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### INDOMETHACIN AFFECTS CELL PROLIFERATION AND PROTEASOME ACTIVITY IN NEURONAL CULTURES

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**Background**: Indomethacin (INDO) is used for of intraventricular hemorrhage prophylaxis and closure of ductus arteriosus in premature infants. Cellular and molecular actions of INDO on brain cells remain unclear. We examined INDO effects on cellular parameters (growth, proliferation, respiratory burst).

**Methods**: Assays for MTT proliferation, proteasome function (chymotrypsin activity), viability testing, and respiratory burst (flow cytometric analysis) were performed on cell lines (N2a neuroblastoma and N9 microglia) and primary neuronal and astrocyte cultures treated with INDO at 0, 100, 250, 500, 750 and 1000uM.

**Results**: Percent viability of N2a cells was significantly higher at 250uM and lowest at 1mM. Percent viability of N9 microglia significantly decreased from baseline (91%) to 71% at 250uM ($p<0.0001$), precipitously decreasing to 10% at 500uM. Proliferation assays of primary neonatal cells indicate 250–500uM INDO treatment increased astrocyte proliferation while attenuating neuronal proliferation in a dose-dependent manner. Proteasomes play an important role in intracellular degradation of aging and oxidized proteins and in activating signaling proteins like NF-kB. Normal proteasomal function of N2a neuronal cells or N9 microglia was maintained up to 250uM INDO treatment. N9 cells exhibited marked loss of activity at 500uM and 1000uM while N2a maintained activity up to 500uM. Respiratory burst assays on N9 microglial cells showed INDO pretreatment and cotreatment with LPS/IFN-gamma caused a 10% reduction in reactive oxygen species (ROS)-release by N9 cells compared to N9 with LPS/IFN-gamma treatment. Estrogen (1nM)-pretreated N9 cells decreased ROS release by 30%.

**Conclusion**: Cell function and integrity assays indicate a narrow dosage window (250–500uM) where INDO can preserve cellular growth, viability, respiratory burst, and proteasomal function. That estrogen is more efficient than INDO in decreasing ROS production may explain suggested gender differences in efficacy. Results indicate differential susceptibilities of CNS cells, with microglia being most sensitive while neurons and astrocytes tolerate INDO treatment better.

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### COMPARISON OF THE RESPIRATORY WORKLOAD DURING PATIENT TRIGGERED AND CONVENTIONAL MECHANICAL VENTILATION MODES IN PREMATURE INFANTS (UK)

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In a randomised trial, patient triggered (PTV) compared to conventional mechanical ventilation (CMV) was associated with a shorter duration of weaning in premature infants. During PTV, all the spontaneous breaths of the infant are supported by the ventilator and the work of breathing compared to the work of breathing during CMV could be reduced. To test that hypothesis we compared the diaphragmatic pressure-time product (PTPdi), the integration of transdiaphragmatic pressure over time, which reflects the energy expenditure of the diaphragm, in preterm infants supported by PTV and CMV. Twelve infants (median gestational age 27.5 weeks) were studied in the recovery stage of their respiratory illness. All infants were receiving caffeine. The infants were studied both on PTV and CMV in a random order, each for 5 minutes. Transdiaphragmatic pressure (Pdi) was calculated by subtraction of oesophageal from gastric pressure measured using a dual tip pressure catheter. The inspiratory duty cycle was calculated from the airflow signal measured using a pneumotachograph inserted into the distal end of the endotracheal tube. The mean PTPdi per breath cycle expressed over one minute was calculated during the last minute of each five minute period. The PTPdi on CMV was significantly higher than the PTPdi on PTV (median 197.5 cmH2O*sec/min, range 117.9–321.4, versus median 95.6 cmH2O*sec/min, range 51.9–270.9, respectively, $p=0.0022$). In conclusion, the diaphragmatic workload is higher on CMV than on PTV and this may explain the superiority of PTV as a weaning ventilatory mode.

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### ACTIVE SURVEILLANCE OF EARLY-ONSET EATING DISORDERS, PRADER-WILLI SYNDROME AND VITAMIN D DEFICIENCY RICKETS

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**Objective**: CPSP undertook 2-year surveillance of three nutrition and growth-related conditions: early-onset eating disorders (EOED), Prader-Willi syndrome (PWS) and vitamin D deficiency rickets (VDDR). Canadian incidence estimates were needed to test hypotheses that EOED cases are being missed because current diagnostic criteria do not apply to children aged 5–12 years, that early identification of PWS and related-obesity as well as increasing cases of VDDR are public health issues.

**Methods**: Over 2400 paediatricians reported cases of uncommon high morbidity/mortality diseases by mailing a check off form monthly. For each report, a detailed questionnaire was completed. The response rates were 83% for initial reports and 96% for questionnaires.

**Results**: The EOED study confirmed 138 cases in children aged 5–12 years. The female to male ratio was 8:1 compared to 10:1 in older age groups. Food avoidance was a predominant clinical feature. Children displayed preoccupation with food and fear of gaining weight. The mean weight loss was 7.8 kg. The majority had no comorbid psychiatric diagnosis; however, 44% had a positive psychiatric family history and 54% experienced changes in social situations. Bradycardia was the most common medical complication. The PWS study identified 35 genetically confirmed cases, diagnosed at a mean age of three years. Of the 28 with available data, 25 had infantile feeding problems and 43% had gained excess weight between 1–6 years of age. The VDDR study confirmed 104 cases. Ninety were infants and toddlers with intermediate and dark skin who had been exclusively breastfed without appropriate vitamin D supplementation. The most frequent signs and symptoms at diagnosis were: skeletal deformity, seizures, failure to thrive, fractures, and delayed milestones.

**Conclusions**: The findings indicate a need for creating developmentally appropriate EOED diagnostic criteria, for identifying PWS early and for heightened awareness of rickets prevention among health care providers

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### INCREASED AQUAPORIN 4 EXPRESSION IN MOUSE BRAIN AFTER HYPOXIA AND REOXYGENATION WITH ROOM AIR OR 100% O2

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**Background** Aquaporins (AQP) are membrane proteins, mediating water movements across lipid bilayers. AQP4 the brain aquaporin, is believed to be involved in development of cerebral edema after brain injuries. Hypoxia and reoxygenation is a known clinical situation provoking cerebral edemas with possible serious consequences.

**Objective** We analyzed AQP4 protein concentration in brain homogenates from mice exposed to hypoxia and reoxygenated with either room air or 100% oxygen. Brains from untreated mice were used as controls.

**Methods** Four to seven weeks old mice were exposed for two hours to hypoxia with 4% oxygen. The mice were randomized to 30 minutes of reoxygenation with either room air(n=17) or 100% oxygen(n=13). The untreated controls(n=10) came from the same cages as the ones exposed to hypoxia. Three hours post-reoxygenation the mice were sacrificed and the brains were rapidly frozen and stored at −70 C . Small samples of brain homogenates were mixed according to examination group, and the resulting three batches were analyzed for AQP4 protein level by Western blotting. Only the average scores in the three batches were registered. These scores were weighted according to the number of mice in each group. A univariate analysis of variance (ANOVA) was conducted to explore the overall differences in aggregated scores between groups.

**Results** In both groups of mice exposed to hypoxia we measured an increase of about a doubling in AQP4 protein level compared with controls ($P<0.05$). We found higher AQP4 levels when 100% oxygen was used for reoxygenation compared to room air ($P<0.05$). Conclusion In mice exposed to hypoxia and reoxygenation we have found an increase in the level of aquaporin 4 in brain homogenates. The highest level of AQP4 was measured in the group of mice reoxygenated with 100%O2. This finding may explain edema development after exposure to hypoxia, and contribute to the choice of reoxygenation regime after a period of hypoxia.

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### INTESTINAL FLORA AND FECAL CALPROTECTIN IN VLBW INFANTS

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**Background**: The early bacterial colonization of the intestine post partum may affect gut inflammation and risk for disease. Very low birth weight infants (VLBW, < 1500 g) are at risk for gastrointestinal disease (e.g. NEC) and for poor growth. We have previously shown that fecal calprotectin (f-calprotectin), a marker of intestinal inflammation, was decreased in VLBW infants receiving antibiotics. Very little is known about the normal development of intestinal flora in VLBW infants. The aim of this study was to investigate the intestinal flora in VLBW infants and to determine if there is any association between fecal flora and f-calprotectin.

**Methods**: Bacterial culture and analysis of f-calprotectin were performed on 147 stool samples collected from 33 Swedish VLBW infants during the first 10 weeks of life.

**Results**: The proportion of samples with counts of > 100 000 CFU/g feces was 68% for lactic acid bacteria (LAB), 37% for other gram positive bacteria (GP) and 37% for gram negative bacteria (GN). LAB and GN counts in feces increased with postnatal age ($p=0.001$ and $p<0.001$). There was a positive correlation between LAB and GN counts ($r=0.32$, $p<0.001$) and between LAB and GP counts ($r=0.44$, $p<0.001$). In post-meconium samples, there was a positive correlation between GN and f-calprotectin ($r=0.40$, $p<0.001$) and between GP and f-calprotectin ($r=0.22$, $p=0.024$). Antibiotic use was associated with lower GN counts (odds ratio 4.5 [2.1–9.8] for counts < 100 000 CFU/g).

**Discussion**: Compared to previous studies of VLBW infants, the proportion of LAB was surprisingly high. This may be explained by the fact that all infants in our study were fed breast milk. Non-LAB-bacteria (GN and GP) increase intestinal inflammation as measured by f-calprotectin and this may possibly have negative health consequences. Our results suggest that antibiotics reduce f-calprotectin by reducing GN. Probiotics may be effective in this risk population.

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### LOW DOSE DEXAMETHASONE IN CHRONICALLY VENTILATOR-DEPENDENT INFANTS AND SURVIVAL FREE OF CEREBRAL PALSY IN EARLY CHILDHOOD. THE DART STUDY

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**Background**: Postnatal corticosteroids to treat ventilator-dependent infants are associated with adverse neurological sequelae in childhood, especially cerebral palsy, but may save lives. Corticosteroids may be beneficial in infants at very high risk of chronic lung disease (CLD).

**Aims**: To determine if a low-dose, short course, of dexamethasone given after the first week of life affects survival free of cerebral palsy (CP) at 2 years of age.

**Methods**: Very preterm (<28 weeks) or extremely low birthweight (birthweight <1000 g) ventilator-dependent infants after the first week of life and in whom the clinician thought steroids were clinically indicated were eligible. Infants were randomly allocated to receive either a 10-day tapering course of low-dose dexamethasone (0.89 mg/kg total over 10-days), or an identical volume of saline placebo. Surviving children were assessed at 2 years of age, corrected for prematurity, by paediatricians and psychologists blinded to treatment group allocation.

**Results**: Thirty-five infants were randomly allocated to each group. The rate of CLD in the control group was >80%. Mortality rates were not substantially different between the 2 groups (dexamethasone 11%, controls 20%; OR 0.52, 95% CI 0.14, 1.95; P=0.32), and the rates of cerebral palsy were 11% in the dexamethasone group and 17% in the control group. There was not a clear-cut difference in the combined rate of death or cerebral palsy (dexamethasone 23%, placebo 37%; OR 0.50, 95% CI 0.15, 1.60). The reduction in the combined outcome of death or cerebral palsy in this cohort at very high risk of CLD was consistent with the results of a recent metanalysis of randomised trials of postnatal corticosteroids (Pediatrics 2005:115; 655–661).

**Conclusions**: The long-term effects of dexamethasone in infants at high risk of CLD may not be universally detrimental.