

Surfactant Metabolism in the Neonate

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Key Words

Surfactant metabolism · Neonates · Respiratory distress syndrome · Stable isotopes · Congenital diaphragmatic hernia · Meconium aspiration syndrome

Abstract

With the use of stable isotope-labeled intravenous precursors for surfactant phosphatidylcholine (PC) synthesis, it has been shown that the de novo synthesis rates in preterm infants with respiratory distress syndrome (RDS) are very low as are turnover rates. This is consistent with animal data. Surfactant therapy does not inhibit endogenous surfactant synthesis, and prenatal corticosteroids stimulate it. With the use of stable isotope-labeled PC given endotracheally, surfactant pool size was estimated. It turned out to be low in RDS, as expected. Similar studies were performed in term neonates with severe lung diseases. In general, patients with lung injury show a lower surfactant synthesis. The controversy around surfactant in congenital diaphragmatic hernia (CDH) persists: studies on CDH with and without extracorporeal membrane oxygenation yielded different results. In severe meconium aspiration syndrome surfactant synthesis was found to be decreased but surfactant pool size was maintained. It is possible and safe to

study surfactant metabolism in human neonates with the use of stable isotopes. This can help in answering clinical questions and has the potential to bring new in vitro and animal findings about surfactant metabolism to the patient.

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Introduction

Avery and Mead [1] showed in 1959 that pulmonary surfactant deficiency is a major factor in the pathophysiology of respiratory distress syndrome (RDS). In 1980 Fujiwara et al. [2] administered exogenous surfactant successfully to preterm infants with RDS for the first time. This was followed by numerous clinical trials which demonstrated a decrease in death rates and complications. Data which suggest that a disturbed surfactant metabolism plays a role in several other neonatal lung diseases, such as congenital diaphragmatic hernia (CDH), meconium aspiration syndrome (MAS), and surfactant protein-B (SP-B) deficiency, are now accumulating. Surfactant therapy may have a therapeutic role in the management of these clinical conditions.

This review will focus on the surfactant metabolism in human preterm and term neonatal lung diseases. The role

of surfactant in RDS, CDH, MAS, and SP-B deficiency will be discussed. First, we will briefly review the functions and composition of surfactant and the normal cellular metabolism of surfactant.

Surfactant Function and Composition

The primary function of surfactant is to decrease the surface tension at the air-liquid interface in the alveoli and distal bronchioli, to promote lung expansion during inspiration and to prevent alveolar collapse at end expiration. Apart from these biophysical properties, surfactant also plays an important role in pulmonary host defense [3].

Surfactant is a complex mixture of lipids (~90%) and proteins (~10%), which is strikingly comparable across various species including humans [4]. Of the surfactant lipids, 80–90% are phospholipids, of which phosphatidylcholine (PC) is quantitatively the most important, accounting for 70–80% of the total. Other lipids include phosphatidylglycerol (PG), phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, sphingomyelin, cholesterol, triacylglycerols, and free fatty acids. Approximately 60% of the PC contains two saturated fatty acids (sat PC, disaturated PC; DSPC) of which dipalmitoyl (16:0/16:0; DPPC) is the most abundant. DPPC is the principal surface tension-lowering component of surfactant.

Four surfactant proteins have so far been identified [5]. SP-A and -D are hydrophilic, and SP-B and -C are hydrophobic. They are either exclusively lung associated or predominately found in the lung. SP-A is the most abundant surfactant protein and is essential for the formation of tubular myelin. It functions as a regulator of phospholipid insertion into the monolayer, and modulates the uptake and secretion of phospholipids by type II cells. However, mice that lack SP-A indeed have no tubular myelin but have normal lung function and surfactant metabolism even during exercise [6]. Furthermore, together with SP-D, SP-A has an important role in lung defense [7]. SP-A and -D bind pathogens and facilitate their clearance [7]. The absence of SP-D results in increased surfactant lipid pools in the airspaces and emphysema in lungs of mice [8]. SP-B plays a role in formation of tubular myelin and together with SP-C it promotes rapid phospholipid insertion into the air-liquid interface. SP-B also influences the molecular ordering of the phospholipid layer. Infants with a genetic absence of SP-B develop lethal respiratory distress after birth, which can

only be treated by lung transplantation [9]. Absence of SP-B causes a loss of lamellar bodies, tubular myelin, and an incompletely processed SP-C [10]. SP-C regulates the phospholipid ordering in the monolayer, enhances the reuptake of surfactant lipids *in vitro*, and may have a role in surfactant catabolism. Mice that lack SP-C have normal surfactant and lung function, and have no abnormalities in SP-B processing [11]. However, the stability of surfactant at low lung volumes is decreased.

Surfactant Synthesis, Secretion and Clearance

Surfactant PC is synthesized from phospholipid precursors (e.g., fatty acids, glycerol, choline, glucose) in the Golgi apparatus [12] (fig. 1). In the fetal type II cell, intracellular glycogen stores appear to be a major source of the glycerol backbone of PC, whereas in the adult lung glucose from the circulation is a major substrate. Choline is mainly derived from the diet. The fatty acids of surfactant phospholipids are synthesized *de novo* in the type II cell, or taken up from the blood, or are derived from recycling of alveolar surfactant phospholipids [12].

Lamellar bodies are condensed, highly structured lipoprotein packages that serve as the intracellular storage form of surfactant. Lamellar bodies are secreted into the alveolar space by fusing of their limiting membrane with the plasma membrane. After secretion the lamellar bodies unravel to form loose membranous arrays and lattice-like structures, the tubular myelin. At alveolar surface expansion during inspiration, surfactant components insert from the hypophase (epithelial lining fluid; (ELF)) into the monolayer. At expiration the alveolar surface reduces and the monolayer is compressed, thereby squeezing out some surfactant proteins, unsaturated PC, and other lipids. By this mechanism, the monolayer comprises mainly DPPC, which is the most important surface-tension-lowering component during compression.

Alveolar surfactant can be cleared by different pathways. Surfactant components can be reutilized through uptake by the type II cell, incorporation into the lamellar bodies and then direct resecretion [13]. Another way is recycling of degraded surfactant components to synthesize new surfactant lipids or proteins. Finally, surfactant can be removed from the lung, either as intact molecules or as degraded products [14]. The efficiency of recycling is age dependent, and has been calculated to be 90% in young pigs [15], >90% in the newborn rabbit and 50% in adult rabbits [16].

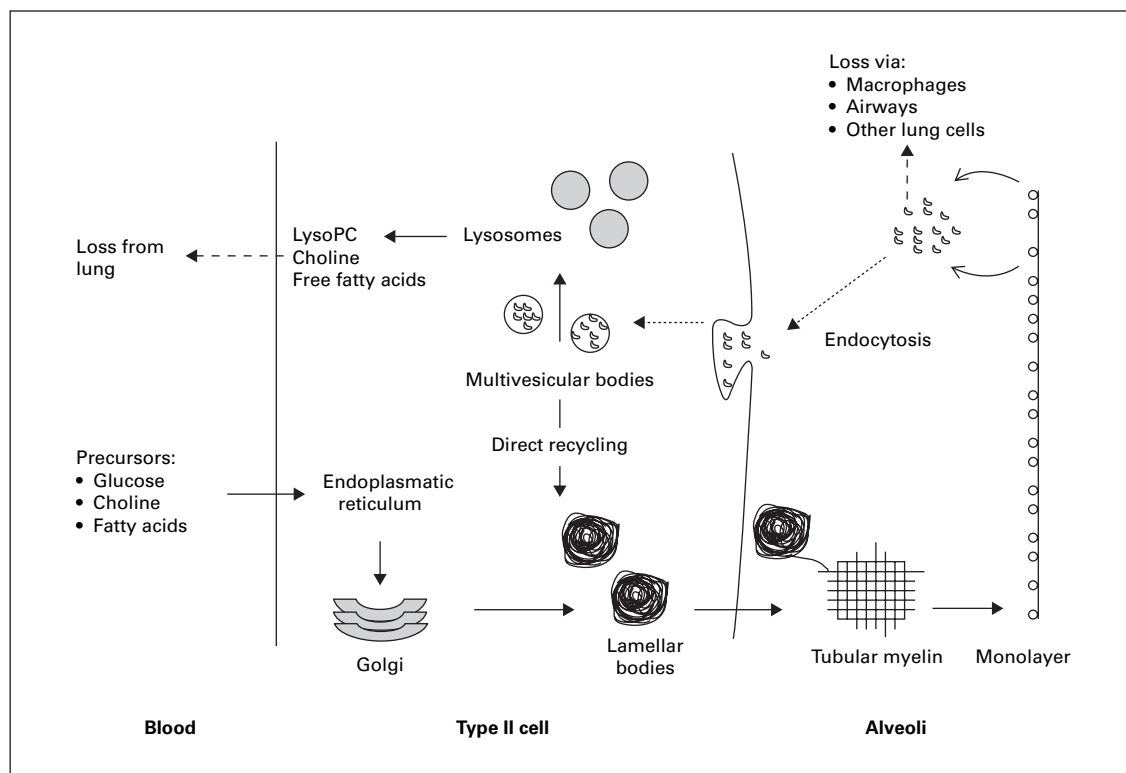


Fig. 1. Schematic illustration of surfactant phosphatidylcholine metabolism.

Surfactant Kinetics

The time required for de novo PC synthesis, secretion, and significant alveolar accumulation has been studied in animals using radioactively labeled substrates [17]. Compared to adult animals, the time to reach peak specific activity is longer in term newborns [18]. Preterm ventilated lambs show a slow movement of surfactant PC from the synthesis sites to the alveolus similar to term ventilated lambs [19].

Recently, stable isotope techniques were used to study surfactant metabolism in human infants. Labeled precursors were infused intravenously and incorporation of label was measured by mass spectrometry in surfactant PC isolated from tracheal aspirates.

In preterm infants with RDS who received a 24-hour intravenous infusion of the stable isotope [U-¹³C]glucose, the palmitic acid of surfactant PC became labeled after ~17 h and was maximally labeled after ~75 h [20, 21]. By using other precursors such as [U-¹³C]palmitic acid, [U-¹³C]linoleic acid, and [1-¹³C]acetate to study surfactant kinetics, the same results were estimated [22, 23].

In term ventilated neonates without significant lung disease using infusion of [U-¹³C]glucose, [U-¹³C]palmitic acid or [1-¹³C]acetate as precursor, the first appearance of label in surfactant PC was at ~9 h, and the maximal enrichment at ~44 h [22, 24, 25]. As in animal studies, these results of human stable isotope studies show slower surfactant kinetics in preterm infants compared to term infants. The kind of precursor does not affect the results.

The fractional synthesis rate (FSR) of surfactant after infusion of labeled glucose is a measure of the percentage of surfactant that is synthesized from glucose per day. In premature infants who received a 24-hour [U-¹³C]glucose infusion, the FSR was calculated to be ~4%/day [20, 21]. Different values were found for different precursors: preterm infants who received labeled palmitic acid and linoleic acid had an FSR of ~12 and ~25%/day, respectively [23]. FSR in preterm and term infants after infusion of labeled acetate was ~2 and ~15%/day, respectively [22]. When term infants received labeled palmitic acid, the FSR was found to be ~17%/day [24]. After infusion with labeled glucose, term infants had a FSR of ~8%/day [25].

These data show a lower FSR in preterm infants compared to term infants. When [U-¹³C]palmitic acid was used as precursor, a higher FSR was found, reflecting that palmitic acid is a more direct precursor.

The slow secretion and alveolar accumulation of surfactant are balanced in the term and preterm lungs by slow catabolism and clearance. For example, the half-life of radiolabeled surfactant phospholipid given endotracheally to term lambs is about 6 days [26]. However, in premature baboons using the same method the half-life is about 30 h [27], which may be due to more recycling in premature animals, and to species difference. Half-lives calculated in animal studies differ depending on gestational age, postnatal age, the labeled substrate used, and the surfactant pool studied.

Half-life measurements of disappearance of labeled surfactant in human infants have been performed mainly in preterm infants with RDS. Half-lives were longer when disappearance of label in surfactant was measured after the intravenous use of labeled precursors: the half-life of ¹³C-labeled PC-palmitate measured in tracheal aspirates of preterm infants with RDS after labeled glucose infusion was ~96 h [20, 21, 31]. In preterm infants the surfactant half-life after infusion of [U-¹³C]palmitic acid, [U-¹³C]linoleic acid or [1-¹³C]acetate was ~98, ~47, and ~106 h, respectively [22, 32]. Using [U-¹³C]palmitic acid, [U-¹³C]glucose, or [1-¹³C]acetate in term infants, gave a half-life of ~43, ~63, and ~28 h, respectively [22, 24, 25]. Thus, surfactant half-life is different between preterm and term infants, with a lower disappearance of label in the preterm infants reflecting slower surfactant kinetics, especially in RDS.

In two reports, term critically ill infants were studied using different precursors for surfactant metabolism [22, 32]. After infusing labeled fatty acids in a group of critically ill infants with various diagnoses, marked differences in PC kinetics among infants were noticed [32]. Term infants with primary respiratory failure had a surfactant metabolism similar to preterm infants with RDS, suggesting either delayed maturity of the surfactant system or disruption from the underlying disease [22].

With stable isotopes, apparent differences in surfactant metabolism could result from differences in tracer metabolism, from true differences in surfactant synthesis and clearance, or from differences in the size of the unlabeled surfactant pool. That is, a small, unlabeled pool would result in an apparent increase of FSR by an unchanged absolute synthesis of surfactant.

In summary, animal studies show a slower surfactant turnover in newborns compared to adults. In human

studies using stable isotopes the surfactant PC metabolism is even slower in preterm infants with RDS compared to term infants without lung disease. Moreover, term infants with respiratory failure have abnormal surfactant metabolism which resembles that of preterm infants with RDS.

Surfactant Pool Sizes

In most species studied, PC, and DPPC in particular, increase in the last trimester of pregnancy [33]. In amniotic fluid, surfactant concentrations increase, reflecting accumulation of alveolar surfactant. This increase is also reflected in an increasing lecithin/sphingomyelin (L/S) ratio [34]. During and shortly after birth large amounts of surfactant are released into the alveolar space [35]. Since there is no depletion of the intracellular surfactant pool, this increase in alveolar pool shortly after birth is accompanied by an increase in de novo surfactant synthesis. However, incorporation studies, as mentioned above, showed slow surfactant metabolism, which means that it takes a long time before de novo synthesized surfactant is detectable in the alveolar space. Increased lung tissue and alveolar surfactant pools can only be explained by the rapid mobilization of surfactant from other pools (lamellar bodies, small vesicles) within type II cells.

The amount of lung tissue and alveolar surfactant changes with age. Surfactant pools are very high in the newborn period in all species studied to date and decrease subsequently with lung maturity. In humans total amounts of sat PC in alveolar washes at autopsy also decrease with age [36].

In preterm infants with RDS, surfactant pool sizes in the alveolus are low (2–10 mg/kg) [18] due to immaturity of the lungs, especially of the type II cells. In adults pool sizes are smaller because the lung structure, alveolar lining fluid, and surfactant film have adapted maximally to optimal function. The mean amount of total lung sat PC/kg for the adult human was ~22 mg/kg, and ~2 mg/kg in alveolar wash at autopsy [36].

In human infants surfactant pool size can be estimated by measuring the dilution of a labeled surfactant component. Hallman et al. [29] and Griese et al. [37] showed an apparent pool size of ~16 mg/kg in human preterm neonates with RDS, by using PG as label to measure surfactant pool size. Pool size measurements in preterm infants with RDS using endotracheally administered stable isotope (¹³C-DPPC) in combination with a treatment

dose of surfactant (100 mg/kg) showed an endogenous surfactant PC pool size (before treatment) of ~10 mg/kg [30].

In conclusion, surfactant pool size increases in late pregnancy, followed by a gradual decrease after birth to adult values. Among different species humans have the smallest alveolar pool sizes, whereas the amount of surfactant PC in lung tissue is similar across species. The lower alveolar surfactant pool size makes the human lung particularly vulnerable to surfactant dysfunction in case of lung injury. As expected, the surfactant pool size in preterm infants with RDS is ~10–15 mg/kg, much lower than at term. Also as synthetic rates of surfactant in these preterm infants are low, it is clear that the amount of surfactant can only be increased rapidly by exogenous addition of surfactant. Surfactant therapy has been used clinically for about two decades.

Effects of Surfactant Therapy on Surfactant Synthesis in Preterm Infants with RDS

Although the administration of exogenous surfactant has become the routine treatment of RDS in the preterm infant, there is very little information regarding effects of exogenous surfactant on endogenous surfactant metabolism. In healthy adult rabbits *in vivo*, administration of surfactant to the left lung only, resulted in increased incorporation of palmitic acid from plasma into surfactant PC in the left lung but not in the right lung [38], suggesting stimulation of endogenous synthesis. In preterm ventilated lambs, surfactant treatment stimulated [³H]palmitic acid incorporation into surfactant PC after correction for the increased surfactant pool [39]. In 3-day-old rabbits, the administration of surfactant did not, however, influence the incorporation of labeled precursor in the total lung surfactant pool [40].

In preterm infants with RDS, the half-life of PG was independent of the dose of exogenous surfactant (60 vs. 120 mg/kg) [29]. This indicates that the absolute turnover had doubled after two doses of surfactant compared to one dose. In preterm infants, the concentration of SP-A increased after exogenous surfactant while this preparation did not contain any SP-A, which suggests that endogenous SP-A secretion was stimulated and not suppressed [41, 42]. In preterm infants the incorporation of ¹³C from intravenous [U-¹³C]glucose into alveolar surfactant PC palmitate increased after exogenous surfactant treatment [31].

In summary, there is little information from animal and human studies about effects of exogenous surfactant

on surfactant metabolism. Exogenous surfactant treatment in the premature with RDS seems not to suppress endogenous surfactant synthesis.

Effects of Prenatal Corticosteroids on Surfactant Synthesis in Preterm Infants with RDS

The role of corticosteroids on surfactant PC synthesis and structural lung development has been described mainly in *in vitro* and animal studies [43, 44]. In many *in vitro* studies corticosteroids increase the activity of the enzymes involved in surfactant PC synthesis [45–49] and increase surfactant protein synthesis [47]. The rate-limiting enzyme CTP: phosphocholine cytidyltransferase (CT) in the surfactant PC synthetic pathway is enhanced by corticosteroids probably in the following way: corticosteroids stimulate the synthesis of the fibroblast pneumocyte factor in the lung fibroblast. The fibroblast pneumocyte factor stimulates the formation of cytidyltransferase directly in the type II cell, and fibroblast pneumocyte factor induces via fatty acid synthetase the synthesis of fatty acids that can stimulate the activity of cytidyltransferase [49–51] and stimulates surfactant phospholipid synthesis by the type II cell [52]. Corticosteroids can probably also stimulate PC synthesis in the absence of fibroblasts [45].

In *in vitro* experiments with lung slices and isolated type II cells, corticosteroids increase the incorporation of radiolabeled precursors into surfactant PC, reflecting increased PC synthesis [46, 49, 51]. Corticosteroids at low concentrations *in vitro* seem to increase SP-A and -B synthesis and at high concentration inhibit their synthesis [53–55]. The *in vivo* prenatal corticosteroid treatment of preterm lambs increases surfactant protein mRNAs rapidly and surfactant protein amounts in lung tissue [56]. In a study by Kessler et al. [57] in premature baboons, a 72-hour treatment with prenatal dexamethasone did not increase radioactive palmitate incorporation in lung lipids, but it increased total lung phospholipids and alveolar lavage DPPC in lung lavage fluid at birth. Bunt et al. [58] found that in preterm baboons, the synthesis rate of surfactant PC from plasma stable isotope-labeled glucose was doubled after a 48-hour treatment with prenatal corticosteroids.

Data on pool size measurements as an indication of surfactant synthesis *in vivo* are conflicting. In some studies with newborn rabbits, preterm monkeys, and lambs chronically catheterized *in utero*, corticosteroid treat-

ment 2–3 days before delivery increased surfactant PC pool sizes in alveolar lavage and lung tissue significantly after birth [46, 57, 59, 60]. In contrast, other studies in large animals show no prenatal steroid-induced increase of the alveolar [61–65] or total lung sat PC pool size after birth [61–64]. Ballard et al. [66] and Ikegami and colleagues [62–65] did not find an increase in total lung sat PC pool size at birth in preterm lambs when corticosteroids were administered 2–4 days before preterm delivery. However, when corticosteroids were administered 1 week before delivery, the total lung sat PC pool sizes at birth increased significantly [62–66].

Obladen et al. [67] found increased concentrations of both PC and PG in tracheal and pharyngeal aspirates in preterm infants after stressed pregnancies. In preterm infants who had received prenatal corticosteroids, alveolar surfactant showed improved function in vitro during the first few days of life although PC concentrations in tracheal aspirates were not elevated, which would imply qualitative rather than quantitative changes [68]. Aries et al. [69] found increased L/S ratios in amniotic fluid from mothers who had been treated with dexamethasone. We recently found in preterm infants that two doses of prenatal corticosteroids doubled the endogenous surfactant synthesis from the stable isotope-labeled precursor plasma [U - ^{13}C]glucose [70].

In summary, prenatal corticosteroids enhance surfactant PC synthesis in vivo, but de novo synthesis rates remain low, and alveolar pool sizes are not increased within 48 h. In preterm animals with RDS, prenatal corticosteroids improve pulmonary compliance within 15 h [64] accompanied by stimulated structural development [71–76]. Therefore, it could well be that the improved outcome of preterm infants after a relatively short period of exposure to prenatal corticosteroids is more the result of non-surfactant phospholipid mechanisms by which pulmonary function is improved.

Congenital Diaphragmatic Hernia

Although CDH lungs are immature and show morphologically some resemblance to lungs of preterm infants with RDS, it is still unclear whether a primary surfactant deficiency is present in human CDH. Several animal models have been developed to study the pathogenesis of CDH.

In bronchoalveolar lavage (BAL) fluid of surgically created CDH lambs, the amounts of phospholipids, PC, SP-A and -B are decreased compared to controls [77, 78].

However, the amniotic L/S ratio is not different in CDH lambs compared to control lambs [78]. In vitro studies in isolated type II cells of CDH lambs show a decreased incorporation of choline into PC [77], suggesting decreased surfactant synthesis in CDH (table 1).

In the nitrofen-induced CDH rat model, total phospholipids, sat PC, and SP-A in lung tissue are lower compared to control rats (table 1) [79]. By using antibodies towards sphingomyelin and DSPC, the lungs of the nitrofen-exposed rats demonstrated an impaired secretion of sat PC into the alveoli compared to controls [79].

Human studies show contradictory results about the presence of surfactant deficiency in CDH. L/S ratios in amniotic fluid have been reported to be both normal and decreased [80]. SP-A and sat PC concentrations in amniotic fluid were lower in fetuses with CDH who died or required extracorporeal membrane oxygenation (ECMO) [81]. Autopsy studies in CDH infants who died at birth or within the first few days of life showed decreased SP-A in lung tissue [82]. However, we found no difference in L/S ratio, PC, and PG concentrations in BAL fluid from CDH infants compared to age-matched controls [83]. We found a PC concentration of ~3 and ~6 mg/ml ELF in ventilated and ECMO-treated CDH infants, respectively. Cogo et al. [24] measured lower amounts of DSPC and SP-A in tracheal aspirates of CDH infants who did not require ECMO compared to control subjects (~2 vs. ~5 mg DSPC/ml ELF).

Recently, we measured surfactant pool size by administering 2H -DPPC endotracheally [84]. Surfactant PC pool size was ~73 mg/kg in CDH infants requiring ECMO, which was comparable with that of a varied group of infants who were not on ECMO (~69 mg/kg). In addition, PC concentrations in ELF were also similar in both groups (~6 mg/ml ELF). However, Cogo et al. [85] using a similar technique, did find a significantly reduced surfactant pool size in CDH without ECMO compared to controls (34 ± 6 vs. 57 ± 7 mg/kg body weight) in addition to a higher turnover rate.

Also surfactant synthesis was studied in CDH by infusing stable isotope-labeled precursors intravenously. After infusion of [U - ^{13}C]palmitic acid no significant difference was observed in the kinetic indices of surfactant between the CDH and control subjects [24]. This was confirmed in another study by Cogo et al. [86] where net synthesis of sat PC (= FSR \times pool size) was measured by use of dual tracers, intravenously and endotracheally. Both CDH and control infants had a synthesis rate of about 8 mg/kg/day but again turnover was higher in CDH.

Table 1. Review of literature on surfactant changes in animal and human neonates with severe lung disease

	DSPC in BAL or TAS	DSPC in lung tissue	Surfactant proteins	L/S ratio	Precursor incorporation
Congenital diaphragmatic hernia					
Lambs	↓		↓ SP-A/B BAL	=	↓
Rats		↓	↓ SP-A tissue	=	
Human			↓ SP-A tissue, TAS, amniotic fluid	↓/=	=
Meconium aspiration syndrome					
Rats	=	=	↓ SP-A/B BAL	=	
Piglets	=				
Human	=		= SP-A BAL		
Pneumonia					
Rats					↑
Human	↓		↓ SP-A = SP-B BAL		
SP-B deficiency					
Mice		=	no SP-B pro SP-C		=
Human	↑		no SP-B pro SP-C		↑/=

DSPC = Disaturated phosphatidylcholine; L/S ratio = lecithin/sphingomyelin ratio; SP-A/B/C = surfactant protein A/B/C; BAL = bronchoalveolar lavage; TAS = tracheal aspirate supernatant.

In CDH infants requiring ECMO Janssen [25] measured FSR by using [U-¹³C]glucose as a precursor and found it to be lower than in controls (2.4 vs. 8%/day) with a similar half-life of endogenous surfactant of ~65 h. It is unclear why these discrepancies in pool sizes and synthesis rates were found in CDH with or without ECMO. It is unlikely that the use of the different labeled precursors can explain these findings.

In the experimental lamb model prophylactic exogenous surfactant therapy caused an improvement in gas exchange, lung mechanics, and hemodynamics [87]. In the nitrofen-induced rat model exogenous surfactant administration only had a transient effect on lung volume [88].

There are only a few reports of surfactant treatment in human CDH infants [89–91]. Bos et al. [89] showed an improvement of oxygenation in 3 of 5 infants with CDH after surfactant administration. When surfactant was given prophylactically to high-risk neonates with CDH, all 3 infants survived [90]. Surfactant treatment in 9 infants with CDH who required ECMO had no beneficial effect on lung function, morbidity, or survival [91].

Even today it is still not clear if there is a primary surfactant deficiency in CDH. We suggest that there is inactivation of surfactant function due to the intensive

ventilation with its adverse effects [92], which is often necessary in CDH infants. Although, decreased surfactant synthesis due to severe hypoplasia of the lungs (especially in the more severe cases of CDH) cannot be excluded.

Meconium Aspiration Syndrome

Aspiration of meconium into the lungs directly inhibits surfactant function and induces an inflammatory response in the lung with possible detrimental effects on type II cell function, and thereby surfactant metabolism. Surfactant function is inhibited by meconium in a concentration-dependent way [93, 94]. Meconium increases the minimum and maximum surface tensions and lowers the surface-spreading rate of surfactant [93–95].

Several mechanisms are involved in the inactivation by meconium, one of which is the infiltration of phospholipids other than DPPC into the surface film [96]. This forms an unstable surface monolayer that easily collapses at high surface tension. Secondly, the formation of aggregates between surfactant and meconium constituents (usually proteins) may delay the formation of the surface film by competitive inhibition of phospholipid entry into the surface monolayer [97]. A third mechanism is degra-

dation of surfactant lipids and proteins by enzymes (lipases, proteases) [98].

Studies of surfactant concentrations and composition in MAS are scarce. Cleary et al. [99] found decreased SP-A and -B levels in the large aggregates of surfactant in a rat model of MAS. However, phospholipid and DPPC levels did not change significantly after meconium instillation in either lung tissue or BAL. Analyses of BAL fluid from 8 ventilated infants with MAS revealed no difference in phospholipid and SP-A content compared to control subjects [100]. However, concentrations of non-surfactant protein and albumin were more than 3 times those found in normal lung. In MAS infants who required ECMO, surfactant phospholipids, PC, and SP-A in tracheal aspirates increased during the ECMO treatment [101, 102]. One study investigated the surfactant kinetics in the presence of meconium [103]. This *in vitro* study in type II cells of adult rats showed that meconium in low concentrations (1%) increases the PC secretion by type II cells, but had no effect on surfactant PC synthesis. Higher meconium concentrations were toxic to cultured type II cells, though the effect of these higher concentrations on surfactant synthesis is not known (table 1).

We studied surfactant metabolism in MAS infants on ECMO using [U - ^{13}C]glucose as a precursor for surfactant PC palmitate [25]. The FSR in MAS infants was ~ 3.3 %/day (compared to controls 8%/day, $p = 0.058$) and peak enrichment was significantly lower than in controls ($p = 0.027$), suggesting lower synthesis in MAS. PC concentration in ELF in MAS was ~ 4 mg/ml (significantly lower than controls: 12.8 mg/ml). The half-life of endogenous surfactant was 69 h (not different from controls). With endotracheally administered 2H -DPPC as label, surfactant pool PC size was measured to be ~ 50 mg/kg in neonates with MAS requiring ECMO, which was not significantly different from controls or other ECMO infants with persistent pulmonary hypertension of the newborn [84].

Exogenous administration of surfactant in animal models of MAS improves lung function and morphology, especially when surfactant is given at a high dose (200 mg/kg) [104]. Several studies showed an improvement in oxygenation after surfactant therapy, although most infants required 2 or more doses of exogenous surfactant [105]. In a randomized trial 3 doses of bovine surfactant were administered in a higher than standard dose (150 mg/kg) to 20 infants with MAS. Infants treated with surfactant had improved oxygenation, a reduction in severity of pulmonary morbidity, a decrease in ECMO requirement, and a decrease in hospitalization time com-

pared to the control group ($n = 20$) [106]. In another trial surfactant was not given as a bolus, but was continuously infused over a period of 20 min, started within 6 h after birth. Lotze et al. [107] performed a trial in term infants with severe respiratory distress who received 4 doses of bovine surfactant (100 mg/kg/dose) at ~ 30 h after birth. Half of these infants had MAS as the cause of their respiratory failure. They were unable to demonstrate a difference in oxygenation, pulmonary morbidity, or hospitalization, although the need for ECMO in the surfactant-treated group was decreased. From these studies it seems that surfactant therapy in MAS is most effective when given in the early phase of respiratory failure, and at a high dose.

The optimal method of administration of surfactant in MAS is still a point of discussion. Lavage with surfactant could remove meconium, inflammatory cells, edema fluid, proteins, and other debris from the lungs, leaving behind a layer of functional exogenous surfactant [108]. Animal studies showed a beneficial effect from surfactant lavage on pulmonary function, radiographical and histological appearance. There are few reports of lavage therapy in human neonates with MAS [109]. However, the patient groups were small and no control group was included. Recently, a multicenter, randomized controlled trial comparing surfactant lavage with standard treatment of MAS has been reported [110]. A trend towards shorter duration of ventilation and improvement in oxygenation were noted, but the differences were not statistically significant.

In conclusion, surfactant inactivation seems to play a more important role in the pathophysiology of MAS than surfactant deficiency. In addition, in the sickest MAS infants, those who require ECMO, surfactant synthesis is disturbed. However, the exact cause (lung damage or an effect of ECMO) has to be further elucidated. Surfactant therapy seems to be effective in MAS, and should probably be given at a high dose and at an early stage in the development of the disease.

Congenital SP-B Deficiency

Congenital SP-B deficiency is a lethal, inherited disease of the term neonate. It was first recognized in 1993 by Nogee et al. [9] and it is characterized by the absence of SP-B, an aberrant form of SP-C, altered phospholipid composition, and disorganized lamellar bodies and tubular myelin. Clinically the neonate with SP-B deficiency presents with severe progressive respiratory distress

which does not respond to surfactant therapy, corticosteroid administration or ECMO [111]. The infants will die in the first few weeks of life, unless they undergo lung transplantation.

SP-B is a hydrophobic peptide with several functions related to surface tension reduction and metabolism of pulmonary surfactant as described earlier in this article. In humans SP-B is encoded by a 9.5-kb gene that contains 11 exons on chromosome 2 and is expressed exclusively in the epithelium of the lung [112]. Mature SP-B is detectable in human lung after 19 weeks of gestation and is found in amniotic fluid after 31–33 weeks of gestation [113].

The most common mutation described to date is a net 2-bp insertion at codon 121 in exon 4 of the SP-B gene (121ins2), causing a frameshift and premature stop signal for the termination of the translation [114]. Affected patients are homozygous for this mutation and their parents are heterozygous carriers. Other mutations have also been identified resulting in total, partial, or transient SP-B deficiency.

Clark et al. [115] described the creation of homozygous SP-B knockout mice which die within minutes after birth, have poorly compliant lungs, and express aberrant SP-C. Ultrastructural examination of the lungs revealed an absence of lamellar bodies and tubular myelin. However, phospholipid content in lungs from SP-B knockout mice was not altered compared to normal mice. Neither was the incorporation of labeled choline and labeled palmitic acid in lung sat PC of SP-B-deficient fetuses after injection of the labels in pregnant mice [116]. Mice heterozygous for the disrupted gene express 50% of normal SP-B. These heterozygous mice show evidence of air trapping, abnormalities of lung compliance, and are susceptible to hyperoxic lung injury [117]. No changes in the amount of lung PC and incorporation of choline and palmitate into sat PC were found [116]. Lung function studies in human subjects heterozygous for SP-B deficiency revealed no abnormalities [118].

Beers et al. [119] investigated the phospholipid content, composition, and synthesis in lung tissue and lavage fluid acquired at transplantation or postmortem from SP-B-deficient infants. They found elevated phosphatidylinositol and percent DSPC in lung tissue and decreased levels of PG in both lavage and lung tissue compared to normal lungs. The incorporation rate of choline into PC and acetate into phospholipids was increased in SP-B-deficient tissue compared to lung tissue of infants with chronic lung disease [119]. The incorporation of glycerol into PC was not different between the groups. Infants

with chronic lung disease who underwent lung transplantation were used as comparison, as they had undergone the same therapeutic interventions as the SP-B-deficient infants.

Recently, we were able to study surfactant metabolism in 5 infants with an inherited surfactant SP-B deficiency [25]. Our results are in accordance with studies in SP-B knockout mice and in vitro studies in autopsy lungs of SP-B infants [119] in that PC synthesis from labeled glucose was not decreased.

In summary, the exact mechanisms, which lead to these changes of pulmonary type II cells, still have to be elucidated. By studying surfactant metabolism in SP-B deficiency, a better insight into general surfactant metabolism in normal and other disease states may be obtained. Functions of certain surfactant structures may become clearer and a means of treatment might be uncovered.

Conclusion

It is possible and safe to study surfactant metabolism in human neonates with the use of stable isotopes. This may help to answer clinical questions and has the potential to bring new in vitro and animal findings concerning surfactant metabolism to the benefit of the infant.

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