

## ● 253

**INTEROBSERVER RELIABILITY OF SUPERIOR MESENTERIC ARTERY (SMA) AND RENAL ARTERY (RA) BLOOD FLOW VELOCITY (BFV) MEASUREMENTS IN NEONATES.** Fiona Weir, Katherine Fong, Mary Lou Ryan, Terri Myhr, Arne Ohlsson, Department of Newborn and Developmental Paediatrics, Radiology and Perinatal Clinical Epidemiology Unit, Women's College Hospital, University of Toronto, Toronto, Ontario, Canada. Changes in blood flow can cause ischemic/reperfusion organ injuries in neonates. The reliability of Doppler BFV measurements to detect changes in flow to the intestine and kidney is not known. **Objective:** To establish the interobserver reliability for SMA and RA BFV measurements in neonates. **Methods:** 2 observers used a colour and duplex Doppler technique to record at least 5 consecutive waveforms in the SMA and RA and measured peak systolic (PSV), end diastolic (EDV) and time average (TAV) velocities. **Results:** The intraclass correlation coefficient (ICC) for the vessels are shown below. Forty-two stable, non-ventilated neonates were enrolled; birthweight  $2.00 \pm 0.53$  kg, gestational age at birth  $33 \pm 2$  weeks, postnatal age  $10 \pm 11$  days. **Conclusion:** Doppler BFV in the SMA and RA can be measured reliably in neonates. These results can be used for sample size calculations in clinical studies.

VESSEL	DOPPLER MEASUREMENTS	ICC	Lower 95% CI (*one-sided test)
RA (n=39)	PSV, EDV, TAV	0.78, 0.72, 0.72	0.65, 0.55, 0.56
SMA (n=39)	PSV, EDV, TAV	0.75, 0.73, 0.76	0.61, 0.54, 0.62

## ▲ 254

**GENE THERAPY FOR CONGENITAL HYPERBILIRUBINEMIA.**

<sup>1</sup> Martina Wilke, <sup>2</sup> Amelie Bijma, <sup>1</sup> Anja J.M. Timmers-Reker, <sup>3</sup> Pieter J. Bosma, <sup>4</sup> Bob J. Scholte, <sup>1</sup> Maarten Sinaasappel.

<sup>1</sup>Dept. of Gastroenterology, Sophia children's hospital, Rotterdam, <sup>2</sup> EDC Rotterdam, <sup>3</sup>Stic Lab., Academic medical centrum, Amsterdam, <sup>4</sup> Dept. of Cell Biology, Erasmus University Rotterdam.

Most of the congenital errors of liver metabolism affect one single pathway and spare the general function and structure of the liver. Gene transfer into hepatocytes might be an alternative treatment of many inborn errors of the liver metabolism. We study gene therapy of the congenital unconjugated hyperbilirubinemia (Crigler-Najjar type I) in a rat model (Gunn-rat). The gene for UDPGT has been cloned. We have constructed an expression vector containing the B-UGT-1 cDNA. We are busy to characterize the expression of the B-UDPGT cDNA by western-blotting and in a functional assay for bilirubin glucuronidation in microsomes prior to the use for gene transfer into hepatocytes *in vitro* and *in vivo*.

Preliminary experiments have been performed for *ex vivo* gene transfer and hepatocyte transplantation. After isolation the hepatocytes were transfected with the pCMV-LacZ construct using DOTAP in culture and injected into the spleen of rats. The marker gene was expressed by 10% of the cultured cells. Five days after injection blue hepatocytes were observed in the spleen (counter staining with PAS). To improve the viability of hepatocyte *in vitro* we started to culture the cells in suspension by using collagen microspheres. The hepatocytes stay viable for > 10 days in culture. To optimize the gene transfer into the hepatocytes it will be necessary to test the different available transfection systems and to develop new methods for vector delivery.

## ▲ 255

**MEASUREMENT OF CEREBRAL VENOUS SATURATION BY NEAR INFRARED ABSORPTION SPECTROSCOPY**

Charles W. Yoxall, A. Michael Weindling, Nader M.H. Dawani, Ian Peart.

Dept Child Health, University of Liverpool. U.K.

**Method :-** Near Infra-Red Absorption Spectroscopy (NIRAS, Hamamatsu NIRO 500) was performed on the heads of four children undergoing cardiac catheterisation. An increase in cerebral blood volume was achieved by unilateral jugular venous occlusion for about 5 seconds. Cerebral venous oxygen saturation (CSvO<sub>2</sub>) by NIRAS was calculated and compared with direct measurement by co-oximetry of blood from the internal jugular vein.

**Results :-** The median (range) age of the children was 1.6 (0.7 - 14.1) years. The median (range) CSvO<sub>2</sub> by co-oximeter was 55.2% (52.7 - 61) and by NIRAS was 53.6% (51.6 - 57.5). Using a Bland Altman plot the mean difference (range) was 1.92% (-2.9 to 5.9).

**Conclusions :-** NIRAS used in this way seems to provide an accurate non invasive measurement of cerebral venous saturation.

## ▲ 256

**LIVER GLUCONEOGENESIS REGULATION BY TNF $\alpha$**  A. Kemal Topaloglu, Masakatsu Goto, W. Patrick Zeller, Depts. of Pediatrics & Physiology, Loyola University of Chicago., Stritch School of Med., Maywood, IL, USA.

Liver glycolysis/gluconeogenesis dysregulation during sepsis/septic shock has been suggested to be the cause of hypoglycemia in endotoxic shock. TNF $\alpha$  is a mediator in endotoxic shock. However, TNF $\alpha$  effects on liver gluconeogenesis is not well known. We have shown that TNF $\alpha$  increases glucose transporter GLUT mRNA abundance and decreases gluconeogenesis in hepatocytes. Therefore, we hypothesize that TNF $\alpha$  increases glucose entry into hepatocytes and causes the suppression of hepatocyte gluconeogenesis. **Objective:** To investigate TNF $\alpha$  induced glucose entry into the hepatocyte. **Materials and Methods:** Hepatocytes ( $10^6$  cell/ml) were isolated from 24 hour fasted 10 day old Sprague-Dawley rats (23-27 grams) and incubated in RPMI 1640 plus <sup>14</sup>C-2-deoxy-D-glucose (0.2 $\mu$ Ci) and recombinant murine TNF $\alpha$  ( $4.5 \times 10^4$  u/ml). Hepatocyte <sup>14</sup>C-2-deoxy-D-glucose uptake and GLUT1 mRNA were determined after a 4 hour incubation. **Results:** TNF $\alpha$  increased <sup>14</sup>C-2-deoxy-D-glucose uptake ( $0.12 \pm 0.02$  vs  $0.06 \pm 0.01$  mM,  $p < 0.05$ ) and GLUT1 mRNA abundance. **Summary:** Glucose transport and GLUT1 mRNA abundance was increased by TNF $\alpha$ . **Conclusion:** The increased glucose entry may cause suppression of hepatocyte gluconeogenesis.

## ▲ 257

**COMPLEMENT ACTIVATION DURING FIRST HOURS OF LIFE IN PRETERM INFANTS WITH EARLY ONSET INFECTION.** Eugen P. Zilow, Wolfgang Hauck, Gertrud Zilow, Division of Neonatology and Department of Immunology, University of Heidelberg, Germany.

Severe systemic infection is one of the most important causes for mortality and long term morbidity of preterm infants. Clear clinical signs often appear late, when the patients are actually decompensating. Laboratory tests for the detection of neonatal sepsis suffer from inter-observer differences (differential blood count) and time delay in significant increase (CRP = C-reactive protein).

We previously have demonstrated the initial generation of anaphylatoxin C3a by alternative pathway activation of the complement system in severe early onset infection of term infants (Pediatr. Res. 34:199-203, 1993). In the present study we determined complement activation products of the alternative pathway, cytokine levels (IL-6) and CRP in preterm newborns with early onset systemic infection (n=19, gestational age 25-33 weeks, median=29 weeks). The control groups consisted of term (n=32) and preterm (n=20, gestational age 23-35 weeks, median=29 weeks) newborns with no signs of infection within the first week of life. In the first six hours after birth infants with infection showed significantly higher C3a, C3bBbP (alternative convertase), TCC (terminal complement complex) and IL-6 levels compared with the control groups of term and preterm infants. After adequate treatment with antibiotics the parameters rapidly returned to slightly elevated or normal values within the first 48 hours of life. CRP, measured routinely as a marker of infection, showed a delayed increase which continued even after initiation of successful treatment.

Thus CRP as an indicator of presumptive bacterial infection might be of little benefit in the initial course of a septic preterm infant, whereas complement activation products and IL-6 provide very early sensitive parameters of severe infection in both term and preterm infants.

## ● 258

**DECREASED SURFACTANT SYNTHESIS IN RATS WITH CONGENITAL DIAHRAGMATIC HERNIA (CDH) IS DUE TO IMMATURETY OF BOTH TYPE II PNEUMOCYTES AND LUNG FIBROBLASTS.** Luc J. Zimmermann, Hanneke Ijsselstijn, Janine den Ouden, Pieter J. Sauer, Joseph J. Batenburg and Dick Tibboel. Dept of Pediatrics and Pediatric Surgery, Sophia Children's Hospital, Rotterdam and Lab of Veterinary Biochemistry, Utrecht University, Utrecht.

Recent evidence indicates that surfactant deficiency may play a role in the pathophysiology of CDH. CTP:phosphocholine cytidyltransferase (CT) catalyses a rate regulatory step in the *de novo* synthesis of surfactant phosphatidylcholine (PC) in type II pneumocytes (TII cells). Conditioned medium from fetal rat lung fibroblasts stimulated with  $10^{-7}$  M cortisol (FibCM) has been shown to increase surfactant PC synthesis in fetal TII cells through an increase of CT activity. Large left sided CDH with hypoplastic lungs were induced by feeding pregnant rats 100 mg Nitrofen on day 10 of gestation. Day 21 fetal TII cells and lung fibroblasts were isolated from control (no Nitrofen) and CDH fetuses. CT activity was significantly lower in homogenates from TII cells from CDH versus control lungs, whether CT was assayed in the absence ( $0.93 \pm 0.18$  vs  $1.27 \pm 0.28$  nmol/min/mg protein; n=8) or presence of 0.5mM PC/oleic acid (1/1) lipid vesicles ( $1.17 \pm 0.21$  vs  $1.48 \pm 0.24$ ; n=8). Choline [<sup>3</sup>H-Me] incorporation into PC was studied in control TII cells incubated for 24h with FibCM. Compared to minimal essential medium with cortisol, FibCM from control rats significantly stimulated PC synthesis ( $51.4 \pm 3.7$  vs  $60 \pm 3 \times 10^4$  dpm/well; n=10) but FibCM from CDH did not ( $54.7 \pm 3.3$ ; n=10). We conclude that CT activity is significantly decreased in TII cells from CDH. In contrast to FibCM from controls, FibCM from CDH does not stimulate PC synthesis in TII cells. These data suggest that the immaturity of the fibroblasts may be primary responsible for decreased PC synthesis in CDH.