

Abstracts

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Room Air Resuscitation of Newborn Pigs following Cardiac Arrest

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Background: In spite of evidence suggesting that resuscitation with 100% O₂ is detrimental, it is still recommended at least for the most compromised neonates. The clinical studies comparing 21% and 100% O₂ included many infants with mild/moderate asphyxia. This has been a focus of criticism in relation to their good response to room air. **Objective:** To investigate the use of 21% and 100% O₂, respectively, in resuscitation of newborn pigs following cardiac arrest. **Methods:** Noroc pigs (12–36 h old) were anaesthetized and mechanically ventilated. Progressive asphyxia was induced until asystole occurred and then cardiopulmonary resuscitation (CPR) was initiated following neonatal resuscitation guidelines. Ventilation was made by either 21% (n = 16) or 100% O₂ (n = 16). Return of spontaneous circulation (ROSC) was defined as a heart rate ≥ 100 /min. If there was no ROSC after 15 min of resuscitation, the procedure was ended. Time to ROSC for these animals was set at an arbitrary 1,000 s for statistical calculations using the Mann-Whitney test. If successful resuscitation, the animals were observed on the ventilator for an additional 4h before euthanasia was performed. **Results:** Mean time of asphyxia sustained and blood gases at asystole were comparable between groups. All animals except two in the 100% O₂ group achieved ROSC. One animal in the 21% O₂ group suffered bradycardia at baseline, and was thus excluded. For the remaining 15 animals resuscitated with 21% O₂, median time to ROSC [IQR] was 150 [115–180] s. In comparison, animals in the 100% O₂ group achieved ROSC after 135 [113–168] s (p = 0.80). There were no differences in temporal changes in mean arterial pressure, heart rate or arterial blood gases throughout the experiment between the groups. **Conclusion:** Even in this extreme model of perinatal asphyxia, 100% O₂ did not provide faster circulatory recovery compared to room air.

Ethical approval: The experimental protocol was approved by The Norwegian Council for Animal Research.

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Surfactant Treatment in an Experimental Model of Meconium Aspiration Syndrome

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Background: Meconium aspiration syndrome is still a common cause of severe respiratory distress in neonates. Meconium has an inhibitory effect on the surfactant system, and bronchoalveolar lavage and surfactant replacement have been studied in recent years. **Objective:** To evaluate the effect of bronchoalveolar lavage with diluted surfactant followed by a supplementary dose of surfactant in a piglet model of meconium aspiration syndrome. **Methods:** 25 newborn piglets were artificially ventilated with fixed settings throughout the study. After tracheal instillation of meconium diluted to 20% in isotonic saline, the piglets were randomized in the following groups: Group I (n = 9) – control; Group II (n = 6) – bronchoalveolar lavage with diluted surfactant (15 ml/kg); Group III (n = 5) – bronchoalveolar lavage with diluted surfactant (15 ml/kg) followed by a supplementary dose of surfactant (Curosurf® 100 mg/kg); and Group IV (n = 5) – dose of surfactant without bronchoalveolar lavage. Arterial blood gas samples were obtained 30 min and 3 h after meconium instillation. **Results:** The difference between PaO₂ before and after the intervention (Δ PaO₂) was (mean \pm SD) 44 + 27 mm Hg (5.9 \pm 3.6 kPa) and 10 + 11 mm Hg (1.3 \pm 1.5 kPa) for Group III and Group I, respectively (p = 0.006) at 30 min, and 69 + 65 mm Hg (9.2 \pm 8.7 kPa) and 12 + 21 mm Hg (1.6 \pm 2.8 kPa) at 3 h, respectively (p = 0.03). The difference between Group II and control was not statistically significant. The PaO₂ variation in Group IV was very similar to the control group. **Conclusion:** Bronchoalveolar lavage with diluted surfactant followed by a supplementary dose of surfactant improves oxygenation in an experimental piglet model of meconium aspiration syndrome.

Ethical approval: approved by the Ethical Committee of PUCRS.

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DNA Damage and Base Excision Repair Seem Unchanged in Newborn Mouse Lung Compared to Liver after Resuscitation with 100% Oxygen

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Background: There is some evidence that treatment of asphyxiated newborns with supplementary oxygen increases the risk of childhood cancer. Reactive oxygen species can cause accumulation of DNA damage if not prevented by antioxidant mechanisms. Damaged DNA may lead to mutations if not repaired. Base excision repair (BER) is the major pathway for repair of oxidative DNA damage. **Objective:** We hypothesized that asphyxia would render tissues susceptible to oxidative DNA damage when followed by resuscitation with oxygen. We wanted to investigate the effect of 100% oxygen on oxidative DNA damage and BER in a murine model of the hypoxemic newborn. **Methods:** Mice at postnatal day 7 were randomized to receive either 8% oxygen or room air for 60 min in a closed chamber and subsequent reoxygenation with 100% oxygen for 0–90 min. Tissues were harvested immediately post-intervention. Oxidative DNA damage was quantified by HPLC-ECD as the ratio of 7,8-dihydro-8-oxo-2'-deoxyguanosine (8OHdG) per 50,000 2'-deoxyguanosine in nuclear DNA. BER activity in nuclear extracts was assessed by the ability to incise at single base damage residues (Uracil, 5-hydroxycytosine, 8-oxoguanine, AP-site) in duplex DNA oligos. **Results:** In liver DNA there was a linear, time dependent increase in 8OHdG in animals exposed to hypoxia (n = 25, B = 0.011, 95% CI [0.001 to 0.021]; p = 0.037). In lung there was no significant correlation (n = 28, B = 0.005, 95% CI [-0.007 to 0.180]; p = 0.384). Removal of damaged base residues was not significantly changed. **Conclusions:** These results indicate that lung tissue may withstand a rapid transition in available oxygen better than other tissues. BER activity seems unchanged suggesting that asphyxia-induced DNA damage is effectively removed by the normal repair capacity in lung. Approved by NARA Norway.

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ABCA3-E292V-Mutation and Respiratory Outcome in Preterm Infants

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Background: Deficiency of ATP Binding Cassette protein A3 (ABCA3) is a genetic cause of severe respiratory failure in term infants. The ABCA3-E292V-mutation was found to be more common in newborns with respiratory distress syndrome (RDS) and

pneumothorax from different countries (Pediatr Res 2008;63:645). **Objective:** To find out whether this mutation is associated with more severe RDS in preterms. **Methods:** Genotyping of the ABCA3-E292V-mutation from buccal swabs in a prospectively enrolled cohort of 2000 German very low birth weight infants (VLBW = <1,500 g). **Results:** Eight (0.4%) of 2000 VLBW-infants were heterozygous carriers of the ABCA3-E292V-mutation. Outcome data of these infants and 1992 VLBW-infants without the mutation are given in the table.

	ABCA3-E292V carriers (n = 8)	Controls (n = 1,992)	p*
Mean gestational age, weeks	28.6	28.9	0.73
Mean birth weight, g	1,029	1,078	0.69
Mean number of surfactant doses	1.4	0.8	0.47
Mechanical ventilation, %	62	45	0.48
Infants treated with surfactant, %	50	47	1.0
Pneumothorax, %	0	6	1.0
Bronchopulmonary dysplasia (O ₂ at 36 weeks, %)	25	13	0.28
Death, %	13	3	0.21
Death or bronchopulmonary dysplasia, %	38	15	0.10

* Mann-Whitney U-test (gestational age, birth weight, number of surfactant doses) and Fisher's exact test.

Conclusions: The frequency (0.4%) of the ABCA3-E292V-mutation is in the expected range for unselected cohorts of European newborns. Due to the low number of infants carrying the ABCA3-E292V-mutation we did not observe significant differences between cases and controls. However, the high prevalence of mechanical ventilation, BPD and death indicates that the ABCA3-E292V-mutation might be associated with poorer (pulmonary) outcome. Substantially larger study populations will be needed to answer questions about the clinical relevance of rare mutations. The German Neonatal Network (GNN) will provide such a platform.

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Surfactant Protein-D Polymorphism in Term and Preterm Infants

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Background: Surfactant protein D (SP-D) participates in the innate immune system and in surfactant homeostasis. The gene is mapped to chromosome 10 and several single nucleotide variations (SNPs) have been described. We have earlier described increased levels of SP-D in preterm infants who need ventilatory support, probably due to capillary leak. **Objective:** To describe

variations in the surfactant protein D gene and the possible effect on respiratory outcome in newborn infants. **Methods:** We included 214 term and 195 preterm newborn infants. SP-D levels in serum were measured by standard ELISA. Genotyping for seven different SNPs in the SP-D gene was performed. We conducted linkage disequilibrium analysis to estimate haplotype frequencies. **Results:** The genotype Met/Met11 was found in 31%, whereas 18% carried the genotype Thr/Thr11. The mean SP-D level in umbilical cord blood was significantly higher in infants with the genotype Met/Met11 than in the genotype Thr/Thr11 ($p = 0.0059$). In preterm infants the difference was even more evident ($p < 0.00001$). In preterm infants in need of respiratory support with the genotype Met/Met11, a significant rise in SP-D levels on day 4–10 was seen, but not in infants with genotype Thr/Thr11. Preterm infants with genotype Met/Met11 had less need for treatment with oxygen than infants with genotype Thr/Thr11, 43.7 vs. 25.0%. We found a significant effect of haplotypes on the SP-D level on the first day of life and the maximum rise in SP-D, but no significant differences in the estimated haplotype frequencies between term and preterm infants. **Conclusions:** The allele frequency found in term infants is similar to the frequency described in adults. The finding of higher SP-D levels in individuals with genotype Met/Met11 is consistent with earlier findings, and is even more apparent in preterm infants. The results suggest that preterm infants with the genotype Thr/Thr11 are less susceptible to develop respiratory distress syndrome. The higher levels of SP-D found in preterm infants cannot be explained by differences in haplotype.

The study was approved by the local ethical committee of Southern Denmark.

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Expression and Function of Carcinoembryonic Cell Adhesion Molecule 6 in Human Neonatal Lung Epithelial Cells

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Background: The transition to extrauterine life requires the induction of numerous genes in fetal lung, several of which are involved in surfactant synthesis and many others have an unknown function. Carcinoembryonic cell adhesion molecule 6 (CEACAM6), a glycosylated protein expressed in several epithelial cell types and cancer cells, is strongly induced during type II cell differentiation and maturation in an in vitro hormone-stimulated human fetal lung cell model but its regulation and functions are unknown. **Methods:** We cultured primary human fetal lung epithelial cells as well as H441 and A549 adenocarcinoma cell lines and examined responses to differentiation-promoting hormones (dexamethasone 10 nM and cAMP 0.1 mM), adenovirus expressing thyroid transcription factor (TTF-1) and silencing of TTF-1 using small inhibitory RNA. CEACAM6 promoter fragments

linked to firefly luciferase were studied after transient transfection of A549 cells with plasmids using lipofectamine. **Results:** In fetal lung cells, glucocorticoid and cAMP had additive stimulatory effects on CEACAM6 content, increasing the rate of transcription 13-fold and protein content 10-fold at 48 h. CEACAM6 localized to plasma membrane and lamellar bodies of fetal type II cells, and a portion of extracellular CEACAM6 was associated with surfactant. Knockdown of TTF-1 (90% by siRNA) reduced hormone induction of CEACAM6 by 80%. Expression of rTTF1 by adenovirus transduction caused a linear increase in CEACAM6 content to levels greater than those obtained with hormone treatment. In studies with H441 cells, which express TTF-1, and A549 cells (TTF-1 negative), hormone treatment increased and decreased CEACAM6, respectively. Basal content of CEACAM6 in H441 cells was not affected on reduction of TTF-1 by >90% using siRNA. In studies of CEACAM6 promoter in A549 cells, reporter expression was detected with 4 different deletion constructs ranging from 0.3 to 3.0 kb with highest expression at 0.3 kb. Reporter expression was decreased 50% by 8-BrcAMP + isobutylmethylxanthine treatment and was unaffected by exposure to dexamethasone. **Conclusions:** We conclude that CEACAM6 in fetal lung epithelial cells is a target protein for TTF-1, which suggests a role in lung adaptation to extrauterine life that may include surfactant-related functions. Conversely, CEACAM6 expression in some adenocarcinoma cell lines is not dependent on TTF-1 and displays different responses to hormones that may be mediated via proximal promoter elements. Dysregulated CEACAM6 may contribute to tumorigenesis of lung epithelial cells.

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Chorioamnionitis Alters the Response to Surfactant in Preterm Infants

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Background: Surfactant efficacy is reduced by inflammatory mechanisms. This may affect the response to surfactant in infants exposed to chorioamnionitis. **Objective:** To study the association between antenatal exposure to chorioamnionitis and the neonatal response to surfactant in preterm infants. **Methods:** Infants received 1–2 doses of beractant 100 mg/kg according to fixed criteria. FiO₂ requirement and time to extubation were compared between groups based on presence of histologic chorioamnionitis (HC) ± fetal involvement (F), and between infants who developed bronchopulmonary dysplasia (BPD) or died and those surviving without BPD. **Results:** 301 infants were included, with 146 receiving surfactant and 98 receiving two doses. There was no significant difference between HC-, HC+F- and HC+F+ infants in the proportion that received surfactant. HC+F+ was associated with significantly increased FiO₂ requirement during the first 12 h after both surfactant doses. Moreover, time to extubation was significantly prolonged in these infants after the second dose

($p < 0.05$). These effects were not accounted for by differences in gestational age and birth weight. Infants who developed BPD or died exhibited a pattern of increased FiO_2 requirement post-surfactant strikingly similar to that observed after HC+F+. Moreover, in infants who received surfactant, HC+F+ was associated with an increased risk for BPD [OR (95% CI) 3.40 (1.02 to 11.3); $p = 0.047$] and for BPD or death [OR (95% CI) 2.73 (1.00 to 7.42); $p = 0.049$] in multivariate analyses. **Conclusions:** Chorioamnionitis is associated with decreased efficacy of exogenous surfactant in preterm infants. Both surfactant inactivation and increased clearance may play a role. An inadequate surfactant response may be involved in the association between chorioamnionitis, mechanical ventilation and development of BPD.

Ethical approval: The study was approved by the local Institutional Review Board.

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Modern Molecular Techniques to Detect Pathogens Associated with Chorioamnionitis

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Background: Antenatal intrauterine infection is implicated in the pathogenesis of bronchopulmonary dysplasia (BPD). A number of organisms are associated with chorioamnionitis (chorio), but there is little work using nucleic acid testing to correlate the microbiological findings with histopathology and clinical outcome. **Objectives:** To determine the relationship between microbiology, histopathology and BPD in babies < 33 weeks' gestation using modern molecular techniques. **Methods:** This is a prospective observational study on 68 babies born < 33 weeks' gestation between February and August 2008. Gene specific polymerase chain reaction (PCR) to detect *Ureaplasma urealyticum*, *Escherichia coli*, *Chlamydia* spp. and *Listeria monocytogenes* was performed on placenta, cord blood and umbilical cord specimens. Chorio/funisitis was determined histologically by looking for the presence of neutrophils in the various layers of the placenta and cord. BPD was defined as oxygen dependency at 36 weeks' corrected age. **Results:** Of the 68 babies recruited, chorio/funisitis was detected in 27 (40%) and 21 (31%) developed BPD. However, PCR was positive in only 17 (26%) in either placenta or cord blood for presence of infection. 62% of the babies who developed BPD had associated chorio and/or funisitis and one third had both chorio and infection. *Ureaplasma urealyticum* was the most frequently obtained organism (16/68; 23%). *Escherichia coli* was isolated in specimens from one baby, and none of the samples was positive for *Listeria* or *Chlamydia*. **Conclusions:** Chorioamnionitis is frequently associated with preterm deliveries and subsequent development of BPD. As the targeted pathogens were not detected in all episodes of chorio, further work should focus on applying molecular diagnostic techniques to detect and quantify a broader range of microorganisms.

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Protective Role of Fibroblast Growth Factor-10 in TGF β -Mediated Lung Injury

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Rationale: Fibroblast Growth Factor (FGF-10) is required for lung development and differentiation of the distal-alveolar epithelial progenitor cells. Reduced FGF-10 causes neonatal respiratory distress syndrome (RDS). Previously, we showed that excess TGF β is associated with adverse pulmonary outcome in preterm neonates. Whether FGF-10 can protect against injury caused by TGF β remains unknown. **Objective:** To test in a transgenic mouse model whether FGF-10 overexpression can protect against bleomycin-induced TGF β -mediated lung injury and fibrosis. **Methods:** SP-C-rtTA; tet(O)FGF10 double transgenic mice were generated. Mice were treated with bleomycin (BLM) for 1 week. Concomitantly, exogenous FGF10 expression in the alveolar epithelium was induced using doxycycline for 1 week. Animals were examined at different time points. **Results:** 100% of mice in the doxycycline-fed group (BLM/DOX+, $n = 8$) survived as compared to only 38% in the control group (BLM/DOX-, $n = 8$). A significant reduction in fibrosis scoring and collagen was found in BLM/X+ vs. BLM/DOX- mice. Bronchoalveolar lavage fluid (BALF) from both groups showed no significant difference in inflammation. However, active TGF β 1 concentrations in the BALF from BLM/DOX+ vs. control BLM/DOX- group showed a reduction at 3, 7 and 14 days after injury associated with a corresponding decrease in TGF β 1 signaling. **Conclusions:** FGF-10 offers a distinct protective effect by attenuating the TGF β 1 pathway. A FGF10 treatment strategy may provide robust protection to neonatal and other forms of lung diseases caused by TGF β .

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Respiratory Therapy and Outcome of Neonatal Respiratory Failure from 13 Neonatal Intensive Care Units of Hebei Province in 2007–2008

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Background: Both capacity and quality of NICUs in the sub-provincial level III hospitals have been extensively developing in China since 2000. It is of clinical importance to evaluate how ef-

ficiently the advanced therapies for neonatal respiratory failure (NRF) are implemented in resource limited regions. **Objective:** To investigate the incidence, clinical management and outcome of NRF from 13 NICUs of level III hospitals in Hebei province, each serving for a population of 1–5 million. **Methods:** Prospective data collection was performed from July 2007 to June 2008 in all the NICU admissions. NRF (age ≤ 7 days) was defined as requiring intratracheal intubation and mechanical ventilation (MV) or nasal continuous positive airway pressure (nCPAP) according to an established protocol [Qian LL et al. *Pediatrics*. 2008; 121 (5); e1115–e1124]. **Results:** From 9,973 NICU admissions, a total of 1,875 cases of NRF were identified (incidence 18.8%) at a birth weight (BW) range of 600–5,500 g, 64.3% being $< 2,500$ g, and only 2% as extremely low BW ($< 1,000$ g). The male/female ratio was 2.6:1. Among NRF cases, 831 had respiratory distress syndrome (RDS, 44.3%), followed by aspiration of amniotic fluid (AAF, 25.9%), lung infection (7.8%), meconium aspiration syndrome (MAS, 7.7%), persistent pulmonary hypertension (6.3%), and transient tachypnea (TT, 5.0%). nCPAP was much more frequently used than MV (73.3% vs. 48.5%, $p < 0.05$), and was initially provided to 1,108 NRF cases, among which 908 (48.4%) had only nCPAP with 80.8% survival; 875 (46.7%) required MV with 59.8% survival. The other 92 (4.9%) met the inclusion criteria but MV was not established because of the parents' decision. The overall mortality rate before discharge was 31.4% (range 9.1–62.7%). In all survivors before discharge, mean length of hospital stay and costs were 14.6 ± 7.8 days and $11,540 \pm 6,902$ Chinese Yuan. Surfactant was given to 533 (28.4%) of NRF cases, mainly for RDS (468, 87%), as well as for AAF (51, 9.5%), MAS (12, 2.2%), and lung infection (2, 0.4%), mostly as rescue (99.7%) and rarely as prophylaxis (0.3%); 80.7% was given within 24 h after birth. Among RDS, 460 (55.6%) received surfactant (range 29.6–80.8%). Very low BW ($< 1,500$ g) accounted for 76.5%, and second dose was given to 6.4%, of the surfactant-treated. In all the NRF cases, male infants were more likely to get surfactant than the females (31.1 vs. 21.4%, $p < 0.05$). **Conclusions:** Along with the limited resources and center variations, nCPAP and rescue surfactant as adjunctive therapy to conventional MV have nowadays been widely accepted for NRF infants in these sub-provincial level III NICUs, comparable to that of the provincial neonatal centers in 2004–2005. These data may serve as a baseline for further study of advanced respiratory therapies in NICUs of emerging regions, and warrant further quality improvement aiming at reduction of NRF mortality.

Ethical approval was obtained from the ethic committee of Children's Hospital of Fudan University and adopted by all the participating centers according to Chinese regulation for clinical investigation. (Supported by CMB 03–786, NSFC 3061120518, and a TEGAE training program in Hebei Province).

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Pulmonary Function and Exercise Capacity in 7–9 Year Old Children with Bronchopulmonary Dysplasia

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Background: Subjects of very low birth weight with bronchopulmonary dysplasia (BPD) have significantly poorer lung function than term-born children. **Objective:** To determine effects of severe BPD on lung function, exhaled nitric oxide, exercise capacity and atopy diathesis in ex-preterms with severe BPD compared with healthy peers. **Methods:** In a prospective study, children born with a gestational age of less than 30 weeks with severe BPD ($> 30\%$ oxygen at 36 weeks and discharged home receiving supplemental oxygen) were followed up to 7–9 years. Participants ($n = 11$; mean gestational age 24.8 weeks) and healthy term control subjects ($n = 9$) were recruited for lung function testing (spirometry), exhaled nitric oxide measurement, fitness assessment (6-minute walk test) and measurement of atopic diathesis. **Results:** The age of participants at the time of assessment did not differ between the groups (7.8 ± 0.9 year in BPD group vs. 7.3 ± 0.8 year in healthy term controls; $p = 0.20$). Healthy term controls were taller (132 ± 6 cm in healthy term controls vs. 121 ± 6 cm in the BPD group; $p = 0.02$), however, weight did not differ between groups (24 ± 7 kg in BPD group vs. 28 ± 4 kg in healthy term controls; $p = 0.20$). Severe BPD was associated with lower forced expiratory volume (FEV-1) (BPD group, 78% predicted vs. controls 89% predicted; $p = 0.04$), lower FEF-25–75 (56% predicted vs. 87% predicted; $p = 0.003$) and lower exercise capacity (6-minute walk test, 524 ± 52 m in healthy term controls vs. 462 ± 68 m in BPD group; $p = 0.04$). No differences between the groups were found in forced vital capacity (FVC), exhaled nitric oxide and atopic diathesis. **Conclusion:** In our group of school-aged children with severe BPD discharged on home oxygen therapy who underwent lung function, atopy, exhaled nitric oxide and fitness assessment, we demonstrated significant impairment in FEV(1), FEF (25–75) and the distance walked in the 6-minute walk test.

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Enrichment of Myristic Acid in Neonatal Surfactant Phosphatidylcholine Is Differentially Modified by Exogenous Fatty Acids

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Background: Palmitoyl-myristoyl-PC (PC16:0/14:0) and palmitoyl-palmitoleoyl-PC (PC16:0/16:1) are enriched in mammalian lung surfactant, and in some cases their concentrations are greater than those of 'classical' phospholipid components (dipalmitoylphosphatidylcholine [PC16:0/16:0], phosphatidylglycerol). In humans, PC16:0/14:0 increases from 34 weeks' gestational age on-

wards but shows considerable interindividual variability. Functionally, PC16:0/14:0 triggers macrophage differentiation and enables them to regulate T-lymphocyte proliferation. While it is increased during alveolarization and decreased in chronic lung injury, regulation of its concentration is unknown. While type II pneumocytes are capable of synthesizing fatty acids (FA), triglycerides (TAG) from lung lipid interstitial cells (LIC) and from plasma are additional and important FA sources of surfactant PC. **Objective:** To investigate the regulation of FA composition in neonatal surfactant PC by intra- and extrapulmonary TAG supply. **Methods:** The profile changes of surfactant PC composition and of pulmonary TAG-FA were followed from d1-d42 in rats (from placental through milk to adult chow feeding). We then compared the effects of high (rat milk) vs. low (carbohydrate-based chow) myristic acid (C14:0) supply on surfactant PC profile in 18–24d old rats. Uniqueness of C14:0-effects was further controlled by feeding with 2% synthetic trimyristin [C14:03] compared to (trilaurin [C12:03], tripalmitin [C16:03], triolein [C18:13] and trilinolein [C18:23]). **Results:** PC16:0/14:0 correlated directly with C14:0 and C12:0 of lung TAG. Any increase in surfactant PC16:0/14:0 was at the expense of PC16:0/16:0. Exogenous C14:0 did not increase C14:0 in plasma or liver PC nor in liver TAG. In plasma only chylomicrons were enriched in C14:0. Nutrition with synthetic TAG showed that among the exogenous FA, C14:03 was most effective in changing the surfactant PC profile. As a consequence PC16:0/14:0 became a major (mean and SD: 45 ± 3 vs. $9 \pm 1\%$) and PC16:0/16:0 a minor (29 ± 2 vs. $46 \pm 1\%$) surfactant component, with no obvious effects on respiration or vitality. Importantly, C12:03 also increased PC16:0/14:0 ($15 \pm 1\%$). C18:23 was the only TAG that increased PC16:0/16:0 ($55 \pm 1\%$) which was at the expense of monounsaturated PC16:0/16:1 (13 ± 1 vs. $26 \pm 1\%$) and palmitioyloleoyl-PC (2.9 ± 0.1 vs. $5.4 \pm 0.3\%$). Exogenous C16:0 and C18:1 showed no significant effects. **Conclusions:** Changes in the PC composition of mammalian lung surfactant are intimately linked to the supply with neutral lipids, particularly those containing myristic, lauric and linoleic acid. Differences in maternal FA supply and metabolism may in part explain the greater variability of surfactant composition in human neonates compared to laboratory rats. The increase in PC16:0/14:0 concentration in response to exogenous C14:0 may be important to alveolar macrophage function in vivo.

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CURPAP Study – an International Randomised Controlled Trial to Evaluate the Efficacy of Combining Prophylactic Surfactant and Early Nasal Continuous Positive Airway Pressure in Extremely Low Gestational Age Neonates

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Background: Prophylactic surfactant (PS) is recommended in extremely low gestational age neonates (ELGANs) at high risk of respiratory disease. Early nasal continuous positive airway pres-

sure (CPAP) has been recently proposed as a first line respiratory support in such patients. **Objective:** To evaluate the efficacy of two strategies of post-delivery stabilization and early respiratory care in spontaneously breathing ELGANs: PS followed by nasal CPAP, versus nasal CPAP followed by rescue surfactant (RS) treatment if needed. **Methods:** Newborns with gestational ages of 25⁺⁰ to 28⁺⁶ weeks who were breathing spontaneously were randomized immediately after birth into either: Group 1 – PS, followed by nasal CPAP as soon as possible or Group 2 – early stabilization on nasal CPAP with early RS. Surfactant (Curosurf[®]) was administered using a first dose of 200 mg/kg. The primary endpoint was the rate of mechanical ventilation (MV) in the first 5 days of life. **Results:** 208 infants were enrolled in 24 European NICUs. The incidence of MV in the first 5 days was similar in the two groups (Group 1: 31.4%, Group 2: 33.0%; RR 0.95; 95% CI 0.64 to 1.41). 21.9% and 21.4% of infants, respectively, required oxygen treatment or respiratory support or had died at 36 weeks' postmenstrual age. The incidence of pneumothorax was 6.7% in Group 1 and 1% in Group 2 (RR 6.82; 95%CI 0.86 to 53.75). There were no differences in the incidence of other complications. **Conclusions:** Prophylactic surfactant followed by nasal CPAP and nasal CPAP followed by early rescue surfactant had a similar efficacy in terms of need for MV during the first 5 days of life and the major complications of prematurity in spontaneously breathing ELGANs. The pulmonary outcome was good in both arms. Further studies may clarify the position of early nasal CPAP combined with surfactant in the post-delivery management of ELGANs.

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Lung Recruitment Manoeuvre in Preterm Infants Undergoing Volume Guarantee Ventilation Improves Efficacy of Surfactant Replacement

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Background: Over or under-recruitment of the preterm lung leads to inefficacy of surfactant replacement, with prolonged ventilation and O₂ dependency. A lung recruitment manoeuvre (LRM) performed during ventilation to optimize alveolar stability avoids over-distension and reduces O₂ requirement. In animal studies, surfactant administration to the 'closed' lung (before LRM) seems to be more efficacious than in the 'open' lung (only after LRM) or without LRM. **Objective:** To study ventilation and oxygenation state in preterm infants treated with surfactant plus LRM in assist/control (A/C) + Volume Guarantee (VG) ventilation. **Methods:** 16 preterm infants, in A/C + VG (Vt = 6 ml/kg), after surfactant therapy (200 mg/kg of porcine surfactant, Curosurf[®], Chiesi, Italy) for RDS, randomly received a LRM in the first 3 h of life (Gr A) or not (Gr B). LRM = increments of positive end-expiratory pressure (PEEP) (steps of 0.2 cmH₂O every 5 min) monitoring the falling FiO₂ needs and rising SpO₂. When FiO₂ need fell to 0.30 we reduced PEEP to set the lung filling on the deflation limb of the pressure/volume (P/V) curve above the lower inflation point. When SpO₂ fell and FiO₂ increased we re-in-

cremented PEEP until stable SpO₂ was achieved. **Results:** The two groups were similar (mean ± SD; gestation 25 ± 2 vs. 25 ± 2 weeks; birth weight 747 ± 233 vs. 737 ± 219 g; CRIB score 9.8 vs. 8.1; FiO₂ at start 0.56 ± 0.28 vs. 0.52 ± 0.21; GrA vs. GrB, respectively). GrA received the LRM at 86 ± 69 min of age and it lasted for 61 ± 18 min and no adverse events occurred. GrA vs. GrB respectively showed different: 1) max PEEP reached during LRM (6.2 ± 0.3 vs. 5.3 ± 0.3 cm H₂O; p = 0.001), 2) time to lowest FiO₂ (94 ± 24 vs. 435 ± 219 min; p = 0.003) and 3) O₂ dependency duration (29 ± 12 vs. 45 ± 17 days; p = 0.04). There were no differences in rates of bronchopulmonary dysplasia, retinopathy of prematurity or death. **Conclusion:** In the 'closed' preterm lung with respiratory distress syndrome, surfactant followed by early LRM led to an earlier achievement of the lowest FiO₂ in the first 12 h of life, which was significantly lower than that previously needed for adequate oxygenation and shorter O₂ dependency. LRM enhances the clinical effect of surfactant replacement during mechanical ventilation.

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Dexamethasone Potentiates Interleukin-6 Induced Surfactant Protein-B Expression in H441 Cells

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Background: The respiratory distress syndrome (RDS) is a major problem after preterm birth but is ameliorated by antenatal steroids and/or chorioamnionitis. Surfactant protein-B (SP-B) is decreased in infants with RDS. Both antenatal dexamethasone administration and chorioamnionitis reduce the incidence and severity of RDS. Concentrations of the proinflammatory cytokine interleukin-6 (IL-6) are elevated in chorioamnionitis activating the intracellular STAT (signal transducer and activator of transcription)3 signalling pathway. **Objective:** In this study we investigated the potential synergistic effect of dexamethasone and IL-6 on SP-B gene expression and STAT3 phosphorylation in H441 cells, a cell line with characteristics of Clara cells. **Methods:** H441 cell cultures were incubated with doses of recombinant human IL-6 (5 to 50 ng/ml) and/or dexamethasone (10⁻⁷ M). Cell cultures were analysed after 1 min till 60 min of incubation for SP-B mRNA by real time PCR and for phosphorylated STAT3 protein in Western blots. **Results:** Dexamethasone alone increased SP-B mRNA levels by 16.5-fold (p < 0.05 versus media) and IL-6 alone by 2.3-fold (p < 0.05 versus media). After 48-hour exposure of cells to dexamethasone IL-6 caused a greater increase in SP-B mRNA levels (28.1-fold) compared with IL-6 or dexamethasone alone (p < 0.05). While IL-6 alone stimulated tyrosine phosphorylation of STAT3 in a time and dose dependent manner, dexamethasone alone had no effect on STAT3 phosphorylation. However, dexamethasone potentiated IL-6 induced phosphorylation of STAT3 4-fold (p < 0.05). The synergism of dexamethasone and IL-6 on SP-B mRNA levels and the effect of dexamethasone on IL-6 induced STAT3 phosphorylation could be blocked by an

inhibitor of the STAT cascade. **Conclusion:** The interaction of IL-6 and dexamethasone on SP-B gene transcription may explain the reduced severity of RDS after antenatal steroids and/or chorioamnionitis.

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Influence of Home Oxygen Therapy on Outcome of Infants of Less than 29 Weeks' Gestation with Bronchopulmonary Dysplasia

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Background: Bronchopulmonary dysplasia (BPD) is the commonest chronic disease of preterm infants, with those being discharged on home oxygen therapy at high risk for developmental delay and slow weight gain. **Objectives:** (1) To assess perinatal factors contributing to the development of BPD requiring home oxygen therapy and (2) to compare growth and development of these infants at two years corrected age with infants discharged home breathing room air. **Design/Methods:** Infants with gestational age (GA) <29 weeks discharged on home oxygen therapy were compared with their peers matched by gestational age and gender. Growth charts from Centers for Disease Control and Prevention 2000 and Denver Development Screening Test II were used to assess their growth and development. BPD was defined as oxygen requirement (SpO₂ 85 to 95%) at 36 weeks' postmenstrual age. During the 5-year period (2002–2006), 329 infants with GA <29 weeks were born, 257 (78%) survived to discharge. 162 (63%) of survivors developed respiratory distress syndrome (RDS), 46 (18%) BPD and 26 (10%) were discharged home on oxygen therapy. Infants with severe white matter lesions (intraventricular hemorrhage of grade IV or cystic periventricular leukomalacia) and congenital myotonic dystrophy were excluded. **Results:** 21 infants (9 boys and 12 girls) with BPD discharged on home oxygen therapy were followed up longitudinally to 2 years corrected age and compared with their matched controls. There were no significant differences between the groups regarding birth weight (820 vs. 853 g), labour beginning with premature rupture of the membranes, clinical chorioamnionitis, antenatal steroids, abdominal delivery, Apgar score at 1 and 5 min, resuscitation procedures at birth, incidence of RDS, severe neonatal infection and requirement of mechanical ventilation. At 24 months corrected age weights of infants discharged on oxygen were on the 15th, length on the 35th and head circumference on the 38th percentiles (–10, –15, and –5 percentiles compared to the control group; NS). In both groups developmental delay was found in 29% of infants, however, blindness, strabismus and partial deafness were more common in the study group (1 vs. 0; 5 vs. 3 and 2 vs. 0, respectively) and cerebral palsy was more common in the control group (1 vs. 2). **Conclusions:** (1) No differences in perinatal factors between infants with BPD requiring home oxygen therapy and infants breathing room air at discharge were found. (2) Home oxygen therapy did not significantly compromise growth velocity and development at 2 years corrected age.

Is a Low Dose Dexamethasone Regimen Effective in Extremely Low Birth Weight Babies with Evolving Bronchopulmonary Dysplasia?

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Background: Dexamethasone (DEX) facilitates extubation of extremely low birth weight (ELBW) babies with evolving bronchopulmonary dysplasia (BPD). There are only a few studies comparing different doses of DEX, and as steroids are associated with an increased risk of cerebral palsy the smallest effective cumulative dose is probably desirable. It has been our standard practice to avoid postnatal DEX where possible, and when it is essential, use a very low dose regimen of DEX, starting at 0.05 mg/kg/day and decreasing over 7 days. **Objective:** To describe the use and efficacy of a very low dose DEX regimen in ventilator-dependent babies with developing BPD. **Methods:** 16 babies who received low dose DEX to wean from mechanical ventilation were retrospectively reviewed. Clinical course and ventilation history were recorded, along with details of DEX duration and dosing. Sequential Oxygenation Indices (OI) were calculated from blood gases and ventilator settings before and after the time of DEX administration. **Results:** Mean (SD) gestation was 25 (1.3) weeks and birthweight 679 (126) g. Mean (SD) total DEX dose was 0.234 (0.056) mg/kg over 7 days. The median (IQR) age for starting DEX was 30 (20–37) days, with initial FiO₂ of 0.49 (0.37–0.58). Of the 16 babies, 11 were extubated by the end of the DEX course, with 1 extubation failure and 1 death. OI decreased significantly during DEX therapy: median (IQR) OI 3 days before DEX was 10.4 (9.0–13.4); on day of starting DEX OI was 14.9 (7.7–18.0) and on day 3 of DEX therapy OI had decreased to 6.0 (4.4–7.5); ($p < 0.01$). **Conclusion:** A 7-day course of DEX starting at a dose of 0.05 mg/kg/day facilitates extubation in ELBW babies and may help minimise side effects. Further randomised trials are justified to explore the role of very low dose DEX in evolving BPD.

Perfluorocarbons Prevent Hyperoxia Induced Arrest in Alveolarisation

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Background: Up to 40% of very low birth weight infants develop bronchopulmonary dysplasia (BPD). New BPD is characterized by an arrest in alveolarisation, causing a reduced gas exchange area. Beside inflammatory processes, relative hyperoxia seems to play an important role in development of BPD. Up until now, no therapy to prevent new BPD is available. Perfluorocarbons (PFCs) reduce oxygen-induced injury and exhibit anti-inflammatory properties [Burkhardt et al., *Am J Physiol Lung Cell Mol Physiol* 2008;294:L1043–L1048]. No data are available concerning the effect of PFC upon alveolar development. **Hypothesis:** Intratracheal administration of PFCs prevent the hyperoxia-induced arrest in alveolarisation in an animal model of new BPD. **Methods:** Newborn Wistar rats were kept under hyperoxia (FiO₂ > 0.80) or normoxia during the first 5 days. Animals in the PFC group received a daily intratracheal administration of 50 µl perfluorodecalin and control animals an air bolus. Thereafter, all animals were kept at room air until a postnatal age of 5 weeks. Lungs were fixed, stained and alveoli were counted by the physical dissector method using a stereological approach. **Results:** Hyperoxic exposure reduced the number of alveoli by $50.7 \pm 6.1\%$ (mean + SE; $p < 0.0001$). Under hyperoxic conditions, animals treated with PFC had significantly ($p < 0.01$) more alveoli than control animals (6.7 vs. 4.2×10^6). Under normoxic conditions there were no differences in alveolar count between PFC and control animals. **Discussion:** Intratracheal administration of PFC reduces the hyperoxia induced arrest in alveolarisation. The effect is most likely mediated by an inhibition of the hyperoxia-induced increase in TNF α . The inhalation of PFC could be a promising strategy to prevent new BPD, however, further experimental research is required to prove this.

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