

## 259

### CEREBRAL HEMODYNAMICS AND OXYGENATION AFTER REMOVAL OF CEREBRAL SPINAL FLUID FROM A SUBCUTANEOUS VENTRICULAR CATHETER RESERVOIR IN INFANTS WITH A POST HEMORRHAGIC VENTRICULAR DILATATION

A A E van Alfen-van der Velden<sup>1</sup>, J C W Hopman<sup>2</sup>, J H G Klaessens<sup>2</sup>, K D Liem<sup>1</sup> <sup>1</sup>University Medical Centre Nijmegen, Nijmegen, Netherlands; <sup>2</sup>University Medical Centre Nijmegen, Clinical Physics, Nijmegen, Netherlands

**Background:** Removal of cerebral spinal fluid (CSF) is the common treatment of post hemorrhagic ventricular dilatation (PHVD) in newborn infants. Puncture from a subcutaneous ventricular catheter reservoir (SVCR) allows easy CSF removal with minimal discomfort for the patient.

**Aims:** To assess changes in cerebral oxygenation and hemodynamics after serial CSF removal from a SVCR in infants with PHVD. **Methods:** 6 infants (GA 216–258 days, BW 1490–2901 gram) were studied during CSF drainage at day 1, 3 and 7 after SVCR placement. The amount of CSF removal was 5.5–9.6 ml/kg. Changes in concentration of oxyhemoglobin (AcO2Hb) and deoxyhemoglobin (AcHHb) were continuously measured using near infrared spectrophotometry. The difference in AcO2Hb and AcHHb, indicated as AcHbD, represents changes in cerebral blood oxygenation. Concentration changes in total hemoglobin (AcTHb), calculated as the sum of AcO2Hb and AcHHb, reflects changes in cerebral blood volume. Changes in cHbD and cHbT were calculated between t=0 (start of CSF removal) and at 15, 30 and 60 minutes after CSF removal. Changes in mean cerebral blood flow velocity (ACBFV) in the internal carotid artery were intermittently measured using Doppler ultrasound. Physiologic variables (heart rate, arterial oxygen saturation, respiration rate and arterial blood pressure) were recorded.

#### Results:

Time (min)	AcHbD (µmol/100g)	AcTHb (µmol/100g)	ACBFV (%)	
Day 1	15	0.51 (0.15,0.72)*	0.65 (0.59, 0.72)*	9.6 (8.9,15.4)*
	30	0.47 (0.06,0.80)	0.65 (0.43,0.74)*	10.9 (12.5,30.2)*
	60	0.41 (0.20,0.82)*	0.64 (0.26,0.73)*	36.5 (8.0,32.9)*
Day 3	15	0.07 (0.10,0.27)	0.45 (0.34,0.56)*	9.8 (9.2,13.2)*
	30	0.10 (0.24,0.22)	0.37 (0.33,0.52)*	20.9 (2.0,39.0)*
	60	0.02 (0.19,0.14)	0.41 (0.20,0.46)*	-2.7 (10.1,5.7)*
Day 7	15	-0.10 (0.27,0.09)	0.31 (0.02,0.63)	1.3 (4.3,11.6)
	30	0.09 (0.26,0.09)	0.38 (0.02,0.69)	4.2 (22.3,13.7)
	60	0.06 (0.06,0.80)	0.36 (0.22,0.79)	13.2 (1.2,23.9)

Values are median (interquartile range). \* significant changes (p <0.05, Wilcoxon-signed ranks test). There were no significant changes in physiological parameters.

**Conclusion:** CSF removal from a SVCR results in improvement of cerebral perfusion, particularly the first day after SVCR placement. Cerebral blood oxygenation is only significantly increased at the day of SVCR placement. These changes are probably related to a reduction in intracranial pressure.

## 260

### ROLE OF CAROTENOIDS IN BRONCHOPULMONARY DYSPLASIA

D van Zoeren-Grobhen<sup>1</sup>, L D Dikkeschei<sup>1</sup>, R A van Lingel<sup>1</sup>, J Slootstra<sup>1</sup>, C Bunkers<sup>1</sup>, E S Snink<sup>1</sup>, A Schaafsma<sup>2</sup> <sup>1</sup>Isala Clinics, Neonatal Unit, Zwolle, Netherlands; <sup>2</sup>Friesland Nutrition, Research, Leeuwarden, Netherlands

**Aim:** Antioxidants (e.g. vitamin E and A) play a role in protecting preterm infants from reactive oxygen species related diseases e.g. bronchopulmonary dysplasia (BPD). Despite adequate supplementation of these antioxidants BPD still remains a major problem in preterm infants and other antioxidants i.e. carotenoids may play a role. In adults the carotenoids lycopene and  $\beta$ -carotene have a protective effect in cancer and cardiovascular diseases. Carotenoids are present in human milk, but not in parenteral nutrients and only some  $\beta$ -carotene in preterm formula. In this study we evaluated whether carotenoid levels are related to oxidative stress or the occurrence of BPD.

**Methods:** The levels of  $\beta$ - and  $\alpha$ -carotene, lycopene, vitamin E and A and plasma F2 isoprostanes were studied at days 1, 3 and 7 in a group of 60 preterm infants (mean [SD] g.a. 29.3[1.5] wks, birth weight 1189[354] g; 17 without pulmonary problems (healthy group), 29 with IRDS (group IRDS), 14 with BPD or death due to pulmonary problems (group BPD))

**Results:** In the total group at birth  $\beta$ -carotene,  $\alpha$ -carotene, and lycopene levels were 1/10 of adult values vitamin E and A levels were also low (mean [SD] nmol/l:  $\beta$ -carotene 37.7[23.2],  $\alpha$ -carotene 12.2[7.1], lycopene 44.5[34.1], vitamin E 9.8[3.9]  $\mu$ mol/l, vitamin A 0.51[0.22]  $\mu$ mol/l). Postnatally lycopene and  $\beta$ -carotene levels decreased (MANOVA both p < 0.01), vitamin A remained stable, vitamin E and  $\alpha$ -carotene levels rose (MANOVA both p < 0.01). No differences were found in carotenoid or vitamin levels comparing the healthy, IRDS and BPD groups. Isoprostane levels remained stable during the study period, and did not differ between the groups. No correlation was found between isoprostane and carotenoid levels.

**Conclusions:** We found no evidence that carotenoids play a role in reducing the incidence of BPD in this small group of infants. However the low levels of lycopene and  $\beta$ -carotene without a postnatal increase may indicate a deficiency of these vitamins due to parenteral nutrition which may induce other harmful effects. We are expanding the study group and follow up these infants to study long term effects. *Financial support Friesland Nutrition, The Netherlands*

## 261

### EFFECT OF NUTRITION ON CAROTENOID LEVELS IN PRETERM INFANTS

D van Zoeren-Grobhen<sup>1</sup>, L D Dikkeschei<sup>1</sup>, R A van Lingel<sup>1</sup>, J Slootstra<sup>2</sup>, C Bunkers<sup>1</sup>, E S Snink<sup>1</sup>, A Schaafsma<sup>3</sup> <sup>1</sup>Isala Clinics, Neonatal Unit, Zwolle, Netherlands; <sup>2</sup>Isala Clinics, Biochemical Laboratory, Zwolle, Netherlands; <sup>3</sup>Friesland Nutrition, Research, Leeuwarden, Netherlands

**Aim:** Carotenoids are important as pro-vitamin A, for retinal development and as antioxidants. In adults carotenoids protect against reactive oxygen species (ROS) diseases i.e. cancer and cardiovascular diseases. Preterm infants are also at risk for ROS related diseases such as bronchopulmonary dysplasia (BPD). Just as for vitamins E and A adequate supplementation of carotenoids may reduce the incidence of BPD in preterm infants. Carotenoids are present in human milk, but not in parenteral nutrients and most preterm formulas.

**Methods:** The levels of the most occurring carotenoids were measured in 29 preterm infants (mean [SD]: g.a. 29.4[1.6] wks, birth weight 1205[322]g) at postnatal days 1,3,7 and 21. All infants received parenteral nutrition supplemented with vitamin E 1.4 IU/d and A 460 IU/d without carotenoids. Introduction of enteral feeds with human milk or a preterm formula (Frisoprop<sup>®</sup>, containing 65  $\mu$ g/dl  $\beta$ -carotene) was started as soon as possible.

**Results:** In the total group of infants the levels of all vitamins were low at birth: mean (SD): vitamin A 0.48(0.22)  $\mu$ mol/l, vitamin E 9.1(4.0)  $\mu$ mol/l, lycopene 36.1(21.0) nmol/l,  $\beta$ -carotene 35.7(16.9) nmol/l,  $\alpha$ -carotene 10.1(5.5) nmol/l. Postnatally the levels of lycopene declined [day 21 mean(SD) 21.3(9.5) nmol/l, MANOVA p < 0.01],  $\beta$ -carotene remained stable [day 21 8.3(7.2) nmol/l], and the levels of  $\alpha$ -carotene, vitamin A and E rose [day 21: 72.1(76.5) nmol/l p < 0.05, 0.70(0.34)  $\mu$ mol/l, p < 0.01 and 33.6(19.6)  $\mu$ mol/l, p < 0.001 respectively]. No significant differences were found between the infants fed predominant human milk (n=18) or preterm formula (n=10). No correlation was found between levels of the vitamins E, A, lycopene,  $\beta$ - and  $\alpha$ -carotene on day 21 and the duration of parenteral nutrition.

**Discussion:** The low carotenoid levels at birth and the postnatal decline of lycopene and  $\alpha$ -carotene may reduce the antioxidant capacity of the preterm infant. More infants need to be studied to confirm these results. Although human milk contains carotenoids, human milk fed infants did not have higher levels. This can be due to the short period these infants received enteral feeds. *Financial support Friesland Nutrition, The Netherlands.*

## 262

### INTERLEUKIN 10 IS UP-REGULATED BY HUMAN PROTEIN C-CONCENTRATE IN THE EARLY PHASE OF A NEONATAL PIGLET MODEL OF SEPTIC SHOCK

A Veldman<sup>1</sup>, M Nold<sup>1</sup>, C Petry<sup>2</sup>, B Richter<sup>3</sup>, D Fischer<sup>1</sup>, J.W. Goethe University Hospital, Pediatrics, Frankfurt / Main, Germany; <sup>2</sup>J.W. Goethe University Hospital, Pharmacology, Frankfurt / Main, Germany; <sup>3</sup>J.W. Goethe University Hospital, Ped. Surgery, Frankfurt / Main, Germany

**Background:** Protein C (PC) is able to reduce the liberation of pro-inflammatory cytokines in sepsis. While this effect was mainly assessed in in-vitro in monocytes and cultured endothelial cells, there is little data on the regulation of interleukin 10 (IL10) by PC in in-vivo sepsis. Furthermore, the action of PC in neonatal septic shock is unclear.

**Methods:** Endotoxin shock was induced in 10 neonatal piglets under general anesthesia by intravenous application of 500  $\mu$ g/kg E. coli lipopolysaccharides. 5 piglets received human Protein C concentrate (Ceprotin<sup>®</sup>, Baxter) with an initial bolus of 50  $\mu$ g/kg, followed by continuous infusion of 200  $\mu$ g/kg/d. 5 piglets served as control group. TNF  $\alpha$ , Interferon  $\beta$  (IFN  $\beta$ ) and IL10 were measured using porcine immunoassays (TNF  $\alpha$  and IL10: R&D Systems, Minneapolis, USA; IFN  $\beta$ : Biosource, Camarillo, USA) of samples taken at 0,60,120 and 180 min after LPS-exposure.

**Results:** TNF  $\alpha$  rapidly increased > 10 fold in all animals after the LPS exposure. The maximum TNF  $\alpha$  concentration was reached at 60 min and decreased at 120 and 180 min, although the TNF  $\alpha$  levels at 180 min were at least 3 fold above baseline. There was no difference in the course of TNF  $\alpha$  whether the animals received PC or not. IFN  $\beta$  levels remained unchanged in this early phase of septic shock in the animals treated with PC and the controls IL10 levels increased in the control animals but did not reach a 2 fold increase over baseline with a maximum between 60 and 120 min followed by a decrease in IL10 levels. In contrast, the IL 10 levels in the PC treated animals exceeded a 2 fold increase and stayed elevated throughout the study period.

**Conclusion:** In this animal model PC induced a fast and sustained induction of the anti-inflammatory cytokine IL10 in the early phase of neonatal septic shock. The unchanged levels of IFN  $\beta$  in all animals might be attributable to the short study time. Surprisingly, the rapid and marked increase of TNF  $\alpha$  followed by a subsequent decrease was observed to be unrelated to PC treatment in this animal model of LPS induced neonatal septic shock.

## 263

### ASPHYCTIC RENAL DAMAGE IS INCREASED BY THE USE OF PURE OXYGEN UPON RESUSCITATION

M Ventós<sup>1</sup>, J Sastre<sup>2</sup>, M Asensio<sup>2</sup>, C Gomez<sup>2</sup>, A Llorca<sup>2</sup>, J Vilà<sup>2</sup>, C Borras<sup>2</sup> <sup>1</sup>Hospital Virgen del Consuelo, Servicio de Pediatría y Neonatología, Valencia, Spain; <sup>2</sup>Universidad Valencia, Departamento Fisiología, Valencia, Spain

**Background:** Perinatal asphyxia is responsible for multiple organ damage which is increased upon re-oxygenation by the generation of an excess of oxygen free radicals. Proximal tubular damage and the subsequent acute renal failure are common complications of severe asphyxia. Thus, limiting the amount of oxygen supplied upon resuscitation seems an adequate approach to reduce oxidative stress-derived renal damage. We hypothesized that room-air resuscitated infants would exhibit diminished renal damage as shown by specific clinical and biochemical markers.

**Methods:** A prospective randomised clinical trial blinded for the gas source was performed in 39 asphyxiated term newborns. 17 were resuscitated with room air (RAR) and 22 with pure oxygen (OxR). Oxidative stress markers such as reduced and oxidized glutathione (GSH, GSSG), and superoxide dismutase activity (SOD) were determined at birth, and 24 and 48 hours thereafter. Diuresis, N-acetylglucosaminidase (NAG) in urine, creatinine, urea, beta 2 microglobulin and fractional excretion of sodium were determined at 24 and 48 hours of life. 20 non-asphyxiated babies acted as controls. Long-term follow up was completed at one month of postnatal age.

**Results:** As shown in the Table, asphyxiated neonates showed in general altered parameters of oxidative stress and renal damage as compared to normal controls. However, at 48 hours of postnatal age, pure oxygen resuscitated infants show higher degree of oxidized glutathione, a higher SOD activity, and NAG excretion in the urine was significantly higher than in room-air resuscitated infants. Moreover, total blood GSSG correlated significantly with NAG in urine indicating that oxidative stress correlated with renal damage. Significant differences between RAR and OxR groups lasted at least 3 weeks.

**Conclusions:** Room-air resuscitated asphyxiated neonates show significantly lesser proximal tubular renal damage as compared to pure oxygen resuscitated infants in the acute phase of asphyxia. Thus, high oxygen concentrations should be cautiously used in the resuscitation of asphyxiated newborn infants.

	U. vessel		24 hours			48 hours			
	Control (22)	RAR (17)	OxR (22)	Control	RAR	OxR	Control	RAR	OxR
GSH (mcM)	995 (116)	898 (105)	1020 (148)	780 (92)	743 (77)	712 (54)	843 (103)	784 (92)	755 (72)
GSSG (mcM)	25.8 (8.8)	69.6 (10.6)**	60.4 (7.8)**	19.6 (5.6)	68.3 (12.5)**	81.9 (10.4)**	22.5 (7.9)	55.6 (10.3)**	77.4 (10.1)**
SOD (U/g Hb)	1.6 (0.4)	3.3 (0.9)**	3.8 (1.1)**	1.9 (0.5)	3.6 (1.1)**	4.0 (1.3)**	0.9 (0.3)	1.9 (0.5)**	3.4 (1.0)**
NAG (IU/nmol creat)				2.1 (0.9)	22.6 (11.9)**	23.5 (9.5)**	3.4 (1.6)	31.4 (15.0)**	39.7 (17.2)**

## 264

### INCREASED LEVELS OF INSULIN-LIKE GROWTH FACTOR-1 (IGF-1) AND $\alpha$ 2-MICROGLOBULIN ( $\alpha$ 2-MG) IN BALF OF PRETERM NEONATES WITH CHRONIC LUNG DISEASE (CLD)

V. Entós<sup>1</sup>, E Capolungo<sup>2</sup>, P G Matassa<sup>1</sup>, M Martelli<sup>1</sup>, V Vendettuoli<sup>1</sup>, E Zecca<sup>1</sup>, C Romagnoli<sup>1</sup>, F Ameglio<sup>3</sup> <sup>1</sup>Università Cattolica del S. Cuore, Div of Neonatology, Rome, Italy; <sup>2</sup>Università Cattolica del S. Cuore, Inst of Biochemistry and Clinical Chemistry, Rome, Italy; <sup>3</sup>S. Giovanni Calibita Fatebenefratelli/AFAR, Clinical Biochemistry, Rome, Italy

**Background:** Activation of profibrotic mechanisms seems one of the possible causes of CLD. IGF-1 is a growth factor, mainly induced by GH on hepatocytes, which is known to activate fibrogenic mechanisms also by polarizing T-cells towards TH2 compartment. In turn, TH2 lymphocytes release large amounts of profibrotic cytokines such as IL-10 and TGF- $\beta$ .  $\alpha$ 2-MG, the non-covalently bound component of HLA-class I molecules, has been recently reported to be increased in urine of neonates with chorioamnionitis and CLD. **Objective:** To evaluate the concentration of IGF-1 (free and total form), IGF-binding protein-3 (IGFBP-3, a representative protein bound to IGF-1 molecules with inhibitory function) and  $\alpha$ 2-MG in BALF of preterm neonates and to correlate these values with their early clinical course and the evolution in CLD (O2 at 28 days of life). **Design/Methods:** BALF samples were taken during the first 5 days of life in 28 preterm neonates (13 CLD (GA:27.8  $\pm$  1.1; BW:1199  $\pm$  284) and 15 Control (GA:32.1  $\pm$  1.1 p < 0.0001; BW: 1800  $\pm$  288, p < 0.0001) without severe birth asphyxia, chorioamnionitis or postnatal infections. Free IGF-1 and IGFBP-3 were measured by immunoradiometric assay,  $\alpha$ 2-MG by nephelometric modified assay, total IGF-1 by ELISA.

**Results:** ELF concentrations of the  $\alpha$  molecules (calculated by the urea method) in study groups: See table 1. Values are expressed as median (range) A further analysis of correlations between these molecules showed that freeIGF-1 and total IGF-1 were highly correlated (R=0.82) as well as  $\alpha$ 2-MG and IGFBP-3 (R=0.73), I). These data confirm that the ratio free/total IGF-1 is biochemically regulated and suggest a possible common cell production/release of both  $\alpha$ 2-MG and IGFBP-3.

**Conclusions:** These data suggest that the mechanisms leading to CLD are present early after birth.  $\alpha$ 2-MG could be a marker of postnatal inflammation per se. More studies are needed to clarify the regulation of these mechanisms and the cells involved in the synthesis of the molecules analysed in this report.

	Control (N:15)	CLD (N:13)	P=
Free IGF-1 ng/ml	1.6(0.5-3)	2.2(0.9-3.5)	0.02
Total IGF-1 ng/ml	14.4(0.9-59.8)	32.2(16.4-68.9)	0.03
IGFBP-3 ng/ml	1114 (113-2054)	1665 (579-2836)	0.03
$\alpha$ 2-MG $\mu$ g/ml	4.0(1.1-14.1)	9.5(1.7-23.9)	0.04