Midwest Society for Pediatric Research

45th Annual Midwest Society for Pediatric Research
Scientific Meeting

October 14-15, 2004

Eric P. Newman Educational Center
at Washington University Medical Center
St. Louis, Missouri
# PROGRAM-AT-A-GLANCE

## October 14–15, 2004

<table>
<thead>
<tr>
<th>TIME</th>
<th>ACTIVITY AND LOCATION</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>WEDNESDAY, OCTOBER 13</strong></td>
<td></td>
</tr>
<tr>
<td>4:00 pm – 6:00 pm</td>
<td>MWSPR Council Meeting</td>
</tr>
<tr>
<td></td>
<td>McDonnell Pediatric Research Building</td>
</tr>
<tr>
<td><strong>THURSDAY, OCTOBER 14</strong></td>
<td></td>
</tr>
<tr>
<td>7:00 am – 8:00 am</td>
<td>MWSPR Registration and Continental Breakfast</td>
</tr>
<tr>
<td></td>
<td>Lobby</td>
</tr>
<tr>
<td>8:00 am – 11:30 am</td>
<td>MWSPR Plenary Session I</td>
</tr>
<tr>
<td></td>
<td>Seminar Room B</td>
</tr>
<tr>
<td>11:30 am – 12 noon</td>
<td>MWSPR Business Meeting</td>
</tr>
<tr>
<td></td>
<td>Seminar Room B</td>
</tr>
<tr>
<td>12:00 pm – 1:30 pm</td>
<td>Founder and Sutherland Award Luncheon</td>
</tr>
<tr>
<td></td>
<td>Great Room B</td>
</tr>
<tr>
<td>2:00 pm – 4:00 pm</td>
<td>MWSPR Plenary Session II</td>
</tr>
<tr>
<td></td>
<td>Seminar Room B</td>
</tr>
<tr>
<td>4:00 pm – 6:00 pm</td>
<td>Reception and Combined Poster Session</td>
</tr>
<tr>
<td></td>
<td>Great Room B</td>
</tr>
<tr>
<td><strong>FRIDAY, OCTOBER 15</strong></td>
<td></td>
</tr>
<tr>
<td>7:00 am – 8:00 am</td>
<td>MWSPR Registration and Continental Breakfast</td>
</tr>
<tr>
<td></td>
<td>Lobby</td>
</tr>
<tr>
<td>7:30 am – 9:00 am</td>
<td>Biostatistics Training Breakfast</td>
</tr>
<tr>
<td></td>
<td><em>(All welcome - you do not need to be a trainee to attend)</em></td>
</tr>
<tr>
<td></td>
<td>Great Room B</td>
</tr>
<tr>
<td>9:15 am – 10:30 am</td>
<td>Combined MWSPR/Washington University Department of Pediatrics</td>
</tr>
<tr>
<td></td>
<td>Grand Rounds</td>
</tr>
<tr>
<td></td>
<td>Main Auditorium</td>
</tr>
<tr>
<td>10:30 am – 12:00 pm</td>
<td>MWSPR Plenary Session III</td>
</tr>
<tr>
<td></td>
<td>Seminar Room B</td>
</tr>
<tr>
<td>12:00 pm – 1:30 pm</td>
<td>MWSPR Kenny, Metcoff, and Cleveland Clinic Awards Luncheon</td>
</tr>
<tr>
<td></td>
<td>Great Room B</td>
</tr>
</tbody>
</table>
This meeting has been made possible in part through the generosity of our supporters and the research efforts of the faculty, fellows, residents, medical and graduate students. We are very proud of the extent and breadth of our research programs and activities, and we trust that you will enjoy the day’s activities.

We would like to thank the abstract reviewers for their time and effort in the review process in this important endeavor.

Planning Committee

Aaron Hamvas, MWSPR President
Thomas Scholz, MWSPR President-Elect
Howard Kilbride, MWSPR Secretary-Treasurer

Acknowledgements

The Midwest Society for Pediatric Research would like to thank the following organizations for their generous support:

Mead Johnson Nutritionals
Ross Products Division, Abbott Laboratories
8:00 WELCOME AND INTRODUCTION
Aaron Hamvas and Howard Kilbride

8:15 State–of–the–Art Speaker
DEVELOPMENTAL AND PEDIATRIC PHARMACOGENOMICS: WHAT, WHY AND HOW?
Steven Leeder, University of Missouri at Kansas City

Carolyn Cannon and Scott Saunders, Presiding

9:00 GALECTIN-1 EXPRESSION IS INCREASED IN THE SECONDARY ALVEOLAR TIPS OF MOUSE LUNG. JJ Foster and JM Snyder, Iowa City, IA. University of Iowa
Abstract 1

9:15 SU1498 INHIBITS ALVEOLIZATION IN NEWBORN MICE. SJ Cho, MJ Acarregui, JM Snyder, and CS George, Iowa City, IA. University of Iowa
Abstract 2

9:30 EXPRESSION OF EPIDERMAL GROWTH FACTOR-LIKE DOMAIN 7 IN NEONATAL RAT LUNGS DURING NORMOXIA AND HYPEROXIA. D Xu, M Rezaiekhaligh, S Mabry, L Shao, and WE Truog, Kansas City, MO. University of Missouri-Kansas City School of Medicine
Abstract 3

9:45 CHARACTERIZATION OF GENETIC VARIATION IN INTRON 4 OF THE SURFACTANT PROTEIN B GENE. DJ Wegner, J Watkins-Torry, M Trusgnich, C Naeger, H Heins, K Madden, Y Liu, A Hamvas, and FS Cole, St. Louis, MO. Washington University School of Medicine
Abstract 4
10:00 – 10:15 Intermission

Lilly Immergluck and David Rudnick, Presiding

10:15 CHARACTERIZING THE EARLY CRANIAL MESODERM: DEVELOPMENT OF THE ENDOTHELIUM. KR Melton, K Zueckert-Gaudenz, J Griffith, and P Trainor, Kansas City, MO. University of Missouri - School of Medicine

10:30 ADVERSE EFFECTS OF CAFFEINE ON EMBRYONIC CARDIAC FUNCTION DURING EARLY CARDIAC MORPHOGENESIS. ME Saaloukeh, K Tobita, N Momoi, and JP Tinney, Pittsburgh, PA. University of Pittsburgh

10:45 CARDIOMYOPATHIC CHANGES IN OFFSPRING OF DIABETIC RATS. BE Reinking, TD Scholz, JL Segar, and RM Weiss, Iowa City, IA. University of Iowa

11:00 THYROID HORMONE INCREASES EAAT1 EXPRESSION IN RAT HEART. JC Ralphe, K Bedell, JL Segar, and TD Scholz, Pittsburgh, PA and Iowa City, IA. University of Pittsburgh

11:15 AN EDUCATIONAL REMEDIATION PROGRAM BENEFITS CHILDREN WITH SICKLE CELL DISEASE AND CEREBRAL INFARCTS. AA King, M Noetzel, R McKinstry, M Armstrong, D White, and MR DeBaun, St. Louis, MO. Washington University School of Medicine

11:30 MWSPR BUSINESS MEETING
Seminar Room B

12:30 FOUNDER AND SUTHERLAND AWARD LUNCHEON
Supported through a restricted educational grant by Mead Johnson Nutritionals
Great Room B
Award presented by: Laurence A. Boxer, University of Michigan

Founder Award Recipient: Robert P. Kelch, University of Michigan

MWSPR PLENARY SESSION II

Catherine Bendel and Steven Pipe, Presiding

2:00  
State–of–the–Art Speaker
SEX AND THE SINGLE CELL.
Ursula Goodenough, Washington University

2:45  
RELATIONSHIP BETWEEN DAYTIME OXYGEN DESATURATION AND SLEEP RELATED BREATHING DISORDER IN CHILDREN WITH SICKLE CELL ANEMIA. JF Spivey, E Uong, and MR DeBaun, St. Louis, MO. Washington University School of Medicine Abstract 10

3:00  
REDUCED INTENSITY CONDITIONING USING CAMPATH-1H IS SUCCESSFUL FOR STEM CELL TRANSPLANTATION IN NON-MALIGNANT DISORDERS. AV Rao, W Grossman, D Wilson, RJ Hayashi, Y Barnes, J Dipersio, L Yu, and S Shenoy, St. Louis, MO and New Orleans, LA. Washington University School of Medicine Abstract 11

3:15  
EARLY GESTATION DEXAMETHASONE EXPOSURE ALTERS CALCIUM TRANSIENTS IN CORONARY VASCULAR SMOOTH MUSCLE CELLS IN NEWBORN LAMBS. EM Segar, RD Roghair, FS Lamb, TD Scholz, and JL Segar, Iowa City, IA. University of Iowa Abstract 12

3:30  
EARLY GESTATION DEXAMETHASONE EXPOSURE ALTERS CORONARY ARTERY REACTIVITY IN NEWBORN LAMBS. RD Roghair, FS Lamb, MC Bailey, TD Scholz, FJ Miller, and JL Segar, Iowa City, IA. University of Iowa Abstract 13
GESTATIONAL AGE AFFECTS ACTIVATION OF THE MITOGEN-ACTIVATED PROTEIN KINASES AND AKT IN THE CHRONICALLY ANEMIC FETAL SHEEP HEART. AK Olson, JL Segar, and TD Scholz, Iowa City, IA. 

University of Iowa 

Abstract 14

RECEPTION AND COMBINED POSTER SESSION WITH WASHINGTON UNIVERSITY MEDICAL STUDENTS 
Great Room B

MWSPR PLENARY SESSION III 
Friday, October 15, 2004

7:30 am – 1:30 pm

BIOSTATISTICS TRAINING BREAKFAST 
Great Room B

Guest Speaker 

IS THE RANDOMIZED CLINICAL TRIAL THE GOLD STANDARD FOR RESEARCH? 
Steve Simon, University of Missouri at Kansas City

According to the experts in Evidence Based Medicine, the randomized clinical trial is the gold standard of proof in research. Criticisms of the randomized trial, however, have highlighted deficiencies in this approach for some study questions. In this session designed for trainees and young investigators, these criticisms will be discussed using practical examples from published literature.

COMBINED MWSPR/WASHINGTON UNIVERSITY DEPARTMENT OF PEDIATRICS GRAND ROUNDS. 
Main Auditorium

Guest Speaker 

QUANTITATIVE AND QUALITATIVE DISORDERS OF NEUTROPENIA. 
Laurence A. Boxer, University of Michigan
10:30 FOOD AS A RISK FACTOR FOR GASTROESOPHAGEAL REFLUX SYMPTOMS IN ADOLESCENTS. GK Namachivayam, TS Gunasekaran, and S Cannavino, Park Ridge, IL. Advocate Lutheran General Children’s Hospital

10:45 ANALYSIS OF WEIGHT LOSS OF PEDIATRIC PATIENTS INVOLVED IN A HEALTHY LIFESTYLES CLINIC (HLC). SE Yount, RC Taylor, K Stephenson, and ID Schwartz, Columbia, SC. University of South Carolina School of Medicine

11:00 DIFFERENT LEVELS OF DOCOSAHEXAENOIC ACID (DHA) IN FORMULA AFFECT RED BLOOD CELL DHA LEVELS IN TERM INFANTS. BJ Blessinger, R Figueroa-Colon, DA Diersen-Schade, DR Hoffman, CL Harris, and EE Ziegler, Evansville, IN, Dallas, TX, and Iowa City, IA. Research and Development, Mead Johnson Nutritionals

11:15 EGR-1 NULL MICE EXHIBIT IMPAIRED HEPATIC REGENERATION. E Shreyer, Y Liao, O Shikapwashya, and DA Rudnick, St. Louis, MO. Washington University School of Medicine

11:30 THREE-YEAR ASSESSMENT IS PREDICTIVE OF OUTCOME AT 8 YEARS OF AGE FOR ELBW SURVIVORS. HW Kilbride, S Simon, and D Dailey, Kansas City, MO and Nashville, TN. University of Missouri-Kansas City

11:45 INVOLVEMENT OF ENDOTHELIAN RECEPTORS IN NEONATAL MORPHINE WITHDRAWAL. B Puppala, S Bhalla, G Matwyshyn, and A Gulati, Park Ridge, IL and Chicago, IL. Chicago Medical School
12:00  MWSPR KENNY, METCOFF, AND CLEVELAND CLINIC AWARDS LUNCHEON  
Great Room B  
Supported through a restricted educational grant by Ross Products Division, Abbott Laboratories

COMBINED POSTER SESSION  
GREAT ROOM B

1  EPIDEMIOLOGY OF PERSISTENT COAGULASE NEGATIVE STAPHYLOCOCCAL BACTEREMIA IN NEWBORN INTENSIVE CARE INFANTS.  
*AL Anderson Berry,* M McCulloch, B Brinton, AC Rentz, S Firth, C Byington, and R Faix, Salt Lake City, UT.  
*University of Utah*  
Abstract 21

2  INCIDENCE OF CATHETER RELATED BLOODSTREAM INFECTIONS AFTER THE REMOVAL OF PERCUTANEOUS INTRAVENOUS CATHETERS IN PREMATURE INFANTS.  
*RW Brooker* and WJ Keenan, St. Louis, MI.  
*St. Louis University*  
Abstract 22

3  EPIDEMIOLOGY OF BLOODSTREAM INFECTIONS IN THE FIRST YEAR AFTER PEDIATRIC LUNG TRANSPLANTATION.  
*LA Danziger-Isakov,* E Mendeloff, S Sweet, M delaMorena, C Huddleston, and MR DeBaun, Cleveland, OH, Dallas, TX, and St. Louis, MO  
*The Children’s Hospital, The Cleveland Clinic Foundation*  
Abstract 23

4  RENAL FUNCTION IN RARE LIVING RELATED SMALL BOWEL TRANSPLANT CHILDREN.  
*EG John,* A Lumpaopong, S Kecskes, G Arteaga, G Testa, and E Benedetti, Chicago, IL.  
*University of Illinois at Chicago*  
Abstract 24

5  BARRIERS TO SCREENING INFANTS FOR RETINOPATHY OF PREMATURITY AFTER DISCHARGE OR TRANSFER FROM A NEONATAL INTENSIVE CARE UNIT.  
*MA Attar,* M Gates, A Iatrow, M Gates, and LS Bratton, Ann Arbor, MI.  
*University of Michigan*  
Abstract 25
6 THE EFFECTS OF A PRACTICE CHANGE IN THE UMBILICAL ARTERIAL CATHETER REGIMEN FOR LESS THAN 800-GRAM INFANTS. JK Jackson, HW Kilbride, J Raucci, and TL Sandritter, Kansas City, MO. University of Missouri - School of Medicine

7 TRANSIENT HYPERAMMONEMIA IN PRETERM INFANTS WITH HYPOXIA. G Brar, R Thomas, E Bawle, and V Delaney-Black, Detroit, MI. Wayne State University

8 NUTRITION PRACTICE GUIDELINES AND NEONATAL OUTCOMES IN ELBW INFANTS. R Donovan, B Puppala, D Angst, and B Coyle, Park Ridge, IL. Advocate Lutheran General Children's Hospital

9 THE IMPACT OF EARLY AND CONSISTENT OCCUPATIONAL THERAPY WITH PRETERM INFANTS' ORAL FEEDINGS. NM Mohr, SR Lacey, S Teasley, and HW Kilbride, Kansas City, MI and Huntsville, AL. Children's Mercy Hospital and Clinics

10 INITIATION OF ORAL FEEDINGS IN INFANTS LESS THAN 34 WEEKS TO FACILITATE EARLY DISCHARGE FROM NICU. K Macwan, Peoria, IL. UIC College of Medicine at Peoria

11 THE LONG TERM OUTCOME OF FUNDOPPLICATION IN CHILDREN WITH GASTROESOPHAGEAL REFLUX: A RETROSPECTIVE ANALYSIS. H Mousa, F Woodley, M Metheney, and J Hayes, Columbus, OH. Columbus Children's Hospital

12 FACTORS INFLUENCING PARENT ANXIETY LEVELS IN A PEDATRIC EMERGENCY DEPARTMENT WAITING AREA. LJ Holm and LS Fitzmaurice, Kansas City, MO. Children's Mercy Hospital
13 WHAT INFLUENCES PARENTS' DECISION TO LIMIT OR WITHDRAW LIFE SUPPORT? M Sharma, KL Meert, and AP Sarnaik, Detroit, MI. Wayne State University

14 NON-INVASIVE CONTRAST ENHANCED ELECTRON BEAM TOMOGRAPHY: A THREE DIMENSIONAL IMAGING ADJUNCTIVE TEST TO DETECT RENAL VASCULAR AND A-V FISTULA GRAFT ABNORMALITIES. EG John, A Lumpaopang, and C Ruiz, Chicago, IL. University of Illinois at Chicago

15 ETIOLOGY AND TREATMENT OF PEDIATRIC PLEURAL EMPYEMA. MA Jackson, LC Olson, and D Bratcher, Kansas City, MO. University of Missouri-Kansas City


17 COMPARISON OF MULTIDRUG RESISTANCE PROTEIN-1 (MRP-1) AND P-GLYCOPROTEIN (PGP) EXPRESSION IN THE DEVELOPING HUMAN CENTRAL NERVOUS SYSTEM: CELLULAR AND TISSUE LOCALIZATION. MJ Daoood, M Ahdab-Barmada, and JF Watchko, Pittsburgh, PA. Pittsburgh University

18 BILIRUBIN EFFLUX BY BRAIN CAPILLARY ENDOTHELIAL CELL MONOLAYERS IN VITRO: ROLE OF P-GLYCOPROTEIN. D Sequeira, MJ Daoood, JF Watchko, and B Mahmood, Pittsburgh, PA. Pittsburgh University
DEVELOPMENTAL IRON DEFICIENCY IMPAIRS WATERMAZE PERFORMANCE FOR RATS. BT Felt, H Tian, J Shao, JD Barks, and T Schallert, Ann Arbor, MI and Austin, TX. Abstract 39
GALECTIN-1 EXPRESSION IS INCREASED IN THE SECONDARY ALVEOLAR TIPS OF MOUSE LUNG.
JJ Foster1, JM Snyder2, Department of Pediatrics1 and Department of Anatomy and Cell Biology2, University of Iowa, Carver College of Medicine, Iowa City, IA.

Bronchopulmonary dysplasia, a common complication of prematurity, is characterized by inadequate alveolarization. The process of alveolarization, by which the lung forms mature gas-exchanging units, is not well understood. In mice, alveolarization occurs during postnatal days four through twelve, when the formation of secondary septa creates thin walled alveoli. The purpose of this study was to investigate the genes involved in this process. RNA was isolated from dissected tips of secondary septa and whole lung tissue of day six postnatal mouse lung. The tips of secondary septa were obtained via laser capture microscopy of frozen sections. Total RNA was isolated from the tip and whole lung samples and amplified in the same manner. Affymetrix gene profiling was then performed, using mouse U74Av2 GeneChips. The signal for galectin-1 mRNA was six fold higher in the secondary septal tips than in whole lung tissue (p<0.05). Galectins, or S-type lectins, are beta-galactoside-binding proteins involved in the regulation of cell proliferation, differentiation and apoptosis. Galectin-1 is a homodimer of two 14 kDa subunits. It is the most abundant galectin in the lung. To confirm the relative abundance of galectin-1 in secondary septal tips versus whole lung, immunostaining of sections of day six postnatal and adult mouse lung tissue was performed. Staining for galectin-1 was concentrated in the tips of the secondary septa in the day six postnatal tissue. Furthermore, staining was dramatically increased in the day six mouse lung tissue when compared to staining levels in the adult lung sections. Immunoblot analysis of lung homogenates obtained at different stages of lung development demonstrated that a peak of galectin-1 expression occurs at postnatal days six and twelve, corresponding to the time of alveolarization. The increased expression of galectin-1 at the site and time of ongoing alveolarization suggests that it may play a role in this important aspect of lung development. Supported by NIH grants HL-62861 and HL-07638.
SU1498 INHIBITS ALVEOLARIZATION IN NEWBORN MICE.
SJ Cho, CS George, JM Snyder, MJ Acarregui, Carver College of Medicine, University of Iowa, Iowa City, IA.

BACKGROUND: Bronchopulmonary dysplasia (BPD) in premature infants is characterized by inhibited alveolarization and vasculogenesis. Inhibitors of angiogenesis induce emphysema in newborn rats resulting in a phenotype similar to BPD in premature infants. Our goal was to generate a mouse model of inhibited alveolarization that could be employed to explore the mechanisms resulting in, and interventions for BPD. SU1498 is a commercially available compound that inhibits vascular endothelial growth factor receptors.

METHODS: Three day old C3H/HeNrsd mice were injected with a single dose of SU1498 (30mg/kg, SC). Lungs of control (sham-injected) and treated mice were inflation fixed on postnatal day 21. Tissue sections were mounted and morphometric analysis was performed to determine the volume density (VD) of air space, tissue, large blood vessels, conducting airways, and alveolar surface area. Lungs were also harvested for electron microscopic analysis of alveolar structures.

RESULTS: The VD of airspace (63.7±1.9% vs. 53.2±1.2%) and conducting airways (3.0±0.9% vs. 0.9±0.4%) were significantly greater in treated versus control mice (n=8, P<0.05). The VD of large blood vessels was not different between the two groups. The alveolar tissue VD (31.0±1.5% vs. 43.9±1.3%) and the alveolar surface area (318.6±31.6 cm² vs. 238.1±22.8 cm²) were significantly less in treated versus control mice (n=8, P<0.05). Electron microscopy demonstrated a decrease in alveolar wall thickness in the lungs of treated mice. Treatment also resulted in fewer but enlarged capillaries compared to controls.

CONCLUSION: A single of dose of the VEGFR inhibitor, SU1498, to newborn mice results in inhibition of alveolar development at 21 days. This phenotype provides a model for the investigation of mechanisms resulting in inhibited alveolarization. Such investigation may lead to strategies for the prevention or treatment of BPD in prematurely born infants.
EXPRESSION OF EPIDERMAL GROWTH FACTOR-LIKE DOMAIN 7 IN NEONATAL RAT LUNGS DURING NORMOXIA AND HYPEROXIA.

D Xu*, M Rezaiekhaligh*, S Mabry*, L Shao‡, WE Truog*, *Department of Pediatrics, ‡Department of Pathology, Children's Mercy Hospitals and Clinics, University of Missouri-Kansas City SOM, Kansas City, MO.

Purpose of Study: Preterm babies treated with ventilator support and supplemental oxygen frequently develop chronic lung disease (CLD) that has significant mortality and morbidity. Oxygen toxicity plays an important role in CLD etiology. Several lines of evidence have suggested that impairment of pulmonary angiogenesis is implicated in alveolization and the development of CLD. Epidermal growth factor-like domain 7 (EGFL-7) is a recently identified protein secreted from vascular endothelial cells and it regulates vascular tubulogenesis (Nature 2004;428:754). Aim of this study was to measure EGFL-7 expression in the neonatal lung during normoxia and hyperoxia.

Methods: Rat pups at 4 days of age were randomly assigned to normoxic and hyperoxic groups. The rats in the normoxic and hyperoxic groups were treated with room air and 95% O2 for 3, 6, and 10 days, respectively. The lung tissues were collected for total RNA isolation. EGFL-7 mRNA expression was measured by quantitative real-time reverse-transcription polymerase chain reaction (Q-RT-PCR). Separately, human umbilical vein endothelial cells (HUVEC) were cultured in 37°C, 5% CO2 incubator, and were exposed to normoxia (room air) or hyperoxia (95% O2).

Results EGFL-7 mRNA in normoxic neonatal rat lung was consistently expressed from 7 days to 2 months of age (n = 3) at each time. EGFL-7 mRNA expression in the hyperoxic group was significantly decreased after oxygen exposure for 3, 6 and 10 days; it decreased 2.1 fold at day 3 (n = 3); 4.1 fold at day 6 (n = 3); and 3.1 fold at day 10 (n = 3) compared to time-matched normoxic group results, respectively. EGFL-7 mRNA expression in the hyperoxic group returned to nearly normal levels 2 weeks (n = 3) after discontinuing oxygen exposure, and it remained at normal levels during the 2 month recovery period (n = 2-3). In cultured HUVEC, EGFL-7 mRNA expression also decreased 2.6 fold after 95% O2 exposure for 48 hours.

Conclusions: Oxygen exposure is associated with the decrease of EGFL-7 mRNA expression in the neonatal rat lung and the expression level returns to normal after oxygen treatment. These findings imply that reduced levels of EGFL-7 at a critical lung development stage may be a contributing factor in the impairment of pulmonary angiogenesis and alveolization after hyperoxic lung injury.
4
CHARACTERIZATION OF GENETIC VARIATION IN INTRON 4 OF THE SURFACANT PROTEIN B GENE.

Expression of the surfactant protein B gene is required for function of the pulmonary surfactant. The 9.5 kb surfactant protein B gene includes 10 translated and exons and 1 untranslated exon. Genetic variants in intron 4 characterized by insertions or deletions of 11 distinct motifs have been associated with respiratory distress in some populations of infants, adult respiratory distress syndrome, and risk of squamous cell carcinoma of the lung. Due to polymerase enzyme stutter, characterization of allelic variation by direct sequencing has been difficult. To examine genetic variation in intron 4 in a cohort of Missouri infants (n=240), we identified a polymerase enzyme with high fidelity for intron 4 amplification and analyzed product length by agarose gel electrophoretic mobility. In 480 alleles, we found 14.4% (69/480) variant alleles, 9.4% with insertions and 5.0% with deletions. Allelic diversity was significantly greater among African-Americans (n=204, 19.1% insertion alleles, 2.9% deletion alleles) than Caucasians (n=244, 2.0% insertion alleles, 7.4% deletion alleles) (p<.001). Insertion variants were strongly associated with African Americans (28/31), and deletions were associated with Caucasians (15/20) (p<.001). We then cloned fragments from a subset of 42 infants (22 African-American, 19 Caucasian, 1 Hispanic). Automated sequencing of the 32 variant alleles revealed 6 previously unreported variants (all insertions) that accounted for an unexpectedly high proportion (18/32) of variant alleles sequenced. Two different insertion variants shared agarose gel electrophoretic mobility (Mr~570 bps), as did 2 other insertion variants (Mr~600 bps). When analyzed by automated sequencing, these variants differed in motif insertion or deletion or in length of dinucleotide (CA) repeat. The C genotype at genomic position 1580, a C/T nonsynonymous, single nucleotide polymorphism in exon 4, was strongly associated with insertion (28/31 = T)( p<.001). We conclude that genotype at genomic position 1580 and race are associated with intron 4 variation. Characterization of intron 4 by agarose gel electrophoretic mobility alone may fail to detect important differences in genetic variation in intron 4 that contribute to risk for respiratory disease.
CHARACTERIZING THE EARLY CRANIAL MESODERM: DEVELOPMENT OF THE ENDOTHELIUM.

K Melton, K Zueckert-Gaudenz, J Griffith, and P Trainor, Stowers Institute and Children’s Mercy Hospital, Kansas City, MO.

In classic models of craniofacial development, the cranial mesoderm was thought to play only a passive role, receiving signals from the migrating neural crest cells (NCC). Recent studies suggest that the cranial mesoderm can influence NCC identity and migration, suggesting a more active role for the mesoderm in craniofacial development. The goal of our study was 1) To identify genes specific to the cranial mesoderm that may influence NCC development, 2) To characterize their spatial and temporal expression, and 3) To evaluate the effect of identified genes on NCC development. Using an Affymetrix microarray, we have identified 184 genes expressed at a >3 fold difference in the cranial mesoderm when compared to a pooled endoderm/ectoderm sample. A large number of endothelial genes and genes involved in vascular development were identified by the screen, including Vegf-C, Flk-1 and Flt-1, Fli-1, Sox18, VE-cadherin, Esam1, Claudin 5 and Igfbp4, which suggests that development of the endothelium may play an important role in early craniofacial formation. In situ hybridization analysis demonstrates that the endothelial genes show diffuse punctate expression throughout the cranial mesoderm, and focus on endothelial gene Igfbp4 demonstrates that Igfbp4 is dynamically expressed in the developing branchial arches. Functional analysis of Igfbp4 using bead implantation experiments demonstrates that Igfbp4 is upregulated by FGF8 but is not influenced by SHH expression. Further work is underway to evaluate the effect of Igfbp4 and other endothelial genes on NCC development using gene overexpression in Pax3-GFP transgenic mice.
ADVERSE EFFECTS OF CAFFEINE ON EMBRYONIC CARDIAC FUNCTION DURING EARLY CARDIAC MORPHOGENESIS.
M.E. Saaloukeh, K Tobita, N. Momoi, J.P. Tinney, and B.B. Keller, Division of Pediatric Cardiology, Children’s Hospital of Pittsburgh, Pittsburgh, PA.

Caffeine is a naturally occurring product that acts as a mild central nervous system stimulant. In humans the major sources of caffeine are coffee, tea, and soft drinks, as well as cocoa, chocolate, and certain medications. Caffeine is metabolized more slowly in pregnant women and due to the hydrophobic properties of caffeine it can cross the placenta and the brain-blood barrier. Studies in human and animal models have shown that caffeine exposure during pregnancy affects the perinatal cardiovascular system as well as central nervous system and can result in intrauterine growth retardation and stillbirth. Recent studies show that caffeine intake increases risk of first-trimester spontaneous abortion in human. However, the extent and the mechanism by which maternal caffeine intake influences embryonic cardiovascular function during early morphogenesis is not known. We hypothesized that caffeine ingestion during early pregnancy impairs embryonic cardiac function by delaying the onset of heart beat and alters the normal increase in heart rate (HR) resulting in growth delay and first-trimester spontaneous abortion. Eight to 12 week-old pregnant CD-1 mice and 81 embryos were studied under an approved IACUC protocol. Caffeine was dissolve in distilled water and administered daily by gavage at a dose of 120mg/kg from gestational days 0.5 to 10.5. We monitored embryonic heart rate (HR) from gestational days 8.5 to 10.5 at 24 hour intervals using a 40MHz ultrasound biomicroscope. At gestational day 10.5, embryos were fixed and somite number and external morphology was assessed. This period of gestational includes the onset of heart beat of the primitive heart tube through the completion of heart looping. Onset of heart beat was significantly delayed in caffeine group at gestational day 8.5 (heart beat was detected in 41% of caffeine treated embryos versus 79% of sham treated embryos). HR increase was higher in caffeine group at gestational days 9.5 (127±4 in caffeine vs. 112±5 in sham, respectively, p<0.05) and 10.5 (150±4 vs. 140±4). At gestational day 10.5, caffeine treated mice had a significantly higher rate of embryo abortion (10% in caffeine vs. less than 2% in sham, p<0.05). Somite number was similar in both groups, however, body size, head size, and upper extremity length was significantly smaller in caffeine group. Thus, our results confirm that intrauterine caffeine exposure alters embryonic cardiac function (onset of heart beat, normal HR increase), embryo growth, and embryo survival during a critical period of early cardiovascular morphogenesis.
CARDIOMYOPATHIC CHANGES IN OFFSPRING OF DIABETIC RATS.

BE Reinking, RM Weiss, JL Segar, TD Scholz, Department of Pediatrics, Carver College of Medicine University of Iowa, Iowa City, IA.

Background: Infants born to mothers with gestational diabetes are known to have organomegally and asymmetric septal hypertrophy. The purpose of this study is to evaluate the myocardial response in the offspring of severely diabetic rats and characterize the mitogen-activated protein kinase (MAPK) signaling pathways that may mediate these responses. Hypothesis: In a rat model of infant of diabetic mothers (IDM), cardiomyopathic changes develop in the offspring and that the MAPKs will regulate the cardiac responses. Methods: Pregnant rats were given either 50mg/kg of streptozotocin or normal saline intravenously on day 7 of gestation (term=23 d). Maternal blood glucose levels were monitored. Animals were studied on days 18 (E18) and 21 (E21) of gestation, and postnatal days 1 (NB1), 5 (NB5) and 21 (NB21). Hearts were harvested and the ventricles weighed and then frozen in liquid nitrogen. MAPKs measured by Western blot included total and activated (phosphorylated) levels of c-jun-n terminal kinase 1 (JNK1 and pJNK1) and 2 (JNK2 and pJNK2) and extracellular signal-regulated kinase 1/2 (ERK1/2 and pERK1/2). NB1 and NB21 pups underwent echocardiographic study to evaluate left ventricular dimensions and function. Results: The mean heart to body weight ratio (HW/BW) was significantly elevated in the offspring of diabetic mothers when compared to controls due to a significant decline in BW (see Table). No differences were observed between controls and IDM offspring in the levels of ERK1/2, pERK1/2, JNK1, pJNK1 and pJNK2. Total JNK2 differed by age and treatment when analyzed by two-way ANOVA (p<.05). Echocardiographic analysis revealed a greater than two-fold increase in end-systolic LV volume and reduced LV ejection fraction in the NB1 IDM pups compared to controls although cardiac output was unchanged. LV dimensions and function were not different between IDM and control pups at NB21.

Table 1. IDM Presented as Percent of Control (* p< .05 vs control by unpaired t-test)

<table>
<thead>
<tr>
<th></th>
<th>E 18</th>
<th>E 21</th>
<th>NB 1</th>
<th>NB 5</th>
<th>NB21</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>13</td>
<td>38</td>
<td>38</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td>HW</td>
<td>94%</td>
<td>79%*</td>
<td>80%*</td>
<td>65%*</td>
<td>89%</td>
</tr>
<tr>
<td>BW</td>
<td>79%*</td>
<td>68%*</td>
<td>70%*</td>
<td>61%*</td>
<td>78%*</td>
</tr>
<tr>
<td>HW/BW</td>
<td>121%*</td>
<td>131%*</td>
<td>112%*</td>
<td>108%*</td>
<td>117%*</td>
</tr>
</tbody>
</table>

Conclusions: With severe diabetes, IDM had diminished somatic growth that exceeds the decline in heart growth. A cardiomyopathy was present in the immediate newborn period that did not result in activation of the MAPK pathways and resolved by 21d postnatally.
8

THYROID HORMONE INCREASES EAAT1 EXPRESSION IN RAT HEART.

J. Carter Ralph, Kurt Bedell, Jeffrey L. Segar, Thomas D. Scholz, Department of Pediatrics, University of Iowa, Iowa City, IA.

Background: Hyperthyroid induced cardiac hypertrophy is related to increased cardiac workload. These changes are associated with an upregulation of metabolic pathways associated with energy production. The malate/aspartate shuttle, necessary to transfer the reducing equivalents produced by glycolysis into the mitochondria, is increased 33% in hyperthyroid rats. Of the shuttles two inner membrane protein carriers, the aspartate-glutamate carrier is rate-limiting. The Excitatory Amino Acid Transporter, Type 1 (EAAT1) has recently been shown to function as a glutamate carrier in the malate/aspartate shuttle. We hypothesize that EAAT1 is upregulated by thyroid hormone.

Methods: Adult Sprague-Dawley rats were infused with, tri-iodothyroxine (T3), propylthiouracil (PTU), or saline over a period of 8 days. Serum free T3 levels were measured. Rats were euthanized, hearts weighed, and tissue frozen. Northern Blot analysis was performed on total RNA using a unique 350 bp 32-P labeled EAAT1 ribonucleotide probe and normalized to 18S rRNA. A spectrophotometric assay of the malate/aspartate with glutamate and lactate as substrates was performed on isolated mitochondria. Results are displayed as oxidation rate/min/mg mitochondrial protein. Protein lysates from mitochondria were used for immunoblot analysis with human anti-EAAT1 IgG.

Results: EAAT1 steady-state mRNA levels were increased in the T3-treated rats compared to controls (0.031+/-0.005 vs. 0.011+/-0.002; P<0.05), and decreased in PTU-treated rats vs. controls (0.0011+/-0.0002 vs. 0.0015+/-0.0001; P<0.05). EAAT1 mitochondrial protein levels were increased in T3-treated rats vs. controls (8.9+/-0.4 vs. 5.9+/-0.6; P<0.005). EAAT1 protein levels were below detection in PTU-treated rats. Malate/aspartate shuttle activity was unchanged by PTU infusion.

Conclusions: Hyperthyroidism in rats is related to an increase in expression of both EAAT1 mRNA and protein in cardiomyocytes. This 49% increase in the rate-limiting aspartate-glutamate carrier of the malate/aspartate shuttle correlates with the observed increase in shuttle activity. The upregulation of EAAT1 by thyroid hormone may facilitate the cardiomyocyte response to hyperthyroidism and the associated increased metabolic demand placed upon the cell.
AN EDUCATIONAL REMEDIATION PROGRAM BENEFITS CHILDREN WITH SICKLE CELL DISEASE AND CEREBRAL INFARCTS.

A. King, D White, M Armstrong, R McKinstry, M Noetzel, MR DeBaun, Departments of Pediatrics, Psychology, Radiology and Neurology, Washington University and St. Louis Children’s Hospital, St. Louis, MO.

The overall goal of this project was to determine the feasibility of an educational remediation program for children with sickle cell disease (SCD) and cerebral infarcts. Approximately 30% of children with SCD have cerebral infarcts before eighteen years of age, at least 60% of this group have been retained a grade in school, and 79% will have a cognitive deficit. We performed a prospective randomized pilot trial for children with SCD, cerebral infarcts and memory deficits. Participants were randomly allocated to either a control or intervention group to test the following hypothesis: Targeted memory strategy remediation will have a greater improvement in (1) memory skills and (2) academic achievement of children with SCD, cerebral infarcts, and memory deficits than in the same group of children who receive general tutoring. Both the control and intervention groups received general tutoring for four semesters; additionally, the intervention group received specific remediation strategies to improve their memory skills. In the first year, tutors met with students for one hour per week; this was increased to two-one hour sessions per week during the second year. Parents were asked to spend at least the same amount of time with their children each week. The primary outcome measure was assessment of memory performance before/after completion of the intervention. Secondary outcomes were Wechsler Individual Achievement Tests (reading, math, spelling). Nine of eleven children completed the two-year program. Children in the intervention group significantly improved their abilities in two measures of memory: delayed cued memory (19 point improvement, p=.01) and working memory (31 point improvement (2SD) in the digits backward measure, p=.04). Both groups had a range of improvement (1-26 points) in academic achievement tests, but these gains did not reach statistical significance. Unfortunately, most parents did not or could not follow through with tutorials or practicing memory drills at home. In conclusion, we have provided preliminary results that suggest memory remediation may be a feasible method to improve memory skills for children with SCD and cerebral infarcts and provide evidence for further studies in this area of investigation.
RELATIONSHIP BETWEEN DAYTIME OXYGEN DESATURATION AND SLEEP RELATED BREATHING DISORDER IN CHILDREN WITH SICKLE CELL ANEMIA.

J. Spivey, E. Uong, M.R. DeBaun, Department of Pediatrics, Allergy/Pulmonary, and Hematology/Oncology, St. Louis Children’s Hospital, St. Louis, MO.

Recent studies strongly suggest that nocturnal oxygen desaturation in children with sickle cell anemia is associated with an increase incidence of pain and stroke. However, no relationship has been established between daytime oxygen desaturation and sleep disturbance. To determine the relationship between daytime oxygen desaturation and sleep related breathing disorder in children with sickle cell anemia we evaluated 17 patients with HbSS disease referred to the sleep laboratory at St. Louis Children’s Hospital. All patients were referred for daytime oxygen desaturation defined as less than 94% on room air by pulse oximetry. Each patient underwent an overnight recorded polysomnogram with sleep guidelines and parameters following the general consensus statement and standards set by the American Thoracic Society. Results showed 82% of the patients had nocturnal hypoxemia with overnight mean oxygen saturation less than 94% with values ranging from 75-95% and a mean oxygen saturation of 89%. 18% of the patients had an oxygen saturation less than 85% overnight, and 65% of the patients had a formal recommendation of nocturnal supplemental oxygen use after the polysomnogram. 53% of the patients had a diagnosis of Obstructive Sleep Apnea Syndrome (OSAS) with an Apnea Index range of 0.1-7.5 and a Respiratory Disturbance Index range of 4-41.4. Our preliminary results suggest that patients with sickle cell anemia have an increased risk of sleep related breathing disorder including OSAS and nocturnal hypoxemia. Patients with low daytime oxygen desaturation are at risk for sleep related breathing disorders. Further prospective evaluations are underway to validate these findings and to elucidate the etiology of sleep disturbances in this vulnerable population.
REDUCED INTENSITY CONDITIONING THERAPY USING CAMPATH-1H IS SUCCESSFUL FOR STEM CELL TRANSPLANTATION IN NON-MALIGNANT DISORDERS.

A. Rao1,2, R. Hayashi1,2, W. Grossman1,2, D. Wilson1,2, Y. Barnes2, J. DiPersio3, L. Yu4,5, S. Shenoy1,2, 1Washington University School of Medicine, 2St. Louis Children’s Hospital, 3Barnes Jewish Hospital, St. Louis, MO 4Louisiana State University, 5Children’s Hospital of New Orleans, New Orleans, LA.

Stem cell transplantation (SCT), indicated for many non-malignant disorders, is limited by donor availability, graft rejection (GR), toxicities of conditioning, morbidity and mortality (TRM), and graft versus host disease (GVHD). To overcome these barriers, we tested a novel conditioning for SCT. It was designed to support engraftment by deleting host immune reactive lymphocytes and macrophages. Campath-1H (anti-CD52 mab) was given on days -21, -20, and -19 (total dose 48 mg), fludarabine (day -8 to -4) (total 150 mg/m2) and melphalan on day -3 (140 mg/m2). Stem cell sources were related/unrelated bone marrow (BM) (8), peripheral blood (PB) (5) and umbilical cord blood (UCB) (3). GVHD prophylaxis was cyclosporine (tapered after 3 months), methylprednisone (tapered after day +28) and methotrexate on days +1, +3, and +6 (except in UCBT). End points studied were engraftment and TRM. Sixteen patients (1.5-40 yrs) with aplastic anemia (5), Hurler’s (2), sickle cell anemia, XLAAD, histiocytosis (3), thalassemia, adrenoleukodystrophy, Evan’s syndrome and dyserythropoietic anemia were transplanted. Median follow-up was 219 days (66-845). The regimen was tolerated well. All patients that survived >1 month engrafted. Neutrophils (ANC >500/dL) engrafted at 12.5 days; platelets (>50,000/dL) at 21 days (median). Skin GVHD developed in 3 patients and resolved early. Eight patients are off; 4 are tapering immune suppression. All survivors have either stable disease or are cured. One survivor had a normal pregnancy. Two patients died prior to engraftment from previously acquired Pseudomonas infection. Two died of CMV disease and intracranial hemorrhage/refractory thrombocytopenia respectively after engraftment. Other complications were bacterial and viral infections occurring within 100 days. Profound lymphopenia was present 1 month. NK cells recovered by 3 months, CD8+ cells by 6 months, CD4+ and B cells 6-9 months after transplant. Immunoglobulin (Ig M and A) levels reflected B cell numbers; Ig A recovered later than IgM. In summary, successful engraftment despite varied stem cell sources was achieved without significant GVHD using this regimen. Lymphopenia resulted in a significant infection risk early post transplant, requiring close surveillance and intervention. Thus, this transplant regimen is well tolerated and may preserve fertility, making it a promising alternative to conventional SCT.
EARLY GESTATION DEXAMETHASONE EXPOSURE ALTERS CALCIUM TRANSIENTS IN CORONARY VASCULAR SMOOTH MUSCLE CELLS IN NEWBORN LAMBS.

EM Segar¹, RD Roghair², FS Lamb², TD Scholz², JL Segar², Department of Pediatrics², Carver College of Medicine, University Of Iowa, and West High School¹, Iowa City, IA.

Background: Fetal programming of adult diseases, including coronary artery disease, may be a consequence of fetal exposure to increased levels of maternally derived glucocorticoids. In an established ovine model, early gestation glucocorticoid exposure alters postnatal coronary artery vascular reactivity. This programming effect may be related to modifications in intracellular calcium regulatory responses. Hypothesis: To determine if early gestational glucocorticoid exposure alters agonist-induced cytosolic calcium concentrations in coronary artery vascular smooth muscle (VSM).

Methods: Dexamethasone (dex, 0.28 mg/kg/d IV for 48 h) was administered to pregnant ewes at 27-28 days gestation (term 145 d). Ewes were allowed to deliver and the offspring and control lambs studied at a postnatal age of 12 ± 3 d (n=3 for each group). Intracellular calcium responses of primary cultured coronary and carotid VSM cells to a variety of agonists were determined in Fura-2 (a fluorescent Ca2+ indicator) loaded cells using a calcium imaging system (n = 8 - 40 cells for each condition).

Results: Baseline [Ca2+]i was similar in control and dex-exposed coronary VSM. Dex-exposed coronary VSM displayed decreased peak calcium responses to angiotensin II (1uM) and bradykinin (1 uM) compared to controls; peak responses to endothelin-1 (1 uM) and the thromboxane agonist U46619 (10 UM) were similar in both groups. KCl (90mM) caused an increase in [Ca2+]i (322 ± 35 nM) in control VSM but had no effect on dex VSM. The [Ca2+]i response to U46619 in control but not dex VSM was attenuated by the calcium channel blocker nifedipine (10 uM). Conversely, KCl increased [Ca2+]i in dex-exposed carotid VSM, and nifedipine decreased the calcium response to U46619. Dex also enhanced changes in [Ca2+]i in response to angiotensin II and U46619 in carotid VSM. Conclusion: Early gestation glucocorticoids program altered regulation of VSM [Ca2+]i in a vessel-specific manner. In particular, dex diminished apparent function of voltage-gated calcium channels in coronary but not carotid VSM. These observations suggest a mechanism whereby an altered in-utero environment may predispose to coronary artery dysfunction later in life.
EARLY GESTATION DEXAMETHASONE EXPOSURE ALTERS CORONARY ARTERY REACTIVITY IN NEWBORN LAMBS.
RD Roghair¹, MC Bailey¹, FS Lamb¹, FJ Miller Jr.², TD Scholz¹, JL Segar¹,
Department of Pediatrics¹ and Department of Internal Medicine², College of
Medicine, University of Iowa, Iowa City, IA.

BACKGROUND: Exposure of the ovine fetus to glucocorticoids early in gestation programs postnatal elevation of blood pressure and enhanced coronary artery reactivity to second messenger-dependent vasoconstrictors. It is unknown whether the coronary alterations are a direct consequence of the corticosteroid exposure or related to the hypertension that evolves over the first 4 months of life. HYPOTHESIS: Early gestation glucocorticoid exposure enhances newborn lamb coronary artery vascular reactivity in the absence of systemic hypertension. METHODS: Dexamethasone (dex, 0.28 mg/kg/day iv for 48 hours) was administered to pregnant ewes at 27-28 days gestation (term being 145 days). The ewes were allowed delivery, and offspring were studied at a postnatal age of 8 ± 2 days (N = 6). Non-dex exposed age-matched control lambs were used for all comparisons (N = 6). Vascular catheters were placed 48 h prior to recording blood pressures. The contractile responses of circumflex coronary, mesenteric and femoral artery rings were then measured by wire myography.

RESULTS: Exposure to dex was not associated with alterations in mean arterial blood pressure or heart rate at 8 days of life. Coronary vessels from dexamethasone-exposed sheep exhibited enhanced vasoconstriction to endothelin-1 and acetylcholine (both P < 0.05). There was no difference in maximal response of the coronary arteries to potassium chloride or angiotensin II. Dex exposure was associated with attenuation in vasodilatation to adenosine, but not sodium nitroprusside or forskolin. No differences in contractile response were detected between dex-exposed and control mesenteric or femoral arteries.

CONCLUSION: Early gestation glucocorticoid exposure selectively programs postnatal alterations in coronary artery vascular reactivity prior to the development of hypertension. These findings suggest coronary artery dysfunction is a primary programming phenomenon and not secondary to alterations in blood pressure. These coronary vascular alterations may provide a mechanistic link between an adverse intrauterine environment and later coronary artery dysfunction.
GESTATIONAL AGE AFFECTS ACTIVATION OF THE MITOGEN-ACTIVATED PROTEIN KINASES AND AKT IN THE CHRONICALLY ANEMIC FETAL SHEEP HEART

AK Olson, JL Segar, TD Scholz; University of Iowa, Iowa City, IA.

Background: The postnatal heart responds to biomechanical stress by myocyte hypertrophy, whereas the fetal heart may additionally undergo hyperplasia. Before 100 d gestational age (GA), nearly 100% of fetal sheep cardiomyocytes are mitotically active, which decreases to about 20% near term. The mitogen-activated protein kinases (MAPKs) and Akt are hypertrophic signaling pathways in adult hearts. The activities of these pathways are not well characterized in the loaded fetal heart. Objective: To test the hypothesis that activation of myocardial p38, c-jun-n terminal kinase (JNK), extracellular signal-regulated kinase 1/2 (ERK 1/2) and Akt by increased cardiac load resulting from chronic anemia is developmentally regulated in early versus late GA sheep. Methods: Anemia was created in fetal sheep at 98 or 134 d GA (term 145 d) by daily isovolemic hemorrhage (20-30 ml or 60-100 ml, respectively) for 7 d (n = 7 for both ages). Age-matched, non-bled twins served as controls. Right (RV) and left ventricular (LV) MAPK and Akt protein levels were determined by Western blot. Data are given as mean ± SE. Results: In 98 d anemic fetuses, hemoglobin (9.4±0.3 to 4.7±0.3 g/dL) and arterial oxygen content (7.8±0.4 to 3.3±0.3 mL O2/dL) decreased significantly from days 1 to 8 of the study, while total heart weight normalized to body weight was significantly increased (6.69±0.3 vs 5.69±0.3 g/kg) compared to controls (p<0.05). RV and LV total and active protein levels of JNK, ERK 1/2 and Akt were similar between 98 d anemic and control fetuses, as were total levels of p38. Compared to the 134 d anemic fetuses, the 98 d anemic fetuses showed significantly greater RV and LV total and active protein levels of ERK and Akt (p<0.05). Total levels of LV p38 and RV and LV JNK were increased in 98 d versus 134 d anemic fetuses although active levels of RV and LV JNK and total levels of RV JNK were unchanged. Conclusions: In contrast to the late GA fetal heart, activity of MAPK pathways in early GA fetal myocardium is not altered by chronic anemia, indicating MAPKs are not essential for adaptation to an increased load. Volume loading of the fetal sheep heart leads to developmentally regulated cell signaling profiles – possibly related to the high percentage of mitotically capable myocytes in the immature fetal heart.
**FOOD AS A RISK FACTOR FOR GASTROESOPHAGEAL REFLUX SYMPTOMS IN ADOLESCENTS.**

G Namachivayam, TS Gunasekaran, Division of Pediatric Gastroenterology, Advocate Lutheran General Children’s Hospital, Park Ridge, IL, & S Cannavino, Maine East High School, IL.

Gastroesophageal Reflux (GER) is a common GI disorder. We reported a prevalence of 38% of esophageal GER symptoms among adolescents\(^1\) and found cigarette smoking, alcohol and non-steroidal anti-inflammatory drugs (NSAIDs) were risk factors\(^2\). Now we are analyzing if certain foods and drinks are risk factors for GER symptoms in the same age group.

**Aim:** To find out the association between GER symptoms and the following as risk or protective factors: spicy foods, citrus fruit juices, 12 caffeinated and 15 non-caffeinated beverages, obesity, NSAIDs, alcohol, smoking and chewing gum.

**Methods:** A cross sectional survey was done among 14-18 year old students at a high school. The survey instrument contained questions on esophageal (heartburn, regurgitation and dysphagia), respiratory symptoms (cough and shortness of breath) over the past year measured by symptom frequencies on a 6-point scale\(^1\) and questions on the proposed risk factors. The data were entered into a MS Access Database and analyzed using SPSS.

**Results:** Drinking coffee or tea, caffeine containing carbonated drinks (Barq’s root bear, Dr. Pepper, Diet Dr. Pepper) and caffeine-free carbonated drinks (Sierra mist, Barq’s diet root bear, A & W root bear, IBC root bear, Mug root bear, 7-Up, ginger ale, caffeine-free Coke, and Fanta) were found to be risk factors. Spearman’s rho was between 0.01 to 0.30 and p value less than 0.05. Eating spicy foods, drinking citrus fruit juices or chocolate drinks were not risk factors. Subjects with greater BMI tended to have more frequent GER symptoms (rho=0.11, p=0.016). As we showed earlier\(^2\), alcohol, NSAID use and cigarette smoking were found to be risk factors (Odds ratio: NSAIDs – 1.38, cigarettes – 1.76, alcohol – 1.35, p<0.05).

**Conclusion:** Certain carbonated coffee containing and caffeine free drinks were found to be risk factors for GER symptoms. Coffee drinking had a higher risk than tea for GER symptoms. Contrary to our previous study\(^2\), increasing BMI was a risk factor. Use of NSAID, alcohol and cigarette smoking were risk factors for GER symptoms. Chewing gum was not found to be protective for GER symptoms.

**References:****
ANALYSIS OF WEIGHT LOSS OF PEDIATRIC PATIENTS INVOLVED IN A HEALTHY LIFESTYLES CLINIC (HLC).
SE Yount, RC Taylor, K Stephenson, ID Schwartz, Divisions of General Pediatrics and Endocrinology, Palmetto Health and University of South Carolina School of Medicine, Columbia, SC.

Pediatric obesity has become pandemic accompanied by various health risks. Structured, non-uniformed weight (wt) loss programs are being established at pediatric facilities across the country. We have organized a program utilizing a multidisciplinary team of nutritionists, physicians, and PharmD’s called our HLC. **Purpose:** To assess the outcome data as to the efficacy of HLC involving obese pediatric pts. **Methods:** A retrospective chart review of obese pediatric pts enrolled in our university-based HLC. Initial auxologic data were compared with last available data. **Results:** Data were reviewed on 218 obese pts (81M/137F). 137 pts were analyzed who had >1 visit to HLC (47M/90F; AA=76.6%, C=19.7%, H=1.5%, O=2.2%; age 11.4±3.2yrs). F/u visits numbered 2-10 (3.4±1.7; median=3) over 0.5-30 mos (6.3±5.6 mos; median=11.7). 64 pts [24M/40F; AA=81.3%, C=14.1%, H=1.5% (n=1), O=3.1% (n=2) received metformin (MTF). Insurance status: Private=30.7%, Medicaid=54.0%, 15.3%=not documented.

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>Cauc. (n=27)</th>
<th>AA (n=105)</th>
<th>Other (n=5)</th>
<th>All (n=137)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wt Δ (kg)</td>
<td>-2.0±6.3</td>
<td>1.7±6.7</td>
<td>0.1±5.1</td>
<td>0.9±6.7</td>
</tr>
<tr>
<td>Wt Δ + MTF</td>
<td>-5.1±9.0</td>
<td>0.4±5.6</td>
<td>-1.4±3.4</td>
<td>-0.5±6.3</td>
</tr>
<tr>
<td>Wt Δ - MTF</td>
<td>-0.4±2.1</td>
<td>2.1±6.8</td>
<td>2.4±8.1</td>
<td>1.5±6.0</td>
</tr>
<tr>
<td>∆ BMI (kg/m²)</td>
<td>-2.3±5.9</td>
<td>0.1±3.2</td>
<td>0.0±2.4</td>
<td>-0.3±3.9</td>
</tr>
<tr>
<td>Sex</td>
<td>♂ (n=11)</td>
<td>♀ (n=16)</td>
<td>♂ (n=35)</td>
<td>♀ (n=90)</td>
</tr>
<tr>
<td>Wt Δ (kg)</td>
<td>-3.9±8.8</td>
<td>-0.7±3.5</td>
<td>4.1±10.0</td>
<td>2.3±10.1</td>
</tr>
<tr>
<td>Wt Δ + MTF</td>
<td>7.0±10.7</td>
<td>-1.4±2.8</td>
<td>1.5±7.4</td>
<td>-0.6±9.0</td>
</tr>
<tr>
<td>Wt Δ - MTF</td>
<td>1.2±1.6</td>
<td>-1.0±1.9</td>
<td>4.2±11.0</td>
<td>3.7±9.5</td>
</tr>
<tr>
<td>∆ BMI (kg/m²)</td>
<td>-4.1±8.5</td>
<td>-0.8±1.5</td>
<td>0.5±4.3</td>
<td>-0.6±5.9</td>
</tr>
</tbody>
</table>

Only some pt groups had statistically significant decrease in wt, but of questionable clinical significance: C vs Total Group (p=0.04); C♂ vs AA♂ (p=0.02). For pts w/o Rx w/MTF, C had significant wt loss vs Total Group (p=0.03) and vs AA (p=0.02). C♀ had significant wt loss vs C♂ (p=0.04) and vs All females (p=0.03) and vs AA♀ (p=0.01). Addition of MTF, led to further wt loss for C pts of both genders but p=NS due to wide SD. BMI Δ was not significant for any group. **Conclusion:** Our HLC had limited success.
DIFFERENT LEVELS OF DOCSAHEXAENOIC ACID (DHA) IN FORMULA AFFECT RED BLOOD CELL DHA LEVELS IN TERM INFANTS.

BJ Blessinger*, R Figueroa-Colon*, DA Diersen-Schade*, DR Hoffman**, CL Harris*, EE Ziegler***, *Research and Development, Mead Johnson Nutritional, Evansville, IN, **Retina Foundation of the Southwest, Dallas, TX, ***University of Iowa, Iowa City, IA.

DHA is an important component of the brain. Red blood cell (RBC) DHA levels are thought to be related to brain DHA content and to visual acuity in infancy. The DHA content of the RBC of infants is determined by the DHA content of the feeding. To assess the effects of different levels of formula DHA on RBC DHA levels of infants, we conducted a multi-center, double-blind, prospective study. Infants were randomized to one of two infant formulas: Formula Higher DHA with DHA at 0.32% of total fatty acids, similar to worldwide mean levels found in breast milk, and Formula Lower DHA with DHA at 0.15% of total fatty acids, similar to lower levels typically found in breast milk in the USA. Infants were fed study formula from 14 through 120 days of age. Fatty acids in blood lipid fractions were analyzed by capillary gas chromatography at 120 days of age. The table summarizes DHA levels in total-RBC lipids, RBC-phosphatidylcholine (RBC-PC), RBC-phosphatidylethanolamine (RBC-PE), and plasma phospholipids (Plasma-PL) at 120 days (% of total fatty acids, Mean ± SE).

<table>
<thead>
<tr>
<th></th>
<th>Higher DHA</th>
<th>Lower DHA</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n = 30)</td>
<td>(n = 26)</td>
<td></td>
</tr>
<tr>
<td>DHA, Total RBC</td>
<td>6.67 ± 0.13</td>
<td>4.74 ± 0.15</td>
</tr>
<tr>
<td>0.001</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>DHA, RBC-PC</td>
<td>3.73 ± 0.09</td>
<td>2.39 ± 0.10</td>
</tr>
<tr>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>DHA, RBC-PE</td>
<td>10.85 ± 0.21</td>
<td>7.65 ± 0.24</td>
</tr>
<tr>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>DHA, Plasma PL</td>
<td>5.85 ± 0.13</td>
<td>4.01 ± 0.14</td>
</tr>
<tr>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

Both formulas were well tolerated, and infants in both groups had similar growth. We conclude that infants fed formula containing higher levels of DHA have significantly higher circulating levels of DHA. We speculate that greater incorporation of DHA into brain and retinal tissues may result from higher circulating levels of RBC DHA.
EGR-1 NULL MICE EXHIBIT IMPAIRED HEPATIC REGENERATION.

Yunjun Liao, Olga N. Shikapwashya, Eyal Shteyer, and David A. Rudnick, Department of Pediatrics, Washington University School of Medicine, St. Louis, MO.

The liver regenerates itself in response to a wide variety of injuries. The rodent partial hepatectomy model has been a useful tool with which to investigate the signals that regulate this regenerative response. These signals include activation of an immediate-early gene expression program during early liver regeneration which directs growth factor-dependent hepatocellular proliferation and leads to restoration of normal hepatic mass. The early growth response 1 transcription factor (EGR-1), whose expression is known to be regulated in a variety of models of cellular growth and differentiation, has been shown to be induced as part of the immediate early gene expression response during liver regeneration. In the studies reported here the functional significance of EGR-1 expression during liver regeneration was examined by characterizing the hepatic regenerative response to partial hepatectomy in EGR-1 null mice. The results of these studies showed that liver regeneration in EGR-1 null mice is impaired. Although the early signaling events leading up to the first wave of hepatocellular DNA synthesis occurred normally following partial hepatectomy in EGR-1 null mice, subsequent signaling events and cell cycle progression after the first round of DNA synthesis were deranged. This derangement was characterized by increased activation of the p38 mitogen activated protein kinase and inhibition of hepatocellular metaphase-to-anaphase mitotic progression. Together these observations suggest that Egr-1 is an important regulator of hepatocellular mitotic progression through the spindle-assembly checkpoint. In support of this, microarray-based gene expression analysis showed that induction of expression of the cell division cycle 20 gene (CDC20), a key regulator of the mitotic anaphase promoting complex, is significantly reduced in EGR-1 null mice. Taken together these data define a novel functional role for EGR-1 in regulating hepatocellular mitotic progression during liver regeneration.
THREE-YEAR ASSESSMENT IS PREDICTIVE OF OUTCOME AT 8 YEARS OF AGE FOR ELBW SURVIVORS.

H Kilbride¹, Dept of Pediatrics, and S Simon¹, Office of Medical Research, Children's Mercy Hospital¹, Kansas City, MO, and D Daily, Vanderbilt University Medical Center, Department of Pediatrics, Nashville, TN.

Background: Extremely low birth weight infants are at increased risk for neurodevelopmental disabilities. It would be useful to determine if preschool screening could predict which children will be normal at school age so follow-up resources could be directed to those at greatest risk. Objective: To determine if neurodevelopmental assessment at 3 yrs of age is predictive of outcome findings at 8 yrs of age for children who were <801 g BW. Methods: All surviving infants 450 to 800 g BW cared for at hospitals associated with UMKC School of Medicine were enrolled in a multidisciplinary follow-up clinic. Children were evaluated with a standard battery of cognitive, motor, and language tests. At 3 and 8 yrs, outcomes of children were categorized by composite results as having major disability, mild disability, or normal, according to pre-established criteria (Kilbride, Daily, J Perinatol 1998;18:102). The relationship between the neurodevelopmental category at 3 yrs and outcome measures at 8 yrs was assessed by ordinal logistic regression analysis, controlling for confounding variables (GA, BW, SES, and IVH). Results: 149 children were evaluated. Mean BW (range) was 702±80 g (490-800) and GA, 25.8±1.5 weeks (23-31). There was no significant relationship between BW or GA and outcome at 8 yrs. Infants without IVH were statistically more likely to be categorized as normal (51% vs 31%, grade I-II and 27%, grade III-IV, P=.04), and had higher IQ scores (89±15 vs 82±19, grade I-II and 82±17, grade III-IV). IQ scores were also related to SES (high SES, 90±15 and low SES, 82±19, P=.05). Independent of these variables, neurodevelopmental categorization at 3 yrs was highly correlated with 8-yr outcome findings (P <.000). At 3 yrs of age, 20% (43/149) were considered normal, 54% (80/149) mildly disabled, and 19% (26/149) had major disability. For those with a normal assessment at 3 yrs, 84% were considered normal and 16% mildly disabled at 8 yrs. None of these children had major disability. For those with mild disability at 3 yrs, 31% were normal and 10% had major disability at 8 yrs. 81% of those with major disabilities at 3 yrs remained in that category at 8 yrs; the other 19% were categorized as having mild disability. Overall, between 3 and 8 yrs, outcome status improved for 20% and declined for 10% of children. Conclusion: No child identified as normal at 3 yrs was found to have a major disability at 8 yrs of age. An evaluation at 3 yrs of age using composite categorization of cognitive, motor, and neurosensory assessments may accurately predict functional outcome at school age.
INVOLVEMENT OF ENDOTHELIN RECEPTORS IN NEONATAL MORPHINE WITHDRAWAL.

Bhagya L. Puppala¹, Shaifali Bhalla², George Matwyshyn², Anil Gulati²,
Department of Pediatrics and Neonatology¹, Advocate Lutheran General
Children’s Hospital, Park Ridge, IL, Department of Biopharmaceutical
Sciences², University of Illinois at Chicago, Chicago IL.

Purpose: Management of neonatal opioid tolerance and withdrawal symptoms
remains a major clinical challenge in NICUs. Numerous neuromodulators are
involved in tolerance and withdrawal mechanisms. We have previously shown
that central endothelin (ET) receptors are involved in morphine tolerance. The
purpose of this study is to investigate the involvement of central ET receptors in
morphine withdrawal in neonatal rats. Methods: Pregnant rats were divided into
two groups and rendered tolerant to placebo and morphine pellets, respectively,
over a 7-day period. On day 8, both placebo and morphine pellets were removed
and rats were allowed to undergo withdrawal for 24 hours. Rat pups were
delivered by c-section. Neonatal rat brains were dissected and used for analysis.
Changes in G-protein stimulation were determined by using [(35)S]-guanosine-
5′-o-(3-thio)triphosphate ([35S]GTPγS) binding assay in the brain of neonatal
rats undergoing placebo and morphine withdrawal.

Results: Morphine produced
significantly higher (P<0.05) maximal stimulation in neonatal rats undergoing
morphine withdrawal (83.60%) when compared to placebo (66.81%). EC₅₀
values in morphine withdrawal group (6.825nM) were significantly lower
(P<0.05) as compared to placebo (72.917nM). A significant increase in maximal
G-protein stimulation was observed with ET-1 in the morphine withdrawal
group (87.16%, P<0.05) as compared to placebo (74.88%). EC₅₀ values for ET-1
in neonatal rats undergoing morphine withdrawal (93.75nM) were significantly
higher (P<0.05) than placebo (62.5nM). ETₐ receptor antagonist, BMS182874,
did not stimulate GTP binding in placebo brains (EC₅₀>1000nM), but
significantly increased (P<0.05) maximal stimulation of G-proteins in morphine
withdrawal group (86.07%, EC₅₀=31.25nM). ETₐ agonist, IRL1620-induced
stimulation of G-proteins was similar in placebo (73.43%, EC₅₀=13.26nM) and
morphine withdrawal (75.08%, EC₅₀=11.70nM), respectively. Conclusion:
Morphine-induced G-protein activation is increased while ET-1 induced G-
protein activation is decreased in neonates undergoing morphine withdrawal.
ETₐ antagonist increases activation of G-proteins during withdrawal in
neonates. In conclusion we provide evidence for the first time that central ETₐ
and not ETₜ receptors are involved in morphine withdrawal.
EPIDEMIOLOGY OF PERSISTENT COAGULASE NEGATIVE STAPHYLOCOCCAL BACTEREMIA IN NEWBORN INTENSIVE CARE INFANTS.
Ann Anderson-Berry, Mary McCulloch, Britt Brinton, Alison Rentz, Sean Firth, Carrie Byington, Roger Faix, University of Utah, Salt Lake City, UT.

BACKGROUND: Coagulase-negative staphyloccocal (CONS) bacteremia is the most common nosocomial infection in most newborn intensive care units (NBICU). Infected infants have increased mortality, and morbidity compared to uninfected peers. At the University of Utah NBICU increasing numbers of infants have developed persistent (cultures positive for >48 hours after initiation of antibiotics to which organisms are susceptible in vitro) and prolonged persistent (positive for > 96 hours) CONS bacteremia. OBJECTIVE: To determine rates of persistent CONS bacteremia in NBICU infants in 2002-2003 and to compare risk factors for prolonged persistent vs. persistent CONS bacteremia. DESIGN/METHODS: A computerized microbiology database identified positive NBICU blood cultures from 1999-2003. Chart review of infants with CONS bacteremia documented possible risk factors for persistent and prolonged persistent infection in 2002-2003. Discrete variables were analyzed by chi-square for trend. Analysis for normally distributed continuous variables was by t-test, for non-normally distributed continuous variables by Mann-Whitney test. RESULTS: From 2001-2003, the frequency of bacteremia due to all bacteria has remained stable (0.03/1000 patient days, p= 0.9). From 1999-2003, CONS bacteremia increased (0.3-0.8-0.7-0.9/1000 admissions, p<0.01). Persistent CONS bacteremia (culture positive for >48 hours despite appropriate antibiotics) also increased (0.0-0.2-0.3-0.4-0.5/1000 admissions, p<0.01). Analysis of 51 infants was performed to determine risk factors for prolonged persistent CONS. Sixteen had CONS infection for 48-96 hours vs. 35 for >96 hours. Significant differences were found in days of antibiotics prior to onset (p=0.03), feeding intolerance at onset (p=0.03), and ventilator days associated with CONS (p<0.001). No difference in gestational age, day of life (DOL) of bacteremia onset, DOL to full enteral feeds, birth weight, or association with central vascular catheter < 72 hours prior to first positive culture was found. CONCLUSIONS: Persistent CONS has increased in our NBICU from 1999-2003. Significant differences were found between bacteremia < or > 96 hours with respect to prior days of antibiotic exposure, feeding intolerance, and CONS associated ventilator days. Differences were not found in gestational age, DOL onset, birth weight, indwelling central line in the 72 hours prior to CONS onset or time to full enteral feeds.
INCIDENCE OF CATHETER RELATED BLOODSTREAM INFECTIONS AFTER THE REMOVAL OF PERCUTANEOUS INTRAVENOUS CATHETERS IN PRETERM INFANTS.

RW Brooker, St. Louis University, Cardinal Glennon Children’s Hospital

The use of PICC lines in preterm infants increases the risk for systemic bacterial infections. The overall incidence of Catheter Related Bloodstream Infection (CRBSI) after PICC line removal is not well known. A single dose of antibiotics is sometimes used to try to decrease the incidence of CRBSI. Antibiotic prophylaxis to prevent CRBSI on removal of a PICC and increased use of antibiotics may increase resistant strains of bacteria.

A retrospective review of medical records of infants with gestational age less than 29 completed weeks was designed. Patients were hospitalized between January 2003 and December 2003. Infants were excluded if there was death prior to PICC removal. Data for collection were prospectively identified and included total days of PICC life, days of antibiotics through PICC, days of TPN through PICC, BSI within 48 hours of PICC removal, gestational age of infants and their birthweight, method and location of PICC placement, infections noted during PICC life, sepsis evaluations within 48 hours of PICC removal, and catheter tip culture results.

About 80% of babies less than 29 weeks had a PICC placed during their hospital course. 3 infants out of 72 had a CRBSI associated with a PICC. No bloodstream infections were detected within 48 hours after the removal of a PICC. No infants with a PICC had a sepsis evaluation within 48 hours of PICC removal. All infants with a PICC received total parenteral nutrition through the line. Antibiotic administration was generally remote to PICC removal, but some received antibiotics up to the removal of the PICC. Catheter tips were rarely cultured after PICC removal.

PICC removal is a safe procedure without antibiotic prophylaxis, even in the face of a PICC life greater than 20 days. There is not evidence to support the administration of antibacterial prophylaxis for removal of PICC.
EPIEMIOLOGY OF BLOODSTREAM INFECTIONS IN THE FIRST YEAR AFTER PEDIATRIC LUNG TRANSPLANTATION.
LA Danziger-Isakov, The Children’s Hospital, The Cleveland Clinic Foundation, Cleveland, OH; E Mendeloff, Medical City Hospital, Dallas, TX; S Sweet, M delamorena, C Huddleston, MR DeBaun, Washington University, St. Louis, MO.

Background: Significant morbidity and mortality associated with infection occurs in the first year after pediatric lung transplantation. To better understand the clinical significance of bloodstream infections (BSI), we systematically evaluated the epidemiology of BSI in the first year posttransplant. Methods: A retrospective case-cohort study of pediatric primary lung transplant recipients was performed. The incidence of BSI and organisms isolated were determined through medical and laboratory record review. We assessed variation in causative organisms and rate of BSI in three time periods after transplantation: acute (0-30 days), intermediate (31-90 days) and late (91-365 days). Results: From July 1990 to November 2000, 190 pediatric patients received primary lung transplants. Twenty-six percent (49 of 190) of recipients had at least one BSI. The most common organisms isolated were Coagulase-negative staphylococcus (n=25, 28.4%), Pseudomonas aeruginosa (n=14, 16.0%), and Candida species (n=9, 10.2%). The overall rate of BSI was 2.1 per 1000 catheter-days. The highest rate of BSI occurred in the acute period compared to the intermediate and late periods at 5.5, 1.3 and 1.6 per 1000 catheter-days, respectively (P = 0.21). Early BSI was associated with death in the first year after transplant (RR=3.9, 95% CI 1.6-9.4, p = 0.002). Conclusions: BSI occurs frequently after primary pediatric lung transplantation with the highest rate in the first 30 days after transplantation. Early BSI is associated with death in the first year after transplant. Prevention of early BSI could potentially decrease mortality after pediatric lung transplantation.
RENAL FUNCTION IN RARE LIVING RELATED SMALL BOWEL TRANSPLANT CHILDREN.
E John, A Lumpsapong, S Kecskes, G Arteaga, G Testa, E Benedetti,
Department of Pediatrics and Surgery, University of Illinois at Chicago,
Chicago, Illinois

BACKGROUND: Data from United Network for Organ Sharing (UNOS) and Organ Procurement and Transplantation Network (OPTN) reveals that living related small bowel transplant (LRSMT) in children, age less than 5 years, has been performed only 5 patients since 1988 - 2004. From these national data, 4/5 LRSMT were done at The University of Illinois Medical Center. In this study, we present renal function in this group of patients.

METHODS: Retrospective review of renal function was done in 3 patients: 4 LRSMT (1 patient underwent retransplant). Mean aged at LRSMT was 2.31 ± 1.5 years (1-4 years). Mean follow up period was 11.5 ± 8.3 months (4-21 months). All patients had underlying gastronchis and short bowel syndrome. Immunosuppressive regimen included tacrolimus and steroid therapy. Serum electrolyte, glomerular and tubular functions were measured at 1, 3, 6, 9,12 and 18 months after transplant. Creatinine clearance (CrCl) was estimated by Schwartz formula and tubular functions were estimated by calculating fractional excretion of sodium (FENa, normal < 1%), magnesium (FEMg, normal 2-2.5%), urine calcium/creatinine ratio (UCa/Cr, normal < 0.21) and tubular reabsorption of phosphorus (TRP, normal 80-90%). Proteinuria was diagnosed when urine protein/creatinine (Uprot/Cr) > 0.2.

RESULTS: Patient survival was 100 %. One patient lost graft secondary to PTLD at 4 months post LRSMT and underwent combined liver-small bowel transplant 8 months later. CrCl at 1, 3, 6, 9,12 and 18 months was 92.2±16, 98.1±25, 95.2±5, 108.5±2, 112.5 (n=1), and 113.9 (n=1) cc/min/1.73 m² respectively. Proteinuria and tubular dysfunctions were observed after LRSMT and gradually improve. (Uprot/Cr = 1.49 ±1.0, FENa = 2.3±0.9 %, UCa/Cr = 0.20± 0.2, FEMg = 15.7±7.8 %, and TRP = 76.6±19.3 % at 1 month and Uprot/Cr = 1.27± 0.7, FENa = 1.1±1.0 %, UCa/Cr = 0.11±0.1,FEMg = 13.8±11.4 %, and TRP = 87.0±17.1 % at 3 months). All patients had renal tubular acidosis, hypocalcemia, hypomagnesemia and hypophosphatemias and received bicarbonate, calcium, magnesium and phosphorus supplement. Tacrolimus level at 1, 3, 6, 9, 12 and 18 months was 17.0 ± 2, 9.6 ± 3, 11.7 ± 1, 12.8 ± 1, 12.5 (n=1) ng/ml respectively.

CONCLUSION: Abnormal glomerular, tubular function and electrolyte imbalance was observed after LRSMT especially in the early phase. Serial monitoring of renal function and long term follow up is necessary in this patient group.
BARRIERS TO SCREENING INFANTS FOR RETINOPATHY OF PREMATURITY AFTER DISCHARGE OR TRANSFER FROM A NEONATAL INTENSIVE CARE UNIT.
M Attar, M Gates, A Iatrow, S Lang, S Bratton, Department of Pediatrics, University of Michigan, Ann Arbor, Michigan

Neonatal intensive care unit (NICU) practices may influence the delivery of ophthalmology care for infants eligible for back transfer to a referring community hospital. We conducted this study to assess barriers to screening for Retinopathy of Prematurity (ROP) after discharge or transfer from a NICU.

**Study Design:** Retrospective study of 78 infants who needed ophthalmology examinations at the time of their discharge or transfer from the NICU. These infants either needed screening for ROP or had retinal examinations in the NICU and needed further follow-up. **Results:** 64% of infants received appropriate eye care. Infants who did not receive the follow-up care had greater mean gestational age (mean SD; 30.7±2.3 vs. 29.4±2.6 weeks, p=0.02) and birth weights (mean SD; 1581±366 vs. 1329±504 gm, p=0.007) compared to infants who received the recommended care. There were no statistical differences between the two groups when comparing their race, maternal age, maternal marital status, insurance coverage, or site of birth. Forty-three (55.1%) infants were transferred back, 26 (33.3%) infants were discharged home from the NICU, while nine (11.6%) infants were discharged home from the regional center after their transfer to the general pediatrics service. Infants discharged from the NICU were significantly more likely to receive recommended eye care compared to infants transported back to the community hospital or transferred to the pediatric service at the regional center (Relative Risk Ratio 1.5, 95% confidence interval 1.1-2.1, p=0.01). Infants transferred or discharged from the NICU not screened for ROP (n=49) had lower completion rates compared to infants who had their first retinal examination in the NICU and needed follow-up (n=29) (89% vs. 49%, p<0.0001). Infants whose ophthalmology follow up was recommended in the discharge summary and had their appointments arranged prior to discharging them from the community hospital or the regional center were ten time more likely to get the appropriate eye care (Relative Risk Ratio 10.7, 95% Confidence Interval 2.8-40.3, p<0.0001) compared to infants who did not have arrangements and recommendations. Parents were compliant (n=56, 89%) with pre-arranged appointments. **Conclusions:** Infants transferred back or discharged from the NICU before having a retinal examination represent a high-risk group for not receiving eye screening. Scheduling appointments for these infants when they leave the NICU may improve their ROP screening and care.
THE EFFECTS OF A PRACTICE CHANGE IN THE UMBILICAL ARTERIAL CATHETER REGIMEN FOR LESS THAN 800-GRAM INFANTS.

JK Jackson, TL Sandritter, J Raucci, HW Kilbride, Children’s Mercy Hospital and Clinics, UMKC School of Medicine, Kansas City, MO.

Background: We have previously demonstrated through a randomized study that in comparison to a hyptonic regimen, an isotonic amino acid (IAA) umbilical arterial catheter (UAC) solution/flush regimen is associated with less hemolysis, improved nutrition and alterations in fluid/electrolyte balance. We hypothesized that the difference in fluid/electrolyte balance was contributed to by differences in renal function in the 2 groups in relation to the generation of plasma free hemoglobin secondary to hemolysis. Since implementation of findings from a controlled study may be met with unpredictable obstacles and results, we have continued to monitor data as the isotonic regimen was introduced into practice. Objective: To assess the introduction of an IAA UAC infusion/flush regimen into general practice and to evaluate fluid/electrolyte status in infants before (group 1) and after (group 2) implementation in an effort to delineate underlying factors related to fluid/electrolyte balance in infants <800 g BW. Design/Methods: This was a retrospective review of information collected for QI purposes. Patients, < 800 g with UAC in place, were identified from 2 available databases. IRB approval was obtained. The data was analyzed to determine differences in serum sodium (Na), potassium (K), blood urea nitrogen, (BUN), creatinine (Cr); fluid balance, BUN:Cr ratio; and line complications. Based on data from our previous study, 24 infants in each group would provide powers of 99%, 94%, and 94% (α= 0.05) for sodium levels, intakes, and urine outputs, respectively. Results: The data of 33 and 24 infants were evaluated from the time period before and after the change in practice respectively. The new practice was implemented in 86% of cases. There were no group differences in demographics. Mean GA (range) 25 wks (22-28), BW 651 g (435-782), days with UAC 5.7 (1-13). Na level was higher and K level lower in group 2 in the 1st 2 days of life. There was no difference in UAC complications, Cr, BUN levels, urinary output or volume intake. BUN:Cr ratio was higher in group 2 in comparison to group 1 (25±7 vs 21±9, respectively; P<.05). There was also a significant difference in the number of critical high and low Na values between the two groups. Conclusion: The difference in BUN:Cr ratio supports the theory of improved renal function resulting in relative volume depletion in the IAA UAC group. Other group differences were not as pronounced as in the controlled trial, possibly relating to the imprecise implementation of the new practice. These findings support need for monitoring introduction of practices from controlled trials.
TRANSIENT HYPERAMMONEMIA IN PRETERM INFANTS WITH HYPOXIA.
G Brar, R Thomas, EV Bawle, V Delaney-Black, Division of Neonatology, Wayne State University, Detroit, MI.

Purpose of study: Transient hyperammonemia of the newborn can lead to an overwhelming and potentially fatal illness. The etiology of this condition is obscure although hypoxia, immature urea cycle and inadequate hepatic blood flow have all been implicated. We hypothesized that preterm infants with hypoxia would have elevated ammonia levels after birth that normalize within 3-4 weeks. The study aim was to measure serial plasma ammonia levels in preterm infants with hyaline membrane disease (HMD) or perinatal asphyxia to elucidate the role of hypoxia in the pathogenesis of hyperammonemia.

Methods: Infants 24-37 weeks gestation with severe HMD or perinatal asphyxia who had indwelling umbilical catheters and parental consent were enrolled. Hyperalimentation with 10% trophamine was initiated at 24-48 hours of life. Blood samples were drawn at 24 hours, 48 hours and weekly until ammonia levels normalized. Plasma ammonia was analyzed using Vitros AMON Slide Analyzer. A level > 50 umol/l was considered elevated. Pregnancy and neonatal data were collected. Repeated measures ANOVA was employed to examine mean differences in serial plasma levels in each group. All analyses were conducted using SPSS Version 11.5.

Results: The study sample consisted of 20 neonates in the HMD and 6 in the asphyxia group. Infants with asphyxia had significantly lower apgar scores, cord pH and higher ALT levels. In the HMD group, mean plasma ammonia level decreased from 100.68±28.35 umol/l at 24 hours to 88.42±23.61 umol/l at 48 hours (p=0.003) and 56.84±18.42 umol/l at week 2 (p<0.001). In the perinatal asphyxia group, mean ammonia levels decreased from 97.67±10.78 umol/l at 24 hours to 88.67±9.02 umol/l at 48 hours (p=0.003) and 56.83±7.12 umol/l at week 2 (p<0.001). No significant between group differences were seen in mean plasma ammonia. Ammonia levels correlated significantly with pH (neg), pO2(neg) and FiO2 (pos). Multiple regression analyses revealed that both pO2 and pH accounted for ~73% variance of plasma ammonia levels (p<0.001) and pO2 alone accounted for ~52% variance.

Conclusions: Ammonia levels decreased significantly from 24 hours to week 2 in both groups. No significant correlation of plasma ammonia levels was seen with birth weight, gestational age, apgar scores, glucose, calcium, bilirubin, ALT levels and total caloric and protein intake. Hyperammonemia was not associated with neurologic dysfunction. pO2 and pH significantly predicted ammonia levels. Thus, hypoxic state associated with HMD and perinatal asphyxia is associated with transient hyperammonemia that lasts for 2-3 weeks.
Early nutritional intervention, both parenteral and enteral, is becoming a standard of care for the extremely low birth weight infant (ELBW) in many neonatal intensive care units (NICU) across the country. However, there are no published or widely accepted guidelines regarding nutrition support strategies for this population. Most NICU’s have developed their own guidelines and nutritional practices vary widely. In an effort to standardize our practice, we implemented nutrition support guidelines for ELBW infants, initiating both total parenteral nutrition (TPN) and minimal enteral feedings (MEFs) within the first 24 hours of life whenever possible. Objectives: 1) Evaluate the adherence to the nutritional guidelines and 2) Compare pre – and post-guideline outcomes. Materials and Methods: The study was conducted at a Level III NICU from January 2002 until February 2003. Charts of 70 infants born ≤1250gms were reviewed as part of a quality assurance project to monitor adherence to the newly established guidelines. Another 23 charts of ELBW infants who were admitted and cared for in the NICU prior to the initiation of the nutritional guidelines were reviewed as a control group. Student_t-tests were used to compare selected clinical outcomes between infants started on early nutrition support (≤24 hours of life) versus those who were started later. Results: 61.4% and 52.9% of eligible infants were started on TPN and MEF’s, respectively within 24 hours of life. The average time to start TPN was 21.9 hours after the adoption of the guidelines as opposed to 64.4 hours prior to guideline implementation (p<0.01). In the post-guideline group, MEF’s were initiated at mean 27.3 hours of age versus 80.3 hours in the pre-guideline group (p<0.01). Those who were started on early TPN and MEF’s reached full enteral feedings significantly sooner (mean:12.8 and 13.3 days vs. 45.8 and 45.8 days, respectively; p<0.01). Early nutrition support also resulted in earlier regain of birth weight (mean day 12.7 vs. 16.0 for early vs. late TPN; p<0.01 and mean day 13.8 vs. 16.0; p<0.04 for early vs. late MEF’s). While not statistically significant, infants who received earlier nutrition support showed trends toward greater overall weight gains in weeks 2-3 and 3-4 of life and a lower incidence of elevated serum blood glucose for infants who received earlier nutrition support. Conclusions: The implementation of early Nutrition support guidelines influenced the timeliness of initiating nutrition support in our unit. Early initiation of TPN and MEF’s in ELBW infants produces a more rapid regain of initial weight loss, improves weight gain and, enhances earlier achievement of full enteral feedings.
IMPACT OF EARLY AND CONSISTENT OCCUPATIONAL THERAPY WITH PRETERM INFANTS’ ORAL FEEDINGS.
NM Mohr, SR Lacey, S Teasley, HW Kilbride, Department of Physical and Occupational Therapy, Children’s Mercy Hospital and Clinics, Kansas City, MO

Purpose: This study was designed to assess the effectiveness of early occupational therapy intervention on preterm infant’s ability to achieve full oral feedings and be discharged from the neonatal intensive care unit. **Study design and methods:** Forty-one preterm infants were randomized to receive occupational therapy intervention or sham intervention beginning at 30 weeks’ gestational age. An oral stimulation and therapeutic feeding protocol was used for those infants in the experimental group. Sham intervention was used for the infants in the control group and included holding the infant without any provision of oral stimulation or therapeutic feedings. These interventions were provided three times a week for 10-15 minutes each. Outcome measures included: postmenstrual age at first successful feeding, postmenstrual age at full oral feedings, number of infants receiving occupational therapy consultations, postmenstrual age at discharge from neonatal intensive care unit, and total length of hospital stay. **Results:** No significant differences were found in the aggregate; however, significant differences were found in the subgroup of less than 29 weeks’ gestational age. Extremely preterm infants receiving early occupational therapy achieved full oral feedings on average 2.3 weeks earlier than their control counterparts. **Conclusion:** Early limited occupational therapy intervention may accelerate time to full oral feeding in extremely preterm infants.
INITIATION OF ORAL FEEDINGS IN INFANTS LESS THAN 34 WEEKS TO FACILITATE EARLY DISCHARGE FROM NICU.

K. Macwan, M. Shareef, V. Albert, D. Drenckpohl, Division of Neonatology, Department of Pediatrics, UIC College of Medicine at Peoria and Children’s Hospital of Illinois at OSF Saint Francis Medical Center, Peoria, IL.

Background: Although the component of sucking and swallowing is present in the premature neonates at 28 weeks of gestation, the synchrony is irregular and oral feeding is difficult. The oral feedings become coordinated by 32-34 weeks of gestation and so the premature neonates are usually offered oral feeding at 32-34 weeks corrected gestational age. We had several neonates who were discharge home at ≤ 34 weeks of gestation. In order for them to be discharged at ≤ 34 weeks of gestation age, they must have had their oral feedings started earlier then 32 weeks of corrected age. Retrospective study was done to find out the earliest gestational age at which it would be safe to initiate oral feedings for early discharge from NICU.

Methods: We reviewed all the charts for the neonates who were discharge home from our neonatal intensive care unit (NICU) at ≤ 34 weeks corrected gestational age from October 1, 2001 to December 31, 2002. The data was collected for gestational age, birth weight, sex, corrected gestational age, day of life the oral feedings were initiated, discharge weight and length of stay.

Results: We had total 50 patients who were discharged home during the study period who were ≤ 34 weeks of corrected gestational age.

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational Age (Weeks)</td>
<td>30.1 ± 1.7</td>
</tr>
<tr>
<td>Birth Weight (g)</td>
<td>1556 ± 366</td>
</tr>
<tr>
<td>Female/Male (%)</td>
<td>62/38</td>
</tr>
<tr>
<td>Corrected Gestational age oral feedings started</td>
<td>31.3 ± 0.7</td>
</tr>
<tr>
<td>Day of Life oral feedings started</td>
<td>9 ± 11</td>
</tr>
<tr>
<td>Corrected Gestational age discharged</td>
<td>33.3 ± 0.6</td>
</tr>
<tr>
<td>Length of stay (days)</td>
<td>23 ± 12</td>
</tr>
</tbody>
</table>

Conclusion:
Oral feedings can be initiated as early as 30 weeks of corrected gestational age in neonates. Early initiation of oral feedings may shorten the length of stay in NICU.
THE LONG-TERM OUTCOME OF FUNDOPLICATION IN CHILDREN WITH GASTROESOPHAGEAL REFLUX DISEASE: A RETROSPECTIVE ANALYSIS.

H. Mousa, F. Woodley, M. Metheney, J. Hayes*, Division of Gastroenterology, Center for Biostatistics*, Ohio State University, Columbus Children’s Hospital, Columbus, Ohio

Purpose of the Study: To evaluate our 5-year experience with anti-reflux surgery (ARS) and determine the parents’ perspective of the outcome. Methods Used: We conducted a retrospective analysis of the outcome and quality of life as a result of anti-reflux surgery (ARS) performed at Columbus Children’s Hospital during 1998-2003. Charts were reviewed for demographic data and surveys were sent out to 491 patients who underwent fundoplication, 119 were returned as address unknown, 65 people returned the survey for a response rate of 17% (65/372) and 4 expressed the desire not to participate. We assessed the outcome and quality of life on 61 patients; age (mean 4 years, median 1), 37 M / 24 F, 75% Caucasian. The primary reason for ARS was FTT n=11 (18%), breathing problems n=25 (41%), and vomiting n=23 (38%). Nissen was performed on 60 and Thal on 1. Surgery was done by an open approach on 54 (88.5%) and by laparoscopic approach on 7 (11.5%). Among the 61 patients, 21(34%) had neurological developmental delay. Results: Six months after ARS, 32% of patients ate exclusively by mouth compared to 55% prior to surgery (p = 0.001*) and 22% remained on exclusive continuous jejunal feeding compared to 37% prior to ARS (p = 0.09). General overall health score, emotional state and respiratory condition were scored as (poor, fair, good, very good and excellent). Analysis of pre and post ARS values per Wilcox matched pair test revealed significant improvements with p <0.001 in all 3 domains. Limitation of child’s activity was scored between none and severe. Statistical analysis revealed significant improvement (p=0.001). Average number of workdays/month missed due to doctor visits was 19.2 compared to 8 post ARS (p=0.022). Conclusions: 1. Anti-reflux surgery does not improve the oral intake of children with GER. 2. Most children fed via transpyloric access will continue to require this means of feeding after ARS. General quality of life improves after ARS and health care utilization decreases.
FACTORS INFLUENCING PARENT ANXIETY LEVELS IN A PEDIATRIC EMERGENCY DEPARTMENT WAITING AREA.
L Holm, L Fitzmaurice, Division of Pediatric Emergency Medicine, Children’s Mercy Hospital, Kansas City, MO.

Purpose: Bringing a sick child to the emergency department (ED) is inherently stressful. This stress is compounded by the ED waiting area environment, which can be loud and chaotic. The aim of this study was to determine the general stress level of adults accompanying children to a pediatric emergency department (ED). A secondary aim was to determine what factors are associated with these stress levels, with a focus on waiting area factors.

Methods: This descriptive study was conducted with a convenience sample of adults accompanying children to the ED over 7 consecutive days, with study blocks in the daytime and nighttime. Stress levels were measured using the Spielberger state anxiety inventory (STAI). The test is scored from 20 to 80 and has a published mean of 36 in working adults under normal conditions. College students in an experimental exam situation scored a mean of 43. Additional questions eliciting the influence of other, especially environmental factors were asked. Demographic data as well as the perceived length of time in the waiting area were solicited. All responses were measured on a four point Likert scale. Minors, adults who did not read or speak English, and caretakers accompanying children arriving in the department outside the usual triage process were excluded.

Results: A study group totaled of 295 participants with an average age of 32 years, 61% female. Two-thirds identified themselves as a parent of a patient. The mean anxiety level was 41. There was no difference in mean anxiety levels according to gender, age, or relationship to the patient. The mean anxiety level for those with perceived wait times over one hour was significantly higher than those with wait times less than one hour. (49.9 vs 38.9, p .006). Factors which may contribute to stress were elicited using a 4 point Likert scale. Worry over the child’s illness (mean 2.76) and perceived long wait times (mean 2.56) were the most highly ranked causes of stress in the ED, as compared to environmental factors, which ranged from 1.32-2.00.

Conclusion: Long wait times are associated with significantly increased anxiety among adults accompanying children to the ED. Worry over the child’s illness and perceived long wait times were the most influential factors related to causes of stress in the ED, while environmental factors were considered less important. Interventions to minimize perceived wait times and worry over the child’s illness may result in diminished anxiety for adults waiting with their child in an ED.
WHAT INFLUENCES PARENTS’ DECISIONS TO LIMIT OR WITHDRAW LIFE SUPPORT?
M Sharma, KL Meert, AP Sarnaik, Department of Pediatrics, Division of Critical Care Medicine, Children’s Hospital of Michigan, Wayne State University, Detroit, MI.

Purpose: Decisions to limit/withdraw life support from critically ill children are commonly faced by parents and physicians. Previous research regarding parents’ perspectives on end-of-life (EOL) decision-making in the PICU has been limited by retrospective methods and the use of closed-ended questions with predetermined response choices. Deeper understanding of parents’ views will allow physicians to focus EOL discussions on factors important to parents and help to resolve conflicts. We prospectively identified and described parents’ self-reported influences on EOL decision-making for critically ill children.

Methods: Parents of children whose PICU physician had made a recommendation to limit/withdraw life support from their child were eligible for the study. Parents participated in semi-structured audio taped interviews regarding their decision-making process. Interviews were conducted during the child’s PICU stay. Interviews were transcribed verbatim and independently coded by two investigators to identify segments of text that describe factors influencing parents’ EOL decisions.

Results: Fourteen parents (9 mothers, 5 fathers) of 10 children were interviewed during the decision-making process. Factors influencing parents’ decisions to limit/withdraw support included their personal observation of their child’s suffering and decline; previous experience with death/EOL decisions; perceptions of their child’s will-to-survive regardless of the child’s cognitive and developmental state; their child’s previous response to critical care interventions; their need to protect and advocate for their child; and the family’s financial resources and concerns regarding life-long care. Parents expressed an overwhelming desire to do what is best for their child but struggled with feelings of selfishness, guilt and the need to avoid agony and sorrow. Physician recommendations, review of options and joint formulation of a plan helped parents gain a sense of control over their situation. Parents of eight children agreed to limit/withdraw life support and parents of two did not.

Conclusions: Prospective interviews with open-ended questions identified factors influencing parents’ EOL decisions such as their past experience with death and their anticipated emotional adjustments and future resources not previously described in the critical care literature. Inclusion of these factors into discussions with parents may facilitate the decision-making process. More prospective research is needed to further understand what is meaningful to parents during EOL decision-making for children.
NON-INVASIVE CONTRAST ENHANCED ELECTRON BEAM TOMOGRAPHY: A THREE DIMENSION IMAGING ADJUNCTIVE TEST TO DETECT RENAL VASCULAR AND A-V FISTULA GRAFT ABNORMALITIES.

EG John, A Lumpaopong, C Ruiz, Department of Pediatrics, University of Illinois at Chicago, Chicago, IL.

Electron beam tomography (EBCT) is a relatively new technology with several potential advantages over traditional CT such as fast acquisition time, less exposure to radiation, less sedation and volume of dye, and better 3D visualization.

A retrospective study was done to assess the use of 3D EBCT to detect renal vascular abnormalities and patency of arteriovenous-fistula (A-VF) and arteriovenous-graft (A-VG). EBCT was done in 7 children (age 3-18 year, mean age 10.8 years). All had hypertension (> 95%) and high rennin levels. Four of the patients (group I) had normal renal function. One patient (group II) was 20 days post enblock renal transplantation with ascites, hypertension and renal dysfunction. Two patients (group III) had end-stage renal disease, hypertension, and AV-F and AV-G. One of these patients had SLE and difficult IV access. In group I, 2/4 had left ventricular hypertrophy, eye and CNS changes. MAG-3 renal scan (RSC) and renal ultrasound (RUS) were normal in 2/4 group I patients. In group II, 2/4 EBCT showed unilateral renal artery stenosis: one of these children had decreased velocity in one kidney by RUS and the other child had possible renal artery stenosis by RSC. One child in group I with abnormal RUS suggestive of RAS did not have RAS by EBCT and traditional angiography. In group II, patient with transplant, EBCT showed normal renal vasculature and ureterovesicle anastomoses without RAS or leak respectively. In group III, the SLE patient had thrombosis of the inferior vena cava and renal veins and the other patient had patent A-VF with dilated vessels contrary to US finding of thrombosis.

In conclusion, EBCT with its non invasive, maximum intensity projection and 3D images, less radiation and acquisition time is a useful adjunct test to detect RAS and AV-F, AV-G and transplant vascular thrombosis/stenosis in children.
ETIOLOGY AND TREATMENT OF PEDIATRIC PLEURAL EMPYEMA.

MA Jackson, LC Olson, and D Bratcher, Children’s Mercy Hospital and University of MO-Kansas City School of Medicine, Kansas City, MO.

Parapneumonic effusions (PE) complicate pediatric community-acquired pneumonia (CAP) in 40% of cases. It is estimated that empyema forms in more than half of such cases but evidence-based data regarding appropriate treatment is limited. This study evaluates treatment modalities as they relate to duration of hospitalization and cost of care in such cases. A quality improvement based retrospective chart review was undertaken to evaluate outcomes for empyema that were diagnosed between 12/00-3/04 at Children’s Mercy Hospital in Kansas City, MO. Identification of empyema was based on strict criteria that was independently confirmed by 2 evaluating physicians. Cases were included if ultrasound and or CT showed pleural fluid loculation and septation OR pleural fluid was grossly purulent OR bacteria was identified on pleural fluid culture. Data abstracted included demographic data, radiographic imaging studies, pleural fluid analysis, treatment modality, length of hospital stay (LOS), and cost of care. 96 cases of CAP with PE were reviewed; 57 cases were classified as pneumonia, uncomplicated PE, 5 cases as necrotizing pneumonia/abscess but w/o empyema and 34 cases of empyema. Children with empyema ranged in age from 17 months-16 years (mean 5 years) and 20 were boys (59%). In 27 empyema cases, pleural fluid evaluation was performed. Empyema patients more often had neutrophil >90% and glucose <20 mg/dl. Those with LDH >10,000 were more likely to be bacteriologically confirmed (7/11). Bacteriologic diagnosis was confirmed in 38% of cases; gram-positive pathogens predominated and S. pneumoniae was most commonly identified. Ten cases occurred annually and S. pneumoniae remained consistent despite widespread implementation of PCV in our community. Treatment included 4 modalities: Group 1 (n=2): antibiotics (A) only; Group 2 (n=14): A + thoracostomy tube; Group 3 (n=10): A + tube + alteplase fibrinolysis; and Group 4a/b (n=3/5): VATS (early ≤7 d sx; late >7 days sx). Patients in Group 2 had the greatest failure rate and longest stay (11.5 days) as well as cost of care. Patients in Group 3 and 4a had the shortest LOS and cost of care (7 days and median cost $21,062). Empyema was identified in 35% of cases of CAP with PE and the annual incidence remained stable over the last 3 years. Cases caused by S. pneumoniae persist despite PCV and may represent non vaccine strains (type 1 and 3). Among invasive interventions, tube thoracostomy alone had longer LOS and more failures. Early VATS and intrapleural fibrinolysis have shorter stays and cost. More evidence based investigation is necessary to confirm these results; utilization of a strict definition of empyema would facilitate such work.
A COMPARISON OF AUTOMATED AND MANUAL LEUKOCYTE DIFFERENTIAL COUNTS AND THE DETECTION OF LYMPHOID BLAST CELLS FOR DIAGNOSIS AND TREATMENT DECISIONS IN PEDIATRIC ONCOLOGY.

J Huber-Okrainec, J Legassie, V Lewis, R Anderson, W Michaud, & D Strother, McMaster University, Hamilton, ON, Western University, London, ON & Alberta Children’s Hospital, Calgary, AB

The absolute granulocyte count (AGC) is one of the most useful laboratory values used in pediatric oncology. AGCs can be derived from manual or automated leukocyte differential (LD) counts. We wondered whether a primarily automated system could reliably be used for making decisions regarding treatment in pediatric oncology and how this might affect diagnoses of acute leukemia in childhood.

We retrospectively collected all pediatric oncology complete blood cell counts at our institution with matched automated and manual LD counts over a one-month period (n = 439) and conducted correlation analyses. Following an initial analysis of automated compared to manual AGCs, correlation analyses of three sub-groups based on treatment protocols were conducted: AGCs of <0.5 x 10^9/L (n = 110); >0.5-1.0 x 10^9/L (n = 55); and >1.0 x 10^9/L (n = 274). In addition, we analyzed the sensitivity and specificity of the machine to detect blast cells. We subsequently collected all available matched automated and manual LD counts at the time of diagnosis of acute leukemia (ALL and AML) over a two-year period (n = 33) and analyzed the sensitivity and specificity of the machine to detect blast cells in the peripheral blood.

There was a highly positive correlation between automated and manual AGCs (R^2 = 0.879). Sub-group analyses revealed that automated and manual AGCs of 0.00-0.5 x 10^9/L were not correlated (R^2 = 0.565) and of >0.5-1.0 x 10^9/L were not correlated (R^2 = 0.268). There was a strong positive correlation between automated and manual AGCs of >1.0 x 10^9/L (R^2 = 0.826). The sensitivity of the machine to detect blast cells was 37% and the specificity was 74.3%. The sensitivity of the machine to detect blast cells at the time of diagnosis of ALL or AML was 41.4% and the specificity was 66.7%.

Overall, automated AGCs are predictive of manual AGCs in pediatric oncology patients. Automated AGCs of <1.0 x 10^9/L, however, are not satisfactory for decisions regarding treatment. Further, automated detection of blast cells is poor, even at the time of diagnosis of acute leukemia. Therefore, we recommend that both automated and manual LD counts be completed on pediatric oncology patients with automated AGCs of <1.0 x 10^9/L. Automated counts for AGCs >1.0 x 10^9/L are sufficient on their own, however, it is recommended that these samples be manually scanned by a technician for abnormal cellular morphology.
COMPARISON OF MULTIDRUG RESISTANCE PROTEIN-1 (MRP-1) AND P-GLYCOPROTEIN (PGP) EXPRESSION IN THE DEVELOPING HUMAN CENTRAL NERVOUS SYSTEM: CELLULAR AND TISSUE LOCALIZATION.

MJ Daood¹, M Ahdab-Barmada² and JF Watchko¹. ¹Division of Neonatology and Developmental Biology, Department of Pediatrics, University of Pittsburgh School of Medicine, Magee-Womens Research Institute, Pittsburgh, PA and ²WHY-NMD Institute, Pittsburgh, PA.

Background: MRP-1 and Pgp are multidrug efflux pumps that share substantial overlap in substrate specificity including their possible transport of unconjugated bilirubin. Although Pgp is reportedly expressed on endothelial cells and perivascular astrocytes of the blood-brain barrier (BBB) in human newborns, the pattern of MRP-1 expression in the CNS of human neonates has not been characterized. Objective: To test the hypothesis that MRP-1 is expressed in a regionally specific, developmentally modulated fashion in human CNS and compare the pattern of MRP-1 cellular and tissue localization with that of Pgp. Design/Methods: Paraffin embedded postmortem brain tissue sections from infants born at 23-42 weeks gestation were subjected to antigen retrieval in 10 mM sodium citrate at 97°C and immunostained for MRP-1 and Pgp using the monoclonal antibodies MRPr1 (Kamiya) and C219 (Signet) respectively. Immunostaining as a function of age, cell type and brain region was semiquantified. Results: MRP-1 was expressed in choroids plexus epithelium, ependymal cells of the lateral ventricles, oligo-dendroglial cells, neurons in selected brainstem nuclei, and large pyramidal cells of the cerebellum. MRP-1 immunostaining did not change between 23 and 42 weeks gestation. MRP-1 was not observed in capillary endothelial cells or astrocytes. In contrast, Pgp immunostaining was prominent in capillary endothelial cells and perivascular astrocytes of the BBB and observed in choroids plexus epithelium and large pyramidal cells of the cerebellum; Pgp immunostaining increased with gestational age from 23-42 weeks. Conclusions: We conclude that MRP-1 and Pgp are expressed in a regional and cell specific fashion in the human CNS. Pgp is primarily expressed in endothelial cells and perivascular astrocytes of the BBB; cells that do not express MRP-1. MRP-1 is expressed in choroids plexus epithelium and ependymal cells of the ventricles, elements of the blood-CSF barrier. Both Pgp and MRP-1 are expressed in selected parenchymal neurons most notably, large pyramidal cells of the cerebellum. We speculate that the complementary pattern of MRP-1 (blood-CSF barrier) and Pgp (BBB) expression may serve together to limit CNS bilirubin levels during neonatal hyperbilirubinemia. Disclosure: Funded by NINDS (038993), 25 Club of Magee-Womens Hospital, and Mario Lemieux Centers for Patient Care and Research.
BILIRUBIN EFFLUX BY BRAIN CAPILLARY ENDOTHELIAL CELL MONOLAYERS IN VITRO: ROLE OF P-GLYCOPROTEIN.
Deryk Sequeira, Monica J. Daood, Jon F. Watchko and Burhan Mahmood.  
1Division of Neonatology and Developmental Biology, Department of Pediatrics, University of Pittsburgh School of Medicine and Magee-Womens Research Institute, Pittsburgh, PA.

Background: The passage of bilirubin across the blood-brain barrier into the CNS is central to the development of kernicterus. Indirect in vivo evidence suggests that P-glycoprotein (Pgp), a multidrug transporter expressed on brain capillary endothelial cells, may limit the influx and CNS retention of unconjugated bilirubin (Pediatr Res 44:763-766, 1998). This phenomenon has not been studied in brain capillary endothelial cell monolayers in vitro.

Objective: To test the hypothesis that Pgp mediates bilirubin transport across brain capillary endothelial cell monolayers in vitro. Design/Methods: Bovine brain capillary endothelial cells (Cell Systems Corp.) were grown in confluent monolayers at a density of 5 x 10^4 cells/insert in 1 cm^2 Transwell dishes. [3H]-bilirubin (100nM) transport (pnmol/cm^2/min) was tested in both the apical to basolateral [A→B] and basolateral to apical [B→A] directions in the presence and absence of a Pgp inhibitor (cyclosporin A[5uM]). Involvement of a Pgp mediated efflux mechanism is suggested by a B→A/A→B ratio of greater than 1.5 (Pharm Res 16:1206, 1999). Brain capillary endothelial cell Pgp expression was confirmed by Western immunoblotting techniques. Results: Brain capillary endothelial cells express Pgp as seen on Western immunoblots. Bilirubin transport in the B→A direction (0.67±0.05 pmol/cm^2/min) was 6.4 fold higher than the rate for A→B direction (0.11±0.02) suggesting active efflux of bilirubin across brain capillary endothelial cell monolayers. B→A bilirubin transport decreased (0.60±0.07) and A→B bilirubin transport was enhanced (0.17±0.03) in the presence of the Pgp inhibition, with an overall decrease in bilirubin efflux of 27% suggesting that bilirubin transport by brain capillary endothelial cells is mediated in part by Pgp. Conclusions: We conclude that i) bilirubin is transported by brain capillary endothelial cell monolayers in vitro in a net B→A direction (i.e. activie efflux); ii) Pgp plays an active role in this barrier function, and iii) unconjugated bilirubin is a substrate for Pgp. We speculate that i) brain capillary endothelial Pgp limits the CNS passage and retention of unconjugated bilirubin and ii) that low brain capillary endothelial cell Pgp expression, as reported in premature neonates, may enhance brain bilirubin levels during hyperbilirubinemia.

Disclosure: Supported by NINDS (038993), the 25 Club of Magee-Womens Hospital and the Mario Lemieux Centers for Patient Care and Research.
DEVELOPMENTAL IRON DEFICIENCY IMPAIRS RAT WATERMAZE PERFORMANCE.

BT Felt, H. Tian, J Shao, J. Barks, T Schallert, Center for Human Growth and Development, University of Michigan, Ann Arbor, MI; Department of Psychology, University of Texas, Austin, TX.

Background: Iron deficiency (ID), a common nutritional disorder during development, has been associated with cognitive and behavioral impairments. Previous studies suggest developmental ID in rats affects hippocampal dendritic morphology and alters performance in the Morris Water Maze (MWM).

Objective: To explore the nature of MWM deficits in rats that had ID during development. Design/Methods: 8-week old dams were randomized to iron sufficient (IS) or iron deficient (ID) groups. IS or ID diets were given during gestation and lactation. All pups received the IS diet after postnatal day (P) 20. At P35, 8 to 9 IS and ID group males began one of two MWM assessments. 1st: Place learning (standard MWM) and memory (platform removed at 24 hours – probe) were assessed. Rats continued with trials to enhance learning to search the target quadrant then had a second probe trial. 2nd: IS and ID rats had thigmotaxis training before standard MWM assessment and probe. Data (days to criteria, latency, quadrant preference and thigmotaxis) were analyzed using one-way and repeated measures ANOVA (RPM).

Results: 1st: ID group rats had longer latencies on place learning (RPM p<0.001). Two ID rats reached criteria (ID+) but six did not (ID-). ID- rats had significantly more thigmotaxis in place learning trials than IS rats (RPM p<0.002) but ID+ and IS rats did not differ. In the probe, IS rats had greater quadrant preference (30.94 ± 9.03) than ID- rats (-19.62 ± 8.84) and less thigmotaxis. After additional trials, latency was similar for IS and all ID rats. However, IS vs ID- remained different on probe quadrant preference (25.49 ± 5.83 vs -20.46 ± 10.32, p<0.05). 2nd: After thigmotaxis training, IS rats still had shorter latencies than ID rats in the standard MWM (RPM, p<0.001) and greater quadrant preference than ID- rats on the probe (10.29 ± 11.31 vs -33.30 ± 0.01).

Conclusions: Previously ID rats demonstrate poorer MWM performance that appears related to persistent thigmotaxis behavior. Although learning improved for ID rats with additional training, memory for platform location did not. Thigmotaxis training did not impair learning measures for IS rats. The results suggest that developmental ID is associated with poorer ability to leave thigmotaxis and switch-strategies in spatial learning and memory tasks.