematocrit threshold for transfusion has not been defined. We examined the physiological effects of anemia and the acute responses to RBC transfusion in preterm infants with birth weight 500-1300 g, who were enrolled in a trial comparing liberal and restrictive transfusion thresholds (Bell et al. *Pediatrics*. 2005;115:1685). Physiological measurements were performed in 41 infants before and after a transfusion of 15 ml/kg, which was performed because the infant's hematocrit had fallen below the transfusion threshold determined by the study protocol. Cardiac output was measured by echocardiography, and O_2 consumption was performed using indirect calorimetry. As expected, systemic oxygen transport rose in both groups after transfusion. Lactic acid was lower after transfusion in both groups. Oxygen consumption did not change significantly with transfusion in either group. Cardiac output and fractional oxygen extraction fell in the restrictive transfusion group only. These results demonstrate no acute physiological benefit of transfusion in the liberally transfused group. The fall in cardiac output with transfusion in the restrictive transfusion group shows that these infants had increased their cardiac output to maintain adequate tissue oxygen delivery in response to anemia.

Table. *Physiological measurements before and after transfusion (mean ± SD)*

	Liberal Transfusion Group (n = 22)			Restrictive Transfusion Group (n = 19)		
	Pre	Post	P	Pre	Post	P
Hematocrit, %	39 ± 7	48 ± 5	<0.001	27 ± 4	38 ± 4	<0.001
Blood lactic acid, mmol/l	1.1 ± 0.3	0.7 ± 0.1	<0.001	1.1 ± 0.2	0.9 ± 0.2	0.032
Cardiac output, ml/min/kg	291 ± 98	277 ± 91	0.402	301 ± 101	253 ± 66	0.048
O_2 consumption, ml/min/kg	8.1 ± 1.5	8.1 ± 1.1	1.0	10.0 ± 1.7	9.0 ± 1.1	0.279
Systemic O_2 transport, ml/min/kg	44 ± 7	57 ± 12	0.004	33 ± 4	41 ± 5	0.001
Fractional O_2 extraction	0.20 ± 0.08	0.17 ± 0.08	0.250	0.31 ± 0.11	0.24 ± 0.12	0.003

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DISPLAY OF RISK AND PROTECTIVE HEALTH BEHAVIORS ON INCOMING FRESHMEN'S FACEBOOK PROFILES

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Purpose: Adolescent providers frequently assess an adolescent's balance of health risk behaviors (such as alcohol use) and protective behaviors (such as sports involvement) and their impact on an adolescent's overall health. The purpose of this study was to perform a content analysis on Facebook profiles to explore associations between adolescents' displayed risk and protective behaviors. **Methods:** This study analyzed publically accessible Facebook profiles of 18 and 19 year old incoming University of Wisconsin freshmen. Profiles were analyzed for displays of risk behaviors including sex, alcohol, tobacco and drugs; for protective behaviors including sports, the arts (drama, dance, and music), and religion; and for any references to personal health including chronic illness, mental illness, weight concerns and sleep problems. **Results:** Of the 222 evaluated profiles, a content analysis was performed on the 100 profiles that met inclusion criteria. Of these profiles, 97% were 18 years old and 54% were female. One or more risk behaviors were present on 74% of profiles, one or more protective factors were present on 70% of profiles. There was no overall association between the display of risk and protective behaviors. There was a positive association between being female and displaying references to weight ($p=0.05$). In addition, there was a positive association between male gender and the display of references to sports ($p=0.03$). There was also a positive association between the display of references to the arts and sleep problems ($p=0.04$). **Conclusions:** Facebook provides an innovative way to explore the balance between displayed risk and protective behaviors among adolescents. Given the high prevalence of both displayed risk and protective behaviors, adolescents appear to use Facebook as a place to display many types of behaviors. The display of references to personal health is far less common. The significant gender differences in the display of weight concers by females and sports involvement by males appears to follow traditional gender patterns seen in other studies. The relationship between display of references to the arts and sleep problems provides an interesting association that should be further investigated. Further studies should evaluate how risk and protective behavior displays change as students progress through college.

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FOUR YEAR OUTCOMES IN A STEROID FREE GROUP COMPARED TO STEROID GROUP IN PEDIATRIC RENAL TRANSPLANTATION

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Background: The steroid use in the patients with renal transplantation, especially in children leads to growth retardation and obesity along with increased cardio vascular risks. **Objective:** Compare clinical outcomes in steroid free group (SFG) versus steroid group (SG) in pediatric renal transplant recipients receiving maintenance immunosuppression. **Design/Methods:** A retrospective review was done comparing clinical parameters at 1 month, 1 year, 2 years, 3 years and 4 years post-transplant between the two groups (2000–2007). There were 19 subjects included in SG and 40 subjects in SFG. Both the groups received induction therapy with Mycophenolate Mofetil (MMF), Methylprednisolone and Thymoglobulin or Basiliximab. Patients in SFG received weaning dose of steroid for five days after transplantation. Maintenance therapy in SG included MMF/Rapamycin, Tacrolimus/Cyclosporine and Prednisone. Patients in SFG received maintenance therapy with only MMF/Rapamycin and Tacrolimus/Cyclosporine. We reviewed graft survival along with growth, creatinine clearance and metabolic profile in both the groups. The overall means, standard deviation and p-values were calculated by unpaired student t-test to compare the two groups. Significant value was considered $P<0.5$. **Results:** The patient survival in both the groups was 100% at 4 years. Graft survival was good in both groups although the most common reason for graft loss was non compliance in both groups. The growth was better in SFG and the delta height Z- score was found to be statistically significant at 1, 2, 3 and 4 years post transplantation with delta height Z-score of 1.35 ± 0.670 in SFG and -0.21 ± 1.356 in SG. Delta weight and BMI means were significantly lower in SFG. The Cholesterol levels and Blood Sugar levels were lower in SFG group. **Conclusions:** The main benefit of steroid free maintenance protocol compared to steroid protocol in pediatric Renal Transplant is optimal growth with decreased cardiovascular risks.

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SETTING THE STAGE FOR NEWBORN IRON DEFICIENCY

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Background: The antecedents of may illnesses and health vulnerabilities begin in infancy, and often before birth. One common concern in young infants is iron deficiency. Despite U.S. public health efforts to reduce iron deficiency, iron deficiency continues to affect 8–24% of infants in the US. Research has shown that maternal stress experienced during pregnancy may predispose the fetus to a number of health vulnerabilities that may set the stage for postnatal illness. Animal work shows that pregnancy stress may predispose to iron fetal iron deficiency. Thus, the **purpose** of the present study was to examine the impact maternal stress during pregnancy on newborn iron status. **Methods:** 165 mothers and their term babies (92 males; 73 females) were recruited from the Meriter Hospital Birthing Center in Madison, WI, prior to hospital discharge. At birth, a sample of each infant's umbilical cord blood was collected upon consent and subsequently processed to examine iron status markers. We examined whole cord blood and reticulocyte-enriched Zinc Protoporphryin/Heme (ZnPP/H). Enriched ZnPP/H was measured after separating the youngest red cells. All enrolled mothers were asked to fill out a 35-item, retrospective questionnaire to assess the stress they had experienced during their pregnancy. The questionnaire prompted the mother to respond whether or not a particular stressful event had occurred during her pregnancy and to rate the stress impact of the item on a scale of 1–10 (1=did not upset me, 10=extremely disturbed). **Results:** Stress measure total score: M = 46.1; SD=40.5; total score range: 0–201. Stress measure mean: M=1.3; SD=1.2; mean score range: 0–5.74 suggesting that our sample experienced relatively low levels of stress during pregnancy. Despite reporting low levels of stress during pregnancy, hierachical linear regression analyses indicated that above and beyond maternal ethnicity, diabetes, anemia, and age, higher mean scores on the maternal stress measure ($\beta=.28$, $p<.001$) predicted higher enriched ZnPP/H ($F_{1,164}=3.6$; $p<.01$). In addition, after adding the 3 most stressful items into the overall regression model mean stress scores continued to predict newborn enriched ZnPP/H ($F_{1,164}=3.4$; $p<.01$) with mean stress scores accounting for 13.4% of the variance in enriched ZnPP/H. **Conclusion:** High maternal stress is predictive of high reticulocyte-enriched ZnPP/H, suggesting that maternal stress during pregnancy may impair late pregnancy iron delivery to the red cell.

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ELIMINATING HEPARIN DOSING ERRORS IN THE NEONATAL INTENSIVE CARE UNIT

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Background: Heparin is one of eight medicines that account for nearly a third of hospital medication errors, according to the Institute for Safe Medical Practices. Packaging of some heparin products contribute to confusion. Adding confusion and potential for error, is the need to manually dilute heparin in some cases in order to provide preservative free product to infants. Some hospitals now require at least two nurses to check heparin vials before use, however having varied strength heparin available within a neonatal intensive care unit (NICU) allows for the error of the wrong strength to be given, regardless of the level of vigilance of the care providers. **The Purpose of This Study:** was to monitor the safety and efficiency of changing to a single strength heparin product within the NICU. **Methods:** A commercially available, pre-packaged non-sterile 3 ml syringe containing normal saline with 1 unit/ml heparin was brought into the NICU, and replaced all other heparin sources. There was a practice change regarding maintenance of heparin locked midline catheters. Prior to the change, heparin locked midlines were flushed with 10 unit/ml heparin every 6 hr. Following the change, heparin locked midlines were flushed with 1 unit/ml heparin every 4 hrs. Dwell time, and reason for discontinuation of heparin locked midlines was retrospectively reviewed for 110 midline catheters prior to the change and prospectively reviewed for 217 catheters after the change. **Results:** Of the 110 midline catheters heparin locked with 10 unit/ml heparin, 11% become occluded or clotted, compared to 14% occlusion/clotting in the lines heparin locked with 1 unit/ml heparin (NS). The average dwell time was 58 hrs for midlines that were heparin locked with 10 unit/ml heparin, compared to 66 hrs for midlines heparin locked with 1 unit/ml heparin. With increased line access, there was no increase in catheter related infection rate during the second time period. **Conclusion:** Midline catheters can be safely and effectively maintained following heparin lock with 1 unit/ml heparin solution, thereby allowing the use of only one low strength heparin solution within a NICU. Excluding varied concentrations of heparin solutions from the NICU may eliminate the potential for inadvertent delivery of the wrong heparin dose. Using a lower strength concentration of heparin allows for less heparin exposure in infants receiving frequent medication delivery.

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CHILDHOOD BEHAVIORAL PREDICTORS OF ADOLESCENT'S OBESITY

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Background: Obesity among children and adolescents is a serious public health concern in the United States. The prevalence of obesity among children between ages 2 to 18 years increased by threefold from 1970s to 2004 and is still on rise. Further, obesity and its associated morbidity disproportionately affect African American, low income, urban families. Previous research has identified relations between childhood obesity and increased behavioral problems. However, these data have been inconsistent across studies, primarily due to sample size and limitations in study design for example. **Purpose:** To examine the relation between behavior problems at 7 years and adolescent obesity (14 years). **Methods:** Data were obtained from a prospective pregnancy study at our university maternal hospital. BMI and behavior measures were assessed at 7 and 14 years of age. Childhood and adolescent behavior was assessed by standard parent and teacher measures (Achenbach's CBCL and TRF). **Results:** The sample included 432 African American caregiver-child pairs. At age 14, 24% of the teens met criteria for being overweight; 21% were obese and 13% were severely obese. Results showed a positive relation between social and behavior problems at 7 years and teen obesity. After controlling for child gender, caregiver depression, prenatal exposure to cigarettes, child IQ, and maternal weight, teen BMI and obesity were related to several behavior indicators (childhood & teen). Teen BMI was related to caregiver reported Somatic Complains, Social Behavior Problems and Other Behavior Problems as assessed on the Achenbach CBCL. In addition, the higher scores on the CCDS Emotional Numbing and Lack of Belongingness scales were related to elevated teen BMI scores. Caregiver-reported APS scores for GAD, PTSD, and Eating Disorder were also related to teen BMI. Among 7-yr assessments, Social Behavior Problems (CBCL & TRF) reported by teachers and caregivers predicted teen BMI; Teacher Delinquent Behavior Problems and Externalizing Total Score were also predictive. Finally, the 7-yr Devereaux's scales Inappropriate Behavior and Interpersonal Behavior Problems predicted teen Obesity and BMI. **Conclusion:** In this high risk urban sample, behavioral disorders at both early school age and at age 14 years were related to adolescent obesity and BMI. Additional analyses are ongoing to examine the longitudinal impact of childhood obesity on the relation between teen obesity and behavior. These findings could provide an important opportunity for early intervention.

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ZINC PROTOPORPHRYIN/HEME RATIOS (ZNPP/H) AS A POTENTIAL NEWBORN SCREEN FOR IRON DEFICIENCY (ID)

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Background: Iron deficiency (ID), a common nutrient deficiency, impairs brain development. Newborn screening for ID has advantages, as earlier recognition of at-risk children could prevent long-term neurocognitive morbitity. ZnPP/H is an available, cost effective and sensitive biomarker of incomplete iron incorporation into erythrocytes. ZnPP/H, a potential candidate for newborn screening for iron deficiency, is measurable on whole cord blood or filter paper spots. Previous studies rinsed cord blood samples to remove pigments including bilirubin, that interfere with readings. Filter paper newborn screen samples cannot be rinsed and are obtained at 24–48 hrs after birth, when bilirubin levels may be elevated. Our aims were first, to examine the reproducibility of filter paper ZnPP/H measurements and second, to examine methods to limit bilirubin interference with ZnPP/H. **Methods:** We measured reproducibility of cord blood ZnPP/H eluted as hemolyzed blood from filter paper blood spots and whether eluted values correlated to the same whole blood samples. We measured the degree of bilirubin interference with ZnPP/H readings. We evaluated potential candidates to remove bilirubin interference with ZnPP/H, including detergents, albumin, and bilirubin oxidase. **Results:** Filter paper ZnPP/H was reproducible. Filter ZnPP/H correlated to whole or rinsed cord blood, $P<0.0001$, but lines of identity did not pass through zero. Although 5 mg/dL levels of bilirubin were similar to ZnPP/H without bilirubin, 10, 15 and 20 mg/dL increased the levels of ZnPP/H in a dose response fashion ($P<0.0001$). Tween 20 and Triton X-100 increased ZnPP/H baseline variability and did not remove bilirubin interference. Albumin decreased baseline ZnPP/H values to those of rinsed whole blood, but did not remove bilirubin interference. Bilirubin oxidase reagent is inexpensive and stable when refrigerated or frozen in water. Although not returned to baseline, bilirubin oxidase removes bilirubin interference by 70% ($p<0.0001$). **Conclusion:** Filter paper ZnPP/H correlated to intact whole and rinsed ZnPP/H, but bilirubin levels of 10 mg/dL interfere with readings. Bilirubin oxidase removes most bilirubin interference, improving the accuracy of filter paper ZnPP/H. Further studies are needed to validate the use of ZnPP/H from blood on filter paper.

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ADOLESCENTS' DISPLAY OF SEXUAL REFERENCES ON A SOCIAL NETWORKING WEB SITE IS ASSOCIATED WITH INTENTION TO BECOME SEXUALLY ACTIVE

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Purpose: Adolescent sexual initiation is associated with increased risks for sexually transmitted infection and unwanted pregnancy. Intention to become sexually active is among the strongest predictors of sexual initiation. Identifying adolescents who intend to become sexually active would present a key opportunity to provide relevant education and prevention messages. Social networking web sites (SNSs) are popular among adolescents and frequently display sexual references. The purpose of this study is to determine whether displayed sexual references are associated with sexual intention or experience. **Methods:** We identified public Facebook profiles of undergraduate freshmen within one large state university Facebook network. Profile owners who displayed sexual references (Displayers) and did not display references (Non-Displayers) were invited to complete surveys measuring sexual experiences, and sexual intention using the Postponing Sexual Involvement (PSI) scale. A higher PSI score is *inversely* related to intention to initiate sexual intercourse. **Results:** Of the 118 profiles that met inclusion criteria, 85 profile owners completed surveys. Most profile owners were female (56.5%) and Caucasian (67.1%). There were no differences between Displayers and Non-Displayers regarding prevalence of oral sex, anal sex or sexual intercourse. Mean PSI score for Displayers was 6.5 ± 1.6, mean PSI score for Non-Displayers was 10.2 ± 0.6 ($p=0.02$). **Conclusions:** Display of sexual references on college freshmen's Facebook profiles was positively associated with reporting intention to initiate sexual intercourse. Facebook profiles may represent an innovative venue to identify adolescents considering sexual activity who may benefit from targeted prevention or education messages.

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HEALTH CARE WORKER ATTITUDES REGARDING INFLUENZA IMMUNIZATION AND VACCINE RECEIPT

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Background: Yearly influenza immunization is recommended for healthcare workers (HCW); with <50% compliance. HCW acceptance of influenza vaccine is likely related to their attitudes and beliefs about vaccine efficacy and safety; and may influence immunization receipt in their children. **Methods:** A confidential electronic survey of 43 questions was distributed to a random cohort of HCW at a large tertiary children's hospital in March 2009. Attitudes and beliefs toward immunization receipt for themselves and their children were targeted. HCW were divided into 3 groups; physician (33%), nurse (10%), and other HCW (10%). Analysis was performed using SPSS. **Results:** 574/946 surveys were returned; 63/117 (54%) physicians, 135/236 (57%) nurses, and 376/593 (63%) other employees. 82% were female, 83% Caucasian, 53% aged 25–44 years, and 40% had >15 years HCW experience. More physicians believe they are high risk for influenza infection compared to nurses or others ($p<0.0001$), and job description was the only predictor of vaccine receipt. Physicians > nurses > others believe that immunization prevents health care acquired influenza ($p=0.004$, < 0.0001 respectively). Other HCW were most likely to believe that influenza vaccine could cause influenza and physicians were most likely to agree that the vaccine was safe for adults and children ($p<0.0001$).Physicians versus nurses and others were less likely to be concerned about efficacy ($p=0.035$, $p=0.004$) and safety($p=0.001$, $p<0.0001$) of routine childhood vaccines. Physicians (73%) were more likely to agree with a mandatory policy for influenza vaccine compared to nurses (45%) or others (35%) ($p<0.0001$). HCW that received influenza vaccine were more likely to give their child influenza vaccine ($p<0.001$). **Conclusions:** Influenza vaccine receipt was high in our institution. Still, we noted knowledge regarding risk of infection, nosocomial spread and vaccine safety was not universally appreciated. Additionally, physicians are more comfortable with a mandatory vaccine policy, making continued efforts of education regarding vaccine safety and transmissibility paramount to a successful campaign.

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INCIDENCE AND CHARACTERISTICS OF RED MAN SYNDROME IN CHILDREN AND ASSOCIATION WITH GENE POLYMORPHISMS FOR HISTAMINE DEGRADATION

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Background: Vancomycin (vanc) is increasingly used for treatment of antibiotic resistant gram positive infections. Red man syndrome (RMS) is the most common adverse event, typically self-limiting, but can rarely be associated with serious reactions. While it has been well characterized in adults, little is known about this adverse reaction in children. Several single nucleotide polymorphisms (SNP's) in the HNMT gene have been shown to decrease histamine degradation, and may contribute to development of RMS. **Methods:** We conducted a single center study to determine the incidence and characteristics of RMS in the pediatric population and to look for an association with the HNMT C314T variant allele. Hospitalized patients 6 mos– 21 yrs who received vanc were included over a 2-year period. Development of RMS and demographics were identified through chart review and parent/nurse report. Reactions included rash, flushing, itching and/or decline in blood pressure (BP)> 10 mm/hg. PCR was performed on genomic DNA to identify the HNMT C314T allele. SPSS (v16) was used for analysis which included Chi square and Fisher's exact tests. **Results:** RMS was found in 68/311 (21.9%) patients. There was no difference in RMS rates between age groups, ethnicity, or gender. Itching, rash, and flushing occurred in 63%, 50%, and 53% of subjects, respectively. BP changes were not seen. No relation was found between drug allergy, immune suppression or immunosuppressive therapies and RMS ($p=0.09$, 0.4, 0.7 respectively). More patients with RMS received anti-histamines during vanc treatment ($p<0.0001$). However, anti-histamine use prior to vanc did not decrease RMS occurrence ($p=0.16$). No association was found between development of RMS and presence of the HNMT variant allele ($p=0.23$). **Conclusions:** RMS occurred commonly in our pediatric population. Reactions were mild, and treated with anti-histamines; however, anti-histamine pre-treatment did not prevent RMS. While HNMT C314T was not found to be associated with RMS features, future studies should focus on other possible genetic factors that could be associated RMS.

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THE USEFULNESS OF MONITORING TACROLIMUS AND SERUM CREATININE LEVELS FOR EPSTEIN-BARR VIREMIA IN SMALL BOWEL TRANSPLANT PATIENTS

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Purpose: The purpose of this study was to retrospectively review Epstein-Barr Virus (EBV) infections and the relationship to immunosuppression in small-bowel (SB) and liver (LV) transplant patients who received grafts from living related donors. **Method:** Data from 7 female and 4 male patients with an average weight of 11.73 kg was collected. Total of fourteen small bowel transplants were done. Seven patients received both SB & LV transplants. Among 11 patients: 6 were Caucasian, 2 were African-American and 3 were Hispanic. Patients received thymoglobulin/steroid (IV) induction and 5-day post-op course of prednisone. Mycophenolate Mofetil (0.14 mg/kg–4.9 mg/kg) and Tacrolimus (0.05mg/kg-3.1mg/kg) were given for maintenance. EBV counts were collected and analyzed by PCR weekly for 1 month and every 1–2 months thereafter. FK levels and SCr levels were drawn daily while inpatient and every visit once outpatient. P-value <0.01 was considered significant. **Results:** During 12 month follow-up, patients' survival was 73% and graft survival was 71%; 3 grafts were lost to rejection and 1 graft was lost to post-transplant lymphoproliferative disease (PTLD). 6 of 11 of the patients developed EBV infections, 3 of 11 of the patients developed PTLD and 5 of 11 patients responded to decrease in FK and MMF dose. There was a significant positive correlation between both blood FK levels and EBV viral counts ($p<0.01$) and blood FK levels and SCr levels ($p<0.01$). There was no significant correlation between immunosuppression dosage and EBV viral counts or the immunosuppresion dosage and serum creatitine level. **Conclusion:** Our results show positive correlation between serum tactolimus level and EBV viral count indicating FK related nephrotoxicity and severe immnusuppresion. Hence FK and SCr levels can be used to monitor susceptibility to EBV infections and to monitor immunosuppression levels in SB transplant patients. Close monitoring of FKwill help to avoid FK toxicity and its adverse effects including viremia.

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PERMISSION AND ASSENT IN PEDIATRIC RESEARCH

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Purpose of Study: To evaluate the permission and assent process in pediatric research and, if possible, to identify ways of improving the process. **Methods Used:** Permission and assent were assessed in eight pediatric research projects at a large, tertiary care hospital. In a structured interview, parents of children enrolled in clinical research answered questions about the following aspects of the informed-consent process:

(1) Investigator compliance with institutional parental-permission and child-assent policy,
(2) Parental understanding of the child's study,
(3) Parental satisfaction with the permission and assent process, and
(4) Parental education and income levels.

The child-subjects also answered questions about their own understanding of their research project if they were at least seven years-old and if their parents gave them permission to participate in the interview. **Summary of Results:** Twenty-nine parents and nine children were interviewed. The mean investigator compliance score was 9.7 of a possible ten points. Parental satisfaction with the informed-consent process was also high; all the parents interviewed ranked their satisfaction-level as"very satisfied." The range of parental and child understanding of their particular projects was wide. Some respondents (21%) clearly understood even the more confusing features of research while others (28%) did not understand the basic features of study design. The purpose of research, complex randomization procedures, and clinical equipoise were the most difficult concepts for subjects and parents to understand. Methods of improving understanding are identified and discussed. **Conclusions Reached:** Despite investigator compliance with permission and assent standards, parents and child-subjects do not always adequately understand the their research projects. Further research can focus on improving understanding in the basic nature of clinical research. Some interventions have been developed to help adult subjects understand research projects, and these methods should be adopted for pediatric subjects as well, who are potentially more vulnerable.

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DEVELOPING A DIAGNOSTIC TOOL FOR SCREENING IRON DEFICIENCY IN INFANTS

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Purpose: Iron deficiency in infants often results in a multitude of metabolic vulnerabilities later in life. The first step toward analyzing the effects of and treating iron deficiency is to develop an accurate and easily accessible diagnostic tool to screen for iron deficiency thus allowing early treatment in at-risk infants. Whole blood ferritin may more accurately reflect fetal iron stores than the traditional plasma ferritin. Our goal is to use dried blood spots on filter papers (newborn screening cards) through the Wisconsin Newborn Screening Program to test for any iron-based ailments. **Methods:** Enyzme-Linked ImmunoSorbent Assays (ELISA) with 96 ferritin-coated wells were performed on whole blood, erythrocyte, plasma, serum and dried whole blood samples to determine if dried blood spots on newborn screening cards are comparable to whole blood samples collected from cord blood in subsequent iron deficiency tests. **Results:** Significant correlations were obtained for whole blood ($R = 0.65$ and $P = 0.00$) and erythrocyte ($R = 0.70$ and $P = 0.00$) samples with dried blood spots from the screen cards. **Conclusion:** Our data indicate that subsequent tests of iron stores utilizing the iron storage protein, ferritin, in infants can be performed with blood spots on newborn screening cards.

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PARENTAL ATTITUDES TOWARD RESEARCH PARTICIPATION IN PEDIATRIC SICKLE CELL DISEASE

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Socio-cultural attitudes and perceptions are barriers to the recruitment of African Americans for medical research. Yet, no studies currently exist that examine the attitudes and perceptions influencing research participation among individuals with sickle cell disease (SCD) or parents with children affected by SCD. The objective of this study was to examine the broad factors that affect decision-making related to participation in medical research in parents of children with SCD. A 32-item, self-administered survey tool was developed and distributed over a 6-month period to a convenience sample of parents with children followed in the Comprehensive Sickle Cell Program at Children's Memorial Hospital. Standard descriptive analysis was performed and Pearson Chi-Square (Fisher Exact Test) used to determine variables associated with a favorable attitude toward research participation. We performed multivariable logistic regression to determine independent associations with a favorable attitude. A total of 151 surveys were collected from parents (113 born in the US, 38 born outside the US) and analyzed for this pilot study. Overall, 86% of respondents believed that more research needs to be done for SCD. When asked if they would allow their child to participate in a medical research study about SCD, 57% of respondents agreed or strongly agreed, although 27% answered"Don't Know". A favorable attitude toward research participation was associated with having a personal belief that more research needs to be done for SCD (OR 23.4 [4.5–121.9], $p=0.001$), respondent prior exposure to research (OR 3.2, [1.0–10.3], $p=0.043$), and a perceived greater severity of their child's SCD (OR 2.7, [1.0–7.1], $p=0.041$). Attitude toward research participation was not associated with respondent country of birth. Respondents born outside the US, however, were less likely to seek additional knowledge about SCD, were less comfortable discussing their child's SCD with others outside the immediate family, and were less likely to allow their child to participate in research studies that provided reimbursement. In a logistic regression model, only the belief that more research needs to done and the respondents' perception of greater severity of their child's SCD were independently associated with a favorable attitude toward research participation. We conclude that most parents of children with SCD would allow their child to participate in medical research studies. Although non-US born parents differ from US born parents in several aspects, they do not differ in their attitude toward research participation. Knowledge gaps identified by our study also underscore the need for more education about medical research and research participation in our families affected by SCD.

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CITRATE PROPHYLAXIS FOR THE PREVENTION OF NEPHROCALCINOSIS IN ELBW INFANTS: A PILOT TRIAL

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Background: Nephrocalcinosis (NC) develops in nearly 64% of very low birth weight infants and is associated with long term morbidities. Urinary citrate excretion, an inhibitor of renal stone formation, is low in preterm infants and maybe a risk factor in the development of NC. **Objective:** To determine if prophylaxis with citrate prevents NC in extremely low birth weight (ELBW) infants <1000 grams. **Design/Methods:** 19 infants received citrate prophylaxis (Polycitra®) at 2 mEq/kg/day when enteral feeds reached 100 ml/kg/day until discharge. NC- classified as mild, moderate or severe-was evaluated by renal ultrasound at 4, 8 and 12 weeks of age. Urinary calcium/creatinine ratio (Ca/cr) and citrate/creatinine ratio (Cit/cr) were measured. Wilcoxon scores test, t-test and ANOVA were used for data analyses. Multivariate regression models were used to determine significant risk factors. **Results:** 12/19 (63.1%) infants developed NC (group 1) while 4/19 (21.0%) did not develop NC (group 2). One infant (5.3%) died before receiving citrate, one (5.3%) died of necrotizing enterocolitis one week after starting supplementation, and one infant (5.3%) developed autosomal recessive polycystic kidney disease and hence excluded from analyses. Median (25th-75th interquartile range) gravida was higher in group 1 vs. group 2 (2.5; 2–4 vs. 1;1–1.5, $p=0.04$). Use of breast milk was higher in group 1 (Mean ± SD; 16.5 ± 3.6 days vs. 9.5 ± 1.3 days, $p=0.002$). Infants in group 2 received citrate earlier (Mean ± SD; 13.7 ± 2.6 days vs. 20.3 ± 6.5 days, $p=0.07$) and for a longer duration (Mean±SD; 74.7±15.3 days vs. 53.4 ± 11.5 days, $p=0.01$). There were no differences in birth weight, gestational age, days of parenteral nutrition, steroids or diuretic use, days of oxygen supplementation and length of stay between the two groups. In multivariate analyses, only citrate prophylaxis approached significance ($p=0.058$). Urinary Cit/cr ratio increased in group 2 ($p = 0.003$) but not group 1 ($p=0.15$) after starting prophylaxis. Urinary Ca/cr were not different between the two groups at any time. NC was mild in 10/12 (83.3%), moderate in 1/12 (8.3%) and severe in 1/12 (8.3%) infants. NC developed in 2/12 (16.6%) infants by 4 weeks, 11/12 (91.6%) infants by 8 weeks and 1/12 (8.3%) by 12 weeks. **Conclusions:** NC develops by 8 weeks of age in majority of infants. Citrate prophylaxis may prevent development of NC in ELBW infants. A larger trial is needed to address these observations.

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MATERNAL OBESITY AT DELIVERY: A RISK FACTOR FOR NEWBORN IRON DEFICIENCY

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Background: Iron deficiency is the most common nutritional deficiency in the world, plaguing 7% of US infants. Research has indicated that obese adults and children are at increased risk for iron deficiency compared to non-obese counterparts. In addition, studies suggest that obesity is associated with higher rates of iron deficiency anemia due to the inflammatory changes associated with obesity interfering with iron absorption. Whether or not maternal obesity during pregnancy is related to newborn iron-status has yet to be examined. Thus, we hypothesized that maternal obesity would be associated with poorer newborn iron status. **Methods:** 169 mothers and their term babies were recruited from the Meriter Hospital Birthing Center in Madison, WI, prior to hospital discharge. Mothers and infants with risk factors for iron deficiency were over sampled. Maternal characteristics including age, ethnicity, anemia, diabetes, pre-pregnancy and maternal delivery body mass index (BMI), and infant characteristics including sex and BMI were examined as possible predictors and/or covariates. Whole umbilical cord blood and reticulocyte-enriched ZnPP/H were examined as indices of iron deficiency. Enriched ZnPP/H was measured after isolating the youngest red cells. **Results:** To examine group differences in maternal BMI, mothers with a BMI ≤ 30 kg/m^2 were compared to mothers with a BMI >30 kg/m^2. Initial Chi-square analyses indicated that BMI groups did not differ in regard to maternal ethnicity, anemia, or diabetes. A One-way Analysis of Variance (ANOVA) indicated that mothers with a BMI at delivery >30 kg/m^2 gave birth to babies with a higher mean enriched ZnPP/H ($M=131.6$;$SD=41.1$) compared to mothers with a delivery BMI <30 kg/m^2 ($M=118.4$;$SD=42.5$; $F_{1,168}=5.1$, $p<.05$), although the relationship was not linear. **Conclusion**: Women with a BMI >30 kg/m^2 have babies with higher ZnPP/H in reticulocytes, a measurement we previously found was sensitive to early iron deficiency. It is possible that pro-inflammatory changes interfere with maternal iron absorption and/or placental iron transport to the fetus during late pregnancy. Further research is required to investigate the connection between iron status and obesity, and inflammation.

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PATHOLOGY OF AORTIC ARCH IN HYPOPLASTIC LEFT HEART SYNDROME, SURGICAL IMPLICATIONS

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Objective: The success of the Norwood depends in large part upon reconstruction of the aortic arch to provide unobstructed systemic blood flow. Aortic coarctation in hypoplastic left heart syndrome (HLHS) can be a significant problem when a Norwood is performed. Residual coarctation can result from failure to extend the homograft distal enough around the arch, residual ductal tissue or problems related to suture line. Obstruction of the reconstructed aortic arch increases mortality after the Norwood operation. **Methods:** The pathology of the aortic arch in hypoplastic left heart syndrome was studied in 17 autopsy specimens. Nine hearts had undergone Norwood and eight hearts with no surgical intervention with HLHS were selected from formalin-fixed specimens in the Cardiovascular Registry. Aortic coarctation was defined as a localized narrowing (50% or more) in the distal aortic arch. The specimens ranged from stillborn to 5 months of age. 11 with mitral atresia, aortic atresia (65%), 1 with mitral atresia, severe aortic stenosis (6%), 4 with aortic atresia, mitral stenosis (23%) and 1 with severe aortic, mitral stenosis (6%). **Conclusion:** In 8 unoperated specimens, five (62.5%) had significant coarctation of the aorta, being preductal in 4, juxtaductal in 1. In the nine following Norwood 3 (33%) had significant residual coarctation at the distal end of homograft augmentation to the proximal descending aorta. In all 3 the homograft was not extended distal enough into the descending thoracic aorta. To minimize the risk of recurrent aortic arch obstruction in HLHS, ductal tissue encircling the aortic lumen should be resected. The homograft patch should be extended distally to within 2 mm of the first intercostal arteries. This could avoid major aortic arch obstruction.

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MRI VALIDATION OF EFFECTIVE AEROSOL DELIVERY IN A HIGH FREQUENCY OSCILLATOR CIRCUIT IN NEONATAL PIGS

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Background: There are significant challenges in evaluating *in vivo* alveolar drug delivery during mechanical ventilation especially in neonates. We have previously reported effective alveolar delivery of aerosolized Gadopentetate Dimeglumine (Gd-DTPA) using magnetic resonance imaging (MRI) in piglets undergoing conventional mechanical ventilation (CMV) as evaluated by contrast visualization in the lungs and kidneys. Pulmonary deposition of inhaled drugs in neonates undergoing high frequency oscillatory ventilation (HFOV) has not been studied *in vivo*. **Objective:** To evaluate pulmonary delivery of Gd-DTPA following nebulization in a HFOV circuit in piglets using MRI as evaluated by contrast visualization in the kidneys. **Methods:** Four ventilated piglets (3–7 days old, 1.7 to 2.4 kg at birth) were scanned in the Bruker/Siemens 4T MR scanner using T1 weighted spin-echo sequence. Since MR compatible HFOV was not available, aerosolized Gd-DTPA was administered in the HFOV circuit outside the MR suite followed by MR imaging 10–20 min later. Aerosols of Gd-DTPA were generated continuously using the MiniHeart jet nebulizer in the HFOV circuit for different durations (60, 30, 20 and 10 min) to determine the shortest duration of aerosol delivery that would allow sufficient cumulative aerosol delivery to permit MR visualization of contrast in the kidneys. During MRI, manual positive pressure ventilation was provided. **Results:** Enhancement of the collecting system of the kidneys was dramatic and observed as early as 10 min after end of aerosol administration in piglets who received GdDTPA aerosol for 60, 30, and 20 min in the HFOV circuit but not in the piglet who received aerosol for 10 min. Renal concentration of Gd was extracted from the ratio of the signal intensity (SI) as a function of time to the SI at baseline. Gd concentration in kidney increased linearly with time till 40 minutes post Gd with the slope being 0.0034 mM/min. **Conclusions:** We have successfully demonstrated effective pulmonary aerosol deposition in neonatal pigs undergoing HFOV after Gd-DTPA aerosol administration for 20 min or longer as evaluated by contrast visualization in the kidneys. This is likely an underestimate and the use of more sensitive techniques with MR compatible equipment may allow earlier detection of contrast following administration of aerosol for a shorter duration of time. Future studies are needed to quantify efficiency of aerosolized drug delivery in an in vivo closed system.

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IRON BINDING PROTEINS IN HUMAN MILK IN THE FIRST 10 POSTPARTUM DAYS

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Background: Lactoferrin (Ltf), transferrin (Tf) and ferritin (Ferr) are major iron-binding proteins, though their precise role in iron transport in human colostrum or milk is unknown. While it is widely established that Ltf levels decreases in breast milk with increasing postpartum age, the trend of Tf and Ferr has not been investigated in detail during early lactation. **Purpose of Study:** We hypothesized that the magnitude abundance of major iron-binding proteins Ltf, Tf and Ferr in breast milk will decrease with increasing postnatal days and that their levels will directly correlate with milk iron levels. **Methods:** Measurable immunoreactive Ltf, Tf and Ferr concentrations were determined utilizing ELISA and immunoradiometric assays in 196 human milk samples obtained in the first 10 postpartum days (PD) from mothers of 35 term and 37 premature infants. Further, total iron was measured using spectrophotometric assays. **Results:** Milk-borne Ferr abundance levels were significantly greater on PD 0 and 1 compared with that measured between PD 2 through 10 ($p<0.0001$). Ltf and Tf also exhibited a similar trend ($p<0.0001$); levels declined on PD 3. Consistent with literature, iron content of the milk samples also decreased as postnatal day increased ($p<0.0005$). Significant correlations were noted between breast milk iron and Ltf ($p<0.0001$) and Ferr ($p<0.0028$) levels. However, in a multiple stepwise regression, only ferritin was correlated to milk iron. No differences were observed in any of the dependent measures between premature and term groups. **Conclusion:** These results demonstrate that the three iron-binding proteins Ltf, Tf and Ferr may play a significant role in iron transport during early lactation. Further, Ltf and Tf exhibits a sharp decline in their magnitude on PD 3 and Ferr levels dramatically decline on PD2, suggesting that the critical window during early lactation for iron transport proteins could be explained by paracellular transport between mammary epithelial cells. This would require further study.

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INTENSIVE CASE MANAGEMENT (ICM) TO REDUCE PERINATAL HIV TRANSMISSION IN URBAN INNER CITY POPULATION

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Background: HIV infected women face multiple challenges in accessing appropriate services. To provide intensive medical and social services to HIV infected women and their infants during pregnancy and continued care of the mother-infant pair for 18 months following delivery, our team has followed a program of shared personnel and services for the continuum from pregnancy to childhood. **Objective:** To report our experience with ICM from 2001–2007. **Design/Methods:** Pregnant HIV infected women receiving care at Mount Sinai Hospital and its outreach clinics are referred to the HIV maternal child health team comprising of a Nurse Practitioner (NP), Social Worker (SW), Obstetrician and Pediatric infectious disease specialist. The NP under supervision of a physician provides women with primary obstetric and HIV care, focusing on medication compliance and HIV education. The SW arranges transportation, ensures all women attend their medical appointments, assists in obtaining antiviral medications, addresses social service needs, and provides counseling and mental health services. **Results:** During the focus period, 107 HIV infected pregnant women received care. 3 infants tested positive (transmission rate of 2.8%). **Conclusions:** The services of a dedicated and skilled care team can successfully integrate treatment and prevention activities starting in pregnancy and beyond for HIV infected mother and her infant in an urban inner city population.

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IMPACT OF RAPID VIRAL CULTURES ON HOSPITALIZED CHILDREN WITH RESPIRATORY INFECTIONS

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Background: Respiratory viral infections cause a large number of pediatric admissions throughout the winter. Viral antigen testing is used commonly to make a rapid diagnosis; however, the sensitivity is poor. The CDC and WHO recommend a back up or reflex viral culture. Shell vial cultures were implemented for respiratory viral detection in 2007/08. The impact of reflex culture methods on clinical care is unknown. **Methods:** A retrospective case control study was performed on patients admitted from 11/2007 to 1/2008 with negative rapid RSV and/or influenza antigen testing and subsequent hospitalization. A reflex shell vial culture was set up after admission and monitored for growth at 24, 48, and 72 hours. Records of patients with a positive or negative viral culture were reviewed for the following: length of stay, isolation placement, and antibiotic (ab) utilization at admission and discharge. **Results:** Patient records from 100 consecutive positive and negative viral cultures were reviewed. 88% of positive cultures were isolated within 48 hours.

	Positive N=100	Negative N=100	*P* value
Female	49	41	0.26
Age	9 months	4 months	< 0.001
Length of stay	54 hours	52 hours	0.62
Isolation placement	71	67	0.65
Ab at admission	54	51	0.53
Ab at discharge	32	15	0.03

The main reasons for discharge on ab with a positive culture were otitis media (14/32) and pneumonia (12/32).

Conclusion: Reflex viral culture did not impact the management of hospitalized children with viral respiratory illnesses. Total length of stay and antibiotic use were not impacted by these cultures. Even in patients with a positive viral culture, 30% were not placed in appropriate isolation. Surprisingly, more patients with a positive viral culture were discharged on an antibiotic in comparison to those with negative cultures. Future work will be targeted toward educational interventions that aid clinicians in discontinuing antibiotics when viral pathogens are identified.

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WHICH CASES CAUSE MORAL DISTRESS IN THE NICU? FACTORS ASSOCIATED WITH PATIENT REFERRAL TO A COLLABORATIVE CARE ROUND

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Background: Neonatal intensive care units (NICUs) are hotbeds of moral controversy. Nevertheless, NICU professionals rarely consult bioethics committees. Instead, moral controversies tend to be addressed internally, by the staff, within the unit. To facilitate discussion of difficult cases, we have developed a unique approach called Comprehensive Care Rounds (CCRs). Any NICU professional can identify a case for CCR. Physicians, nurses, social workers, and members of the ethics committee attend CCRs. Discussions at CCRs address complex medical, social, or ethical issues. They improve communication, enhance trust, and decrease tension among providers. We wondered what sorts of patients triggered a call by the staff for CCR, so we reviewed all cases referred for a CCR over a 16-month period. **Methods:** We compared the demographic and medical characteristics of all patients who were referred for CCR to those of all patients admitted to our NICU over a 16-month period. **Results:** Forty-five of 1,286 patients (3%) were referred for CCR. The average gestational age of referred patients was 31 weeks (vs 36 for entire NICU). The average length of stay for those referred was 108 days (vs 22 days for entire NICU). The average age at time of referral was 82 days. Eighty-seven percent of those referred were still on ventilators at the time of referral. The main issues generating referrals were related to the management of chronic lung disease and the use of mechanical ventilation, coordination of care among various subspecialties, and discharge planning for technology-dependent children. **Conclusions:** The cases that trigger moral distress in the NICU tend to involve those who have had a long stay and/or remain ventilator-dependent. These patients, although not dying, do not appear to be getting better. Their complex medical problems, uncertain prognoses, and extreme care needs trigger moral distress in their care providers. Similarly, the lack of continuity and shared uncertainty about prognoses lead to tension among health care professionals and between these professionals and parents. The CCR model addresses these issues.

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BRIEF HYPEROXIA: EFFECTS ON NEUTROPHIL FUNCTION

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Background: The safety of oxygen administration for newborn resuscitation has recently been a matter of intense debate. International guidelines recommend the use of 100% oxygen during initial resuscitation of neonates. New data has emerged linking long-term hyperoxia and the development of chronic inflammatory disorders and even brief exposure to 100% oxygen may leads to activation of the pro-inflammatory response. Alternatively, inadequate resuscitation can lead to long-term impairments. No study to date has established the safe duration of hyperoxia. We hypothesized that brief hyperoxia does not activate the inflammatory response. To test this, we focused on the affect of brief hyperoxia on the function of adult neutrophils (PMN) - one indicator of the inflammation. **Methods:** In this case-control study we exposed healthy adult volunteers to 15 min of 100% oxygen at 10L/min flow. PMN were isolated from venous blood samples collected pre-hyperoxia (PRE) and post-hyperoxia (POST). We then investigated 1) PMN inflammatory markers (CD11b and L-selectin expression after chemotactic fMLP stimulation), 2) PMN cytotoxic potential by evaluating the expression of reactive oxygen intermediates (ROI) in PMA-stimulated adult PMN (dihydrorhodamine 123 assay), and 3) evaluated PMN apoptosis (0h, 24h) by annexin V flow cytometric assay. **Results:** 10 subjects (6 females, 4 males) were evaluated. Evaluation of the inflammatory markers in response to fMLP stimulation showed that: 1) L-selectin decreased by 71±28% PRE and 86±9% POST ($p=0.14$) and 2) CD11b increased by 62±37% PRE and 61±57% POST ($p=0.9$). Investigation of PMN cytotoxic function revealed no differences in ROI elaboration with PMA stimulation: 68±89% PRE and 52±41% POST ($p=0.57$). However, apoptosis studies showed an increased percentage of early-apoptotic PMN at 24 h following oxygen exposure: 65±8% PRE and 57±10% POST ($p=0.04$). **Conclusions:** Preliminary results suggest that a brief exposure to hyperoxia may be associated with prolonged PMN survival in adults, although whether such survival correlates with inflammation will require further investigation. Future studies are planned to investigate brief hyperoxia effects in neonates.

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IMPACT OF A PROCEDURE WORKSHOP ON PEDIATRIC RESIDENTS' COMFORT LEVEL WITH PROCEDURES

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Objective: To determine the impact of a clinical skills workshop on the comfort level of pediatric residents on the performance of three different procedures. The procedures selected were lumbar puncture, intravenous (IV) catheter insertion, and venipuncture. **Subjects:** Study subjects were incoming first year pediatric residents of a university-based pediatric residency program for the years 2008 and 2009. **Methods:** 23 incoming pediatric residents participated in a 2 hour procedure workshop. The workshop started with an instructional video, followed by practice of skills on training simulators. Participants were asked to answer a pre-test and post-test, using a 7 point Likert scale, of their comfort level with five aspects of the procedures, as well as four open questions regarding training, previous experience performing the procedure and knowledge of complications. **Results:** Twenty three pre- and post-test pairs were completed. Residents reported a significant improvement in their (1) overall comfort level (median 3 vs. 4, $p<0.001$ by Wilcoxon Signed-Rank Test); and (2) in the level of comfort with all five aspects of the procedure (median 3 vs. 5, $p<0.001$ by Wilcoxon Signed-Rank Test). There was significant correlation between previous experience performing lumbar puncture and overall baseline comfort level (median 2 vs. 3, $p=0.05$ by Mann-Whitney Test). The degree of improvement from baseline comfort levels was similar between those with and those without prior experience ($p>0.05$). **Conclusions:** Pediatric residents who receive specific training and practice on training simulators report higher levels of comfort in their skills to perform certain procedures. Those with no previous experience show equal improvement on their baseline comfort levels compared to those with previous experience.

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EFFECTS OF SURFACTANT ON THE SURVIVAL RATE AND INCIDENCE OF INTRAVENTRICULAR HEMORRHAGE IN INFANTS WITH BIRTH WEIGHT ≤1000 GRAMS DURING THREE DIFFERENT PERIODS IN A SINGLE TERTIARY CENTER

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Objective: To determine the effect of routine use of surfactant in mortality rates and incidence of Intraventricular Hemorrhage (IVH) in extremely premature infants, and to determine any difference in these rates with the use of two different animal-derived surfactants. **Methods:** We reviewed a total of 265 charts of infants born less than1000 grams, in a single tertiary center, during three different years/time periods (1984, 1994, and 2004–2005). Routine use of prophylactic surfactant (Beractant-Survanta®) occurred in 1994. In 2004 we changed to another animal-derived surfactant (Poractant alpha-Curosurf®). In all three periods, cranial ultrasonography was routinely performed at 7 and 28 days of life. Data collected included gestational age at birth, sex, race, birth weight, highest IVH grade, hospital outcome (discharged home, deceased, transfer), and one year survival. **Results:** Chi square analysis of one year survival among extremely low birth weight infants showed significant improvement for both males and females (48% vs 77% vs 79%, $p<0.001$) with highest difference noted among females (57% vs 76% vs 90%, $p<0.001$). These differences were only significant when comparing all three periods, but was not significant when comparing the last two periods only. Race comparison revealed significant improvement on survival rates for non-white females (58% vs 72% vs 98%, $p<0.005$). This improvement remained significant when comparing 1994 vs 2004–2005 periods. No change in the incidence of severe IVH (grade 3 and 4) was observed across the three periods (22% vs 18% vs 22%). **Conclusion:** One year survival rates for extremely low birth weight infants increased significantly with the introduction of surfactant, and have remained stable during the second period despite progress in other areas, such as ventilation and nutrition. However, survival at one year of age for non-white females continued to improve consistently across all three periods. Based on our observations, the type of surfactant used did not influence the outcome in terms of survival or incidence of severe IVH.

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EFFECT OF ANTENATAL BETAMETHASONE VS DEXAMETHASONE ON THE SEVERITY OF INTRAVENTRICULAR HEMORRHAGE IN PREMATURE INFANTS: 10 YEARS EXPERIENCE IN A SINGLE TERTIARY CENTER

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Objective: To determine the effect of antenatal Betamethasone vs Dexamethasone on the severity of intraventricular hemorrhage (IVH) in preterm infants. **Methods:** We reviewed the charts of infants delivered before 32 weeks gestation and birth weight less than 1500 grams who had a diagnosis of IVH. 372 infants were identified in the period between June 1996 and July 2006. 186 charts were available for review. Information collected included gestational age at birth, gender, mode of delivery, birth weight, Apgar score at 1 and 5 minutes, highest grade of IVH, and hospital outcome (discharge or demise). Severe IVH was defined as grade 3 or higher. **Results:** 65% of patients with IVH received antenatal Betamethasone. In those infants with IVH, no significant difference in the rate of severe IVH was observed when comparing patients who received Betamethasone vs those who received Dexamethasone (45% vs 51%, $p=0.59$). There was no significant difference in survival to discharge between the two groups (80% Betamethasone vs 78% Dexamethasone, $p=0.92$). **Conclusions:** From our analysis, survival to discharge and rate of severe IVH are not influenced by the type of antenatal steroid used.