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Dysfunction of Pulmonary Surfactant in Chronically Ventilated Premature Infants

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ABSTRACT

Infants of <30 wk gestation often require respiratory support for several weeks and may develop bronchopulmonary dysplasia (BPD), which is associated with long-term pulmonary disability or death in severe cases. To examine the status of surfactant in infants at high risk for BPD, this prospective study analyzed 247 tracheal aspirate samples from 68 infants of 23–30 wk gestation who remained intubated for 7–84 d. Seventy-five percent of the infants had one or more surfactant samples with abnormal function (minimum surface tension 5.1–21.7 mN/m by pulsating bubble surfactometer), which were temporally associated with episodes of infection ($p = 0.01$) and respiratory deterioration ($p = 0.005$). Comparing normal and abnormal surfactant samples, there were no differences in amount of surfactant phospholipid, normalized to total protein that was recovered from tracheal aspirate, or in relative content of phosphatidylcholine and phosphatidylglycerol. Contents of surfactant proteins (SP) A, B, and C, measured in the surfactant pellet by immunoassay, were reduced by 50%, 80%, and 72%, respectively, in samples with abnormal surface tension ($p \leq 0.001$). On multivariable analysis of all samples, SP-B content ($r = -0.58$, $p < 0.0001$) and SP-C content ($r = -0.32$, $p < 0.001$) were correlated with surfactant function. We conclude that most premature infants requiring continued respiratory support after 7 d of age experience transient episodes of dysfunctional surfactant that are associated with a deficiency of SP-B and SP-C. (***Pediatr Res* 56: 918–926, 2004**)

Abbreviations

BPD, bronchopulmonary dysplasia
SP, surfactant protein
TA, tracheal aspirate

Infants born prematurely, particularly those of ≤30 wk gestation, are at high risk for lung disease. Although the incidence and severity of respiratory distress syndrome has been reduced with antenatal corticosteroid therapy, which hastens lung development, and postnatal replacement surfactant treatment, newborn respiratory distress remains a frequent occurrence in very low birth weight infants and a requirement for respiratory support often continues for many weeks (1, 2). The clinical pattern after the first week of life frequently includes episodes of respiratory deterioration necessitating increased inspired oxygen or ventilatory support (3). In some infants, lung disease progresses to BPD, which is defined as a continuing requirement for supplemental oxygen and/or positive pressure ventilatory support at 36 wk postmenstrual age (4). BPD occurs in approximately 30% of infants with birth weights <1000 g and is associated with long-term pulmonary and/or neurodevelopmental disability or death in severe cases (4, 5). The etiology of this progressive respiratory insufficiency is likely multifactorial, including oxygen toxicity, volutrauma associated with mechanical ventilation, infection, patent ductus arteriosus, and inflammation, and results in arrest of alveolar development and interstitial fibrosis (4).

Pulmonary surfactant is a mixture of lipids and proteins produced by alveolar type II cells. The major phospholipid component, saturated phosphatidylcholine, forms a highly surface-active film in lung alveoli to reduce surface tension and thus maintain alveolar expansion at end-expiration. The surfactant-associated proteins, in particular SP-A, SP-B, and SP-C, interact with surfactant phospholipids to facilitate film formation and stability (6, 7). SP-B is required for a normal surfactant function both *in vitro* and in experimental animal studies (8–14). Moreover, term infants with inherited SP-B

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deficiency have inactive surfactant and severe respiratory distress (15). Infants born prematurely have inadequate surfactant that is deficient in surfactant proteins, however, surfactant production increases during the first postnatal week and mature levels of all surfactant proteins can occur by the third week (16–18). In this study we have examined the status of pulmonary surfactant in premature infants at risk for BPD. We found that most premature infants requiring continued respiratory support beyond the first week of life have episodes of dysfunctional surfactant that are associated with a low content of SP-B and SP-C. A preliminary report of these findings has appeared in abstract form (19).

METHODS

Patient population. Eligible infants for this prospective study were ≤30 wk gestation without congenital anomalies and requiring intubation at birth for respiratory support. Infants were enrolled either on the first day of life ($n = 45$, Hospital of the University of Pennsylvania) or at 7–21 d of age ($n = 23$) as part of a clinical study of inhaled nitric oxide at the Hospital of the University of Pennsylvania and Children's Mercy Hospital, Kansas City. All protocols were approved by institutional review boards and included parental consent. Antenatal and postnatal clinical information was collected, including daily respiratory severity scores (mean airway pressure × fraction inspired oxygen) and occurrence of culture-positive infection. Ventilatory management goals in the newborn intensive care units at the time of this study (July 1997 to December 2001) included minimizing peak inspiratory pressure and oxygen exposure, use of positive end expiratory pressure, limiting tidal volume to 4–6 mL/kg, and maintaining oxygen saturation at 88–95%. Umbilical arterial catheters were generally removed by 7 d of age and Pao_2 determinations were not routinely performed after this time. For this reason, we evaluated respiratory status by severity score, rather than by oxygenation index, as a reflection of the management efforts of the clinician to maintain oxygen saturation within the target range. The standard of care in our units at the time of this study did not yet include frequent use of nasal continuous positive airway pressure to stabilize functional residual capacity. Accordingly, all infants <1000 g birth weight were managed with intubation and ventilation until weight gain was well established.

An analysis of respiratory status, episodes of culture-positive infection, and surfactant function was performed on a subpopulation of 40 infants with three or more (total 193 samples, range, 3–9/infant) samples of surfactant that had a determination of minimum surface tension. Daily respiratory severity scores between postnatal d 7 and extubation (range, d 27–83) were calculated and evaluated for changes in respiratory status. A surfactant sample taken during respiratory baseline was defined as one obtained during a period of two or more days with no consistent change in severity score (≤0.5 unit variation, $n = 73$) both before and after the day of sampling. A surfactant sample during a respiratory deterioration was defined as one obtained during a sustained increase in severity score of ≥1.5 units from the preceding baseline value ($n = 41$). The timing of other samples was judged to be indeterminate with regard to respiratory status, and included collections during active ventilator weaning or at times not meeting the definition of either respiratory baseline or deterioration ($n = 80$). Sepsis was diagnosed by a positive blood culture. Pneumonia and tracheitis were diagnosed by a positive TA culture plus clinical findings: abnormal secretions with increased neutrophils and a predominant organism on Gram's stain for tracheitis and respiratory decompensation plus x-ray changes for pneumonia.

Sample processing. TA samples were collected during airway suctioning of clinically stable, intubated infants as part of routine nursing care. Surfactant isolated from TA of term infants is surface active and has a composition comparable to adult surfactant isolated by bronchoalveolar lavage (17, 20). Collections were performed initially every other day after enrollment (three samples) and then at weekly intervals until extubation. In this sampling procedure, a catheter was placed at the end of the endotracheal tube and three separate 0.5 mL aliquots of normal saline were instilled and collected by suction. The pooled aliquots of aspirate were centrifuged at 500 × *g* for 5 min to remove cells and the supernatants were centrifuged (27,000 × *g* for 60 min) to isolate large aggregate surfactant (pellet) and a supernatant (small aggregate) fraction. Total protein was measured by the Bradford assay and total phospholipid was determined by phosphorus assay of extracted lipids (21, 22).

Surface tension measurements. The surface activity of large aggregate surfactant, resuspended at 1.5 mg phospholipid/mL in buffer, was assessed in a pulsating bubble surfactometer at 37°C (17, 23). We determined minimum surface tension, the time required to achieve minimum surface tension, the maximum surface tension, and surface tension after an initial 10.6 s without pulsation as an estimate of the adsorption rate. A minimum surface tension of ≤5 mN/m under these conditions was defined as normal *in vitro* surface activity.

Biochemical assays. Contents of SP-A, SP-B, and SP-C were assayed by an immunodot procedure as previously described (17, 24). Antibody to mature SP-C was provided by ALTANA Pharma AG (Kontanz, Germany), and was generated against recombinant human SP-C containing phenylalanine substituted for cysteine residues 3 and 4 and isoleucine substituted for methionine at residue 32. Commercial surfactant (a single lot of Infasurf) was used as a standard for assay of SP-B and SP-C, and SP-A standard was purified from human alveolar proteinosis lavage fluid. SP concentrations are expressed as percentage by weight of either total phospholipid or total protein in the surfactant sample. The phospholipid composition of large aggregate surfactant was determined by HPLC and refractive index detection using lipid extracted from pooled, residual samples (21, 25).

Statistics. Clinical characteristics of infants are summarized by frequencies for demographics and other categorical variables, and continuous variables are presented as median and range because data for many of the variables were skewed. All data for surfactant properties were skewed and accordingly values were log-transformed for statistical analysis and results back-transformed for presentation as means and 95% confidence intervals. Because there were multiple surfactant sam-

ples per infant, a mixed-effects linear model was used and was analyzed using SAS Proc Mixed (SAS Institute, Cary, NC) with a compound symmetry covariance structure. The partial correlations for repeated measurements between surfactant proteins and surface properties was performed according to Lipsitz *et al.* (26). Relationships between clinical parameters (respiratory status and infection) and minimum surface tension were evaluated by logistic regression analysis using generalized estimating equation with autoregressive working correlation structure to adjust for multiple measurements from a patient. Based on the generalized estimating equation model, the probability and associated 95% confidence interval (CI) of outcome (respiratory decompensation or dysfunctional surfactant) was predicted for two levels (yes and no) of an explanatory factor (dysfunctional surfactant and infection, respectively). This analytic approach was based on the hypothesis that infection resulted in surfactant dysfunction, which in turn contributed to a deterioration of respiratory status.

RESULTS

Clinical characteristics. Table 1 summarizes clinical characteristics of the infants in this study. Surfactant-related data were obtained from 247 TA samples collected from 68 infants of 23–30 wk gestational age. The median number of TA samples per patient was three, representing only those samples with recovery of a sufficient amount of surfactant for assay of both surface activity and surfactant proteins. There were a similar number of males and females, and most infants received both antenatal betamethasone and postnatal replacement surfactant. Approximately one-third of the infants received a course of postnatal dexamethasone for their continuing lung disease. In general, the duration of dexamethasone treatment was short (median, 4 d), and only 8% of the TA samples in the study were collected during dexamethasone administration. Most of the infants (69%) had a diagnosis of respiratory distress syndrome and/or adverse outcome (BPD or death), reflecting the high degree of prematurity of the population. Those infants without a diagnosis of RDS were intubated for apnea of prematurity and respiratory insufficiency as was the standard of care for very low birth weight infants at the time of the study.

Table 1. *Clinical characteristics of premature infants with tracheal aspirate samples at ≥7 d*

Characteristic		
No. of infants/mothers		68/66
No. of tracheal aspirate samples	247	(1–9/infant)
Gestational age (wk)	26.0	(23.0–30.0)
Postnatal age at study entry* (d)	11	(7–29)
Gender (male/female)		32/36
Antenatal betamethasone treatment	55	(80.9%)
Postnatal surfactant	66	(97.1%)
Postnatal dexamethasone	21	(30.9%)
Age at treatment (d)	22	(3–75)
Length of treatment (d)	4	(1–23)
Respiratory distress syndrome	47	(69.1%)
BPD or death at 36 wk postmenstrual age	47	(69.1%)

Data for continuous variables are median and range.

* Age at first tracheal aspirate sample analyzed.

Postnatal pattern of surfactant function. TA samples were collected at predefined intervals and assessed for surfactant function by pulsating bubble surfactometer. Analysis was restricted to samples collected after the first week of life to avoid interference from exogenous surfactant given shortly after birth. Some samples (25%) had an insufficient amount of surfactant recovered for surfactometer analysis at the predefined phospholipid concentration (1.5 mg/mL). Inadequate recovery of phospholipid was weakly associated ($r = 0.23$) with volume of recovered lavage fluid, suggesting that there were technical issues in the lavage sampling procedure. There was no apparent relationship between amount of recovered phospholipid and clinical status.

For the entire group of 68 infants, 51 infants (75%) had at least one surfactant sample with abnormal function during the time they were intubated. The frequency of abnormal surfactant function by postnatal age is shown in Figure 1*A*. At the intervals between wk 2 and wk 11, 33–55% of infants with a TA sample obtained had a finding of abnormal surfactant function.

To better evaluate the postnatal pattern of surfactant function in intubated infants, we examined surface tension data for a subpopulation of 16 infants who had at least six surfactant samples analyzed over at least a 5-wk period; the mean age at the last sampling was 65 d (range, 39–84 d). Figure 1*B* illustrates three examples of observed patterns. For patient 1, each of the eight surfactant samples had normal *in vitro* function, defined as a minimum surface tension ≤5 mN/m. This pattern was observed in 5 of the 16 infants (31%). Patient 2 had a single episode of surfactant dysfunction represented by two samples with elevated minimum surface tension over a 1-wk period. This pattern occurred in 4 of the 16 infants with repeated samples (25%). All samples of patient 3 had elevated minimum surface tension with increased values on two separate occasions. Seven of the 16 infants had two episodes or a uniform finding of dysfunctional surfactant (44%). In this subpopulation of 16 infants with multiple measurements of surface tension, BPD occurred in three of five infants with all normal surfactant samples (gestational age 26.4 ± 0.3 wk, SEM), two of four infants with one episode of dysfunctional surfactant (gestational age 25.3 ± 0.3 wk), and six of seven infants with two episodes or continuously abnormal surfactant samples (gestational age 24.6 ± 0.6 wk).

Clinical status and surfactant function. To assess the relationship between the respiratory clinical course and surfactant function, we examined clinical and laboratory data for a subpopulation of 40 infants who had three or more surfactant samples analyzed over a 3–12 wk interval. Twenty-seven of these infants had at least one TA sample collected during an episode of respiratory deterioration, defined as a sustained rise in respiratory severity score, with a total of 41 samples. Minimum surface tension was >5 mN/m in 21 of the 41 samples. Of the 73 samples collected during a baseline respiratory period (as defined in "Methods"), 19 had a minimum surface tension >5 mN/m. With logistic regression analysis using generalized estimating equation, there was a significant association ($p = 0.005$) between elevated minimum surface tension value and a deterioration of respiratory status (Fig. 1*C*).

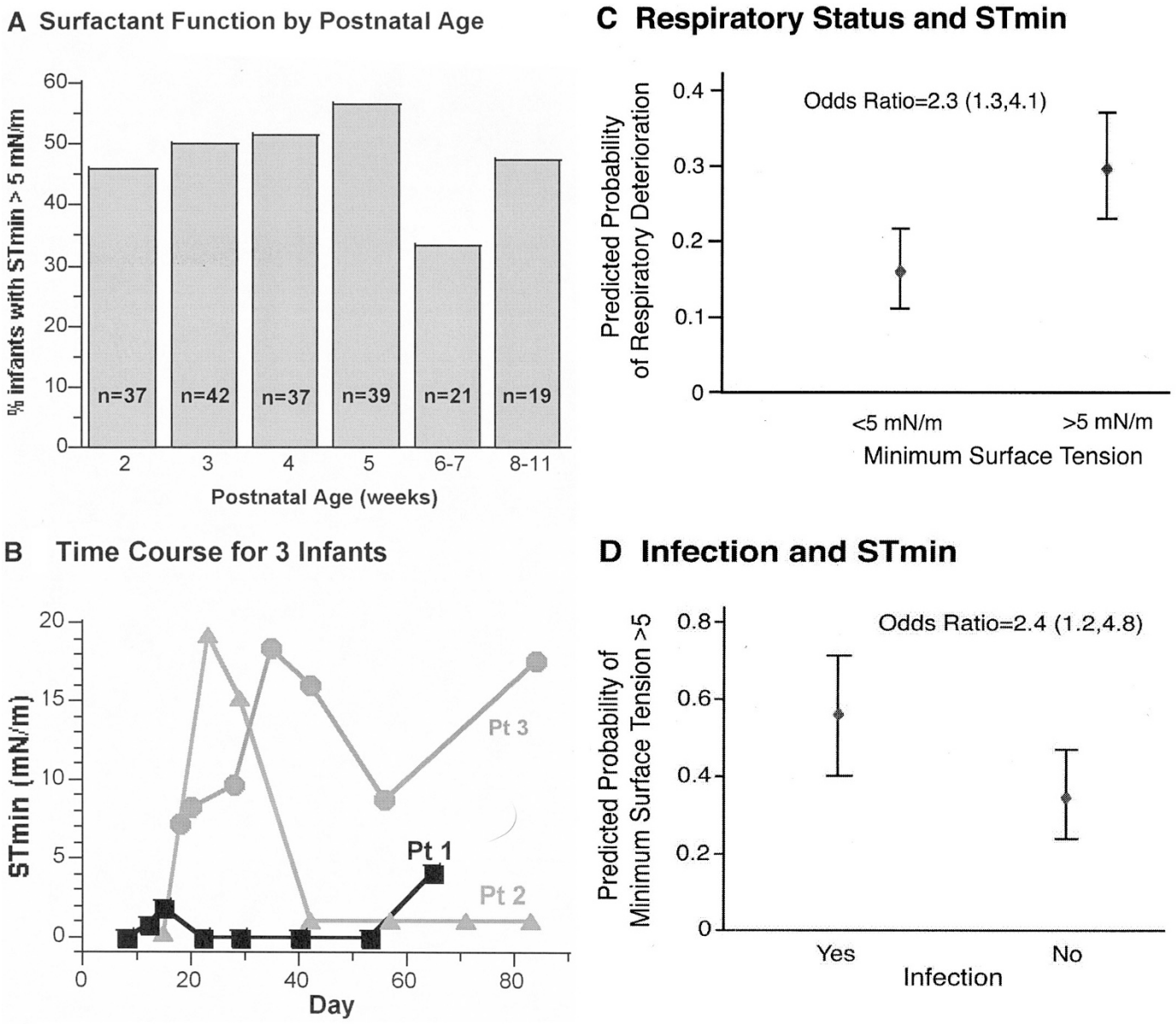

Figure 1. Occurrence of dysfunctional surfactant samples in premature infants. (*A*) Frequency of dysfunctional surfactant by postnatal age intervals; the total number of infants in each group is given within the bar. (*B*) Patterns of surfactant function for three representative infants with repetitive sampling of TA. (*C*) Association between surfactant function and respiratory deterioration. This analysis was performed on a subpopulation of 40 infants with three or more (range, 3–9) samples of surfactant that had a determination of minimum surface tension. By regression analysis, samples with abnormal minimum surface tension (>5 mN/m) are significantly associated (increased predicted probability) with a respiratory deterioration as defined in "Methods." Data are mean, 95% CI for 194 samples. STmin, minimum surface tension. (*D*) Association between infection and surfactant function. This analysis was performed on the 40 infants described in *C*. Fifty-three of the 194 surfactant samples were collected during a period of culture-positive infection (sepsis, pneumonia, or tracheitis). Infection at the time of TA sample collection was significantly associated (greater predicted probability, $p = 0.01$ by logistic regression analysis) with a minimum surface tension >5 mN/m. Data are mean, 95% CI.

Twenty-seven percent of the surfactant samples were by chance collected during a period of culture-positive sepsis, pneumonia, or tracheitis. Minimum surface tension was >5 mN/m in 26 of the 53 samples obtained during an infectious episode compared with 52/141 samples that were collected in the absence of infection. By logistic regression analysis, there was a significant association ($p = 0.01$) between infection and minimum surface tension values >5 mN/m (Fig. 1*D*).

There were 15 surfactant samples from 11 infants that were collected during treatment with postnatal dexamethasone for worsening respiratory status. Ten of the 15 samples taken during dexamethasone administration (67%) had a minimum surface tension >5 mN/m compared with 68/179 (38%, $p < 0.05$) of surfactant samples obtained without dexamethasone therapy. Of note, 3/15 (20%) of the samples during dexamethasone were associated with respiratory decompensations, similar to the frequency for nondexamethasone samples (38/179, 21%).

Surfactant function and composition. Overall, 109 of the 247 surfactant samples (44%) had abnormal *in vitro* function. Table 2 shows the recovery, function and composition of surfactant for samples with normal function (minimum surface

Table 2. *Properties of surfactant from premature infants, according to* in vitro *surface activity*

A. Recovery, function, and protein composition

Variable	STmin <5 mN/m	STmin >5 mN/m	*p* Value
No. of Infants	45	51	
No. of Samples	138	109	
Recovery from tracheal aspirate			
μg phospholipid/mL	85.1 (75.9, 93.3)	72.4 (63.1, 83.2)	0.023
μg phospholipid/mg protein	169.8 (147.9, 190.6)	182.0 (154.9, 213.8)	0.74
Function in bubble surfactometer			
Time to STmin (s)	51.3 (40.7, 64.6)	251.2 (213.8, 295.1)	<0.0001
STmax (mN/m)	42.7 (40.7, 44.7)	49.0 (46.8, 51.3)	<0.0001
STads (mN/m)	25.1 (24.6, 25.7)	28.8 (27.5, 30.2)	<0.0001
Protein composition (%)			
SP-A/phospholipid	2.57 (1.86, 3.55)	1.29 (0.98, 1.66)	0.001
SP-B/phospholipid	0.98 (0.79, 1.20)	0.20 (0.17, 0.25)	<0.0001
SP-C/phospholipid	2.14 (1.55, 3.02)	0.60 (0.42, 0.89)	<0.0001
Total protein/phospholipid	50.9 (45.4, 57.1)	40.0 (34.7, 46.1)	0.02
SP-A/protein	5.2 (3.9, 6.9)	3.3 (2.5, 4.4)	0.001
SP-B/protein	1.95 (1.62, 2.34)	0.51 (0.42, 0.63)	0.001
SP-C/protein	4.3 (3.2, 5.9)	1.4 (0.9, 2.1)	0.0004

Data are back-transformed means and 95% confidence intervals (CI) with *p* values, based on a mixed effect model in the log scale. STmin, minimum surface tension; STmax, maximum surface tension; STads, adsorption surface tension (at 10.6 sec without pulsation).

B. Phospholipid composition (% of total)

Phospholipid species	STmin ≤5 mN/m	STmin >5 mN/m	*p* Value
Phosphatidylcholine	81.1 (78.2, 84.0)	79.7 (66.6, 92.8)	0.69
Sphingomyelin	6.4 (2.9, 9.9)	13.4 (0, 27.7)	0.07
Lysophosphatidylcholine	2.9 (0.8, 5.0)	1.9 (0, 5.6)	0.48
Phosphatidylglycerol	1.6 (0.4, 2.8)	2.0 (0, 4.5)	0.70
Phosphatidylinositol	1.0 (0.2, 1.8)	0.4 (0, 1.0)	0.003
Phosphatidylethanolamine	1.9 (0, 4.0)	0.4 (0, 1.0)	0.01

Analyses of phospholipid composition were performed in triplicate on six pooled, residual surfactant samples, grouped by postnatal age intervals, from 22 samples/12 infants in the STmin ≤5 group and six pooled samples from 21 samples/18 infants in the STmin >5 group. Data for each phospholipid are mean (95% CI) for each sample as percent of total identified phospholipids (set at 100%) and do not include other minor species (*e.g.*, phosphatidylserine) that were not resolved.

tension <5mN/m; range, 0–4.9) and abnormal function (≥5mN/m; range, 5.1–21.7). Recovery of phospholipid in the surfactant pellet was slightly but significantly reduced in samples with abnormal function based on volume of recovered aspirate, but was similar in the two sets of samples expressed per milligram of TA protein. Compared with surfactant with normal function, samples with higher minimum surface tension took longer to achieve minimum tension and had higher values for both maximum surface tension and adsorption surface tension ($p < 0.001$). The contents of SP-A, SP-B, and SP-C, expressed as percentage of either surfactant phospholipids or total protein, were all lower in the abnormal surfactants ($p < 0.001$). Some of the SP data for samples with normal surfactant function (71 samples with STmin <1.0 mN/m from 35 infants) were included in our previous publication (17). Total protein per phospholipid was also decreased in abnormal surfactant samples ($p = 0.02$) by an amount comparable to the reduction in surfactant proteins.

We also determined volume and protein content of recovered TA lavage for samples with normal and abnormal surfactant function. Lavage volume was highly variable and was significantly less in samples with abnormal surface tension (mean, 1.62 mL; range, 0.05–4.80 mL) compared with samples with normal surface tension (mean, 2.23 mL; range, 0.05–5.62; $p < 0.001$). Total protein content of lavage was also reduced in samples with abnormal *versus* normal surfactant (mean, 0.62 mg; range, 0.08–7.02 *versus* mean, 0.97 mg; range, 0.05–5.62; $p < 0.001$). Normalized to total phospholipid in the surfactant pellet, however, total lavage protein was not different for the two groups (mean ± SEM, 7.8 ± 0.8 *versus* 7.4 ± 0.5 μg/μg, respectively).

Analysis of phospholipid composition of surfactant demonstrated the expected high content of phosphatidylcholine with relatively low amounts of other phospholipids (Table 2*B*). The relative contents of phosphatidylinositol and phosphatidylethanolamine were significantly lower and sphingomyelin was increased ($p = 0.07$) in surfactant samples with abnormal function. Notably, relative amounts of phosphatidylcholine and phosphatidylglycerol, which are critical for surfactant function, were not different between the two groups of samples.

The largest difference in surfactant protein composition between normal and abnormal surfactant samples occurred for SP-B. The relationship between SP-B concentration (percentage of phospholipid) and the minimum surface tension value for all surfactant samples is shown in Figure 2*A* for individual data and in Figure 2*B* for data grouped by increments of SP-B content. Minimum surface tension is inversely related to SP-B concentration; all surfactant samples with SP-B content >1.5%

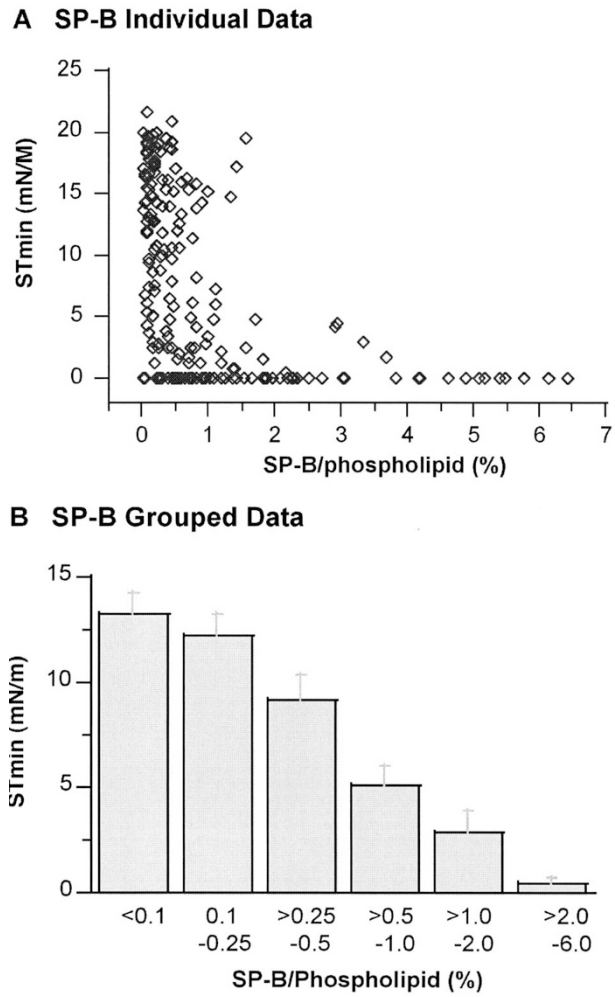

Figure 2. Relationship between surfactant protein B normalized to phospholipid and minimum surface tension for individual (*A*) and grouped (*B*) surfactant samples. In *A*, $r = -0.68$ with a p value of <0.0001. In data not shown, the same univariate analysis was performed for SP-A/phospholipid ($r = -0.34$, $p = 0.004$) and SP-C/phospholipid ($r = -0.48$, $p < 0.0001$). Data in *B* are mean and SEM for SP-B with n values ranging from 28 to 41 per group.

demonstrated normal minimum surface tension. Of the 107 samples with relatively low content of SP-B (<0.5% of phospholipid), 82 (77%) had a STmin value >5 mN/m, however, normal surface function was achieved in 25 of the samples. Comparing these two groups, content of SP-C was higher in samples with normal function (3.0 ± 0.7%, SEM) *versus* 1.6 ± 0.2% ($p = 0.025$) in samples with abnormal function, whereas SP-A content (2.4% *versus* 2.1%) and total protein/phospholipid (44% *versus* 47%) were not different between groups.

Table 3 presents partial correlations based on mixed-effects analyses for surfactant protein content and parameters of surfactant function. Adjusting for effects of SP-A and SP-C, SP-B was negatively correlated with each of the surface tension parameters with correlation coefficients ranging from −0.36 to −0.58. Correlation values were lower for SP-C, and there were no statistically significant correlations for SP-A after adjusting for the other two SPs. In a separate analysis of 15 surfactant samples collected during postnatal dexamethasone treatment, mean SP-B content (1.04%PL) and the partial correlation between SP-B and minimum surface tension ($r = -0.56$, $p = 0.05$) was comparable to the corresponding value for the entire group of samples (Table 3).

SP-B was assayed in the supernatant fraction as well as the surfactant pellet in a subset of 46 surfactant samples from 24 infants. Minimum surface tension values were inversely correlated with the total (supernatant plus pellet) TA SP-B content ($r = -0.68$, $p < 0.05$). The amount of phospholipid associated with the pellet was not different for samples with normal (mean, 72.5 ± 2.1%, SEM) *versus* abnormal (71.0 ± 2.7%) minimum surface tension.

DISCUSSION

Respiratory insufficiency continuing for several weeks is a common occurrence among infants ≤30 wk gestation, however, the status of surfactant in this condition has not been previously investigated. We found that 75% of chronically ventilated premature infants had at least one analyzed surfactant sample with abnormal function *in vitro*. Our observations indicate that approximately one-half of the abnormal surfactant samples were associated with clinically evident respiratory deterioration. Occurrences of dysfunctional surfactant were transient, usually lasting 1–2 wk, consistent with the general

Table 3. *Partial correlations between content of each surfactant protein and* in vitro *surface properties adjusted for other two surfactant proteins*

	STmin (mN/m)	Time to STmin (sec)	STmax (mN/m)	STads (mN/m)
SP-A (% phospholipid)	0.031	0.153	0.119	0.118
	(0.81)	(0.24)	(0.37)	(0.37)
SP-A (% protein)	−0.233	0.060	−0.045	−0.127
	(0.072)	(0.65)	(0.74)	(0.33)
SP-B (% phospholipid)	−0.581	−0.522	−0.478	−0.360
	(<0.0001)	(<0.0001)	(<0.0001)	(0.004)
SP-B (% protein)	−0.561	−0.429	−0.475	−0.404
	(<0.0001)	(<0.0001)	(<0.0001)	(0.001)
SP-C (% phospholipid)	−0.319	−0.284	−0.019	−0.172
	(0.012)	(0.027)	(0.89)	(0.19)
SP-C (% protein)	−0.217	−0.260	−0.181	−0.314
	(0.095)	(0.044)	(0.17)	(0.014)

Data are partial correlation coefficients and p values shown in parentheses from a mixed-effects model where the partial correlation for each SP is determined after adjusting for the other two SPs. STmin, minimum surface tension; STmax, maximum surface tension; STads, adsorption surface tension (at 10.6 s without pulsation).

pattern for treatment and resolution of intercurrent infections that are common in premature infants (27). Based on the current findings, we speculate that episodes of infection are one cause of reduced content of SP-B and SP-C in surfactant, which results in surfactant dysfunction and deterioration of the respiratory status. To our knowledge, this is the first description of surfactant dysfunction, and the role of hydrophobic surfactant proteins, in chronically ventilated premature infants.

There are a number of possible explanations for surfactant dysfunction. Reduced content of saturated phosphatidylcholine or phosphatidylglycerol, or increased concentrations of inhibitory phospholipids such as lysophosphatidylcholine, can impair surface activity (28). We observed only relatively minor changes in phospholipid composition between normal and abnormal surfactant samples. The increased content of sphingomyelin, which has been previously observed with respiratory failure, may reflect release of membrane lipids from inflammatory cells and damaged airway epithelial cells (14, 29, 30). Phosphatidylglycerol, which comprises ~8% of the phospholipids in normal surfactant, was low in surfactant with both normal and abnormal function. Low phosphatidylglycerol content has been previously observed in surfactant from premature infants (31) and may reflect delayed development of biosynthetic capacity and/or effects of lung injury (30, 32). It is possible that phosphatidylglycerol from airway bacteria in infants with pneumonia or tracheitis contributed to the surfactant pool of this phospholipid. None of the observed changes in phospholipid composition is likely to affect surfactant function, particularly in view of unchanged phosphatidylcholine content. The concentration of saturated phosphatidylcholine in surfactant is reduced in both pneumonia and adult acute respiratory distress syndrome (20, 33). Because of the limited amount of surfactant pellet from each TA collection, we performed the measurements of phospholipid composition on pooled residual samples and were not able to assess either saturated phosphatidylcholine or molecular species of phosphatidylcholine. Thus, we cannot rule out the possibility that differences in content of saturated phosphatidylcholine contributed to surfactant function in the infant samples.

Surfactant can be inactivated by serum proteins, and both SP-A and SP-B provide resistance to inactivation (28). We did not investigate for inhibitory proteins in the surfactant preparation, however, the finding of similar, relatively low amounts of nonsurfactant proteins relative to phospholipid in both normal and abnormal surfactants makes it unlikely that there was substantial influx of serum proteins into the airspaces. In addition, the amount of total protein in TA lavage, normalized to surfactant phospholipid in the sample, was not different for normal and abnormal surfactants. In fact, both total protein and recovered lavage fluid volume were ~30% less in TA samples with abnormal surfactant function. The possible reasons for reduced efficiency of the lavage procedure in this group of samples is not known.

The proportion of total lavage phospholipid in the surfactant pellet (large aggregate fraction) is reduced in adult patients with acute respiratory distress syndrome and is inversely correlated with minimum surface tension (34). In our study, however, distribution of phospholipid between large and small (supernatant) aggregate fractions was not different for normal *versus* abnormal surfactant.

The important role of the lipophilic surfactant proteins, in particular SP-B, for surfactant function is well established. *In vitro*, surfactant phospholipids in the absence of surfactant proteins have a slow adsorption rate to the surface film and do not achieve low surface tensions; normal surfactant properties are restored in a dose-dependent fashion by reconstitution of lipids with SP-B, with a nearly optimal response at 0.75% SP-B/phospholipid (12, 13). In animal studies, absence of SP-B by gene ablation or neutralization of SP-B with antibodies causes respiratory distress (8–10). Heterozygous SP-B(±) mice, which have 50% less SP-B protein than wild-type animals, have slightly reduced lung compliance and survive, but are more susceptible to oxygen-induced lung injury. Reduction of SP-B content to 25% of normal in transgenic mice was associated with surfactant dysfunction and respiratory failure, and excess SP-B content preserved pulmonary compliance after exposure of animals to endotoxin (10, 11, 14, 35). Our findings extend this relationship between SP-B content and surfactant function to the clinical situation in infants where all parameters of surfactant function *in vitro* were negatively correlated with the content of SP-B.

Because some samples with low SP-B content achieved a minimum surface tension <5 mN/m, factors other than SP-B concentration likely contribute to surfactant function in these infants. Analysis of data for samples with $<0.5\%$ SP-B/phospholipid indicated that normal minimum surface tension was associated with higher SP-C content. This observation is consistent with the concept that SP-C has a physiologic role in surfactant function and can substitute for SP-B, albeit at lower efficiency, both *in vitro* and *in vivo* (28, 36, 37). Factors other than SP-C content also may have influenced surfactant function in samples with low SP-B. Although total protein content of surfactant was not different for samples with low *versus* high minimum surface tension, it is possible that the amount of specific surfactant-inhibitory proteins differed. It is also possible that differences in phospholipid composition influenced surfactant function, however, we were not able to systematically investigate this aspect with the limited amount of material available.

Surfactant protein deficiency could occur by several mechanisms. SP-B gene expression in cultured lung cells is decreased by the inflammatory mediators tumor necrosis factor-α, transforming growth factor-β, IL-1, lipopolysaccharide, and activators of protein kinase C (37–39). Concentrations of inflammatory cytokines are elevated in TA of premature infants and are associated with development of BPD (40). In rodent models of acute lung injury and infection, the respiratory distress that occurs is associated with surfactant dysfunction and reduced mRNA and protein content of SP-B and/or SP-C (41–43). SP-B content also could be decreased by accelerated degradation secondary to increased proteolytic activity or phagocytosis, and function could be modified through proteolytic cleavage, oxidative events or nitrative reactions.

There are limitations to this study. First, the TA technique collects surfactant present in upper airways, which may differ from alveolar surfactant. However, in a study of normal term

infants, surfactant obtained by TA was surface active and had a similar phospholipid composition as surfactant prepared from bronchoalveolar lavage (20). Collections of TA were made at predefined intervals, rather than during a change in clinical status, and we may therefore have missed episodes of surfactant dysfunction due to the sampling schedule. Accordingly we cannot be precise regarding either the incidence and duration of the episodes of dysfunctional surfactant in chronically ventilated infants or the association with respiratory deteriorations and infection. It is possible that changes in SP-B/C content and surfactant function are secondary to respiratory deteriorations, rather than the cause, perhaps as a consequence of increased oxygen requirement. In this scenario surfactant dysfunction would contribute to continuing and worsening respiratory status. Because we required a positive culture to define infection, we also may have underestimated infectious episodes. Finally, the *in vitro* assay of surfactant function may not necessarily reflect surface properties *in vivo*.

Postnatal dexamethasone was administered to some infants in the study. Because glucocorticoids regulate SP synthesis in fetal lung and also affect respiratory status in intubated infants, we examined separately the data for surfactant samples collected during dexamethasone treatment. There was no apparent effect of corticosteroid exposure, in this relatively small group, on SP-B content or on the dose dependent relationship between minimum surface tension and SP-B. Of interest, however, the frequency of abnormal surface tension in dexamethasone samples was increased compared with nondexamethasone samples, whereas the frequency of clinical respiratory deterioration was the same in the two populations. A possible interpretation of this observation is that dexamethasone treatment improved or stabilized the respiratory status by nonsurfactant related, anti-inflammatory effects such as reduced pulmonary and tracheal edema.

Surfactant dysfunction may contribute to a number of respiratory illnesses. Recently, elevated minimum surface tension values were found in surfactant isolated from children with cystic fibrosis and with pneumonia; surfactant proteins were not examined in that study (33). Adults with acute respiratory distress syndrome have low concentrations of SP-A and SP-B both before and during their lung disease, and minimum surface tension is elevated (30, 44, 45). Interestingly, the pattern and magnitude of the decreases in surfactant proteins-A/B/C per phospholipid in acute respiratory distress syndrome closely parallel our finding for infants with dysfunctional surfactant, with the greatest decrease for SP-B (87% in adults and 80% in infants) (30). Collectively, these findings focus attention on the role of surfactant dysfunction as a contributing factor in both acute and chronic lung diseases. With regard to premature infants, we speculate that episodes of respiratory deterioration result in part from surfactant dysfunction, and that the frequency and duration of these episodes contribute to development of BPD through increased oxygen and ventilatory support with associated inflammatory response.

It is possible that treatment with exogenous surfactant or SP-B/C, or strategies to increase endogenous SP production, would be beneficial in infants with respiratory failure and surfactant dysfunction. In a study of 10 preterm infants at 7–30 d with stable ventilatory requirements, a dose of surfactant produced a transient decrease in fraction of inspired oxygen that was required to maintain appropriate oxygenation (46). We have initiated a study in chronically ventilated infants to examine associations between respiratory deteriorations and surfactant function and composition as well as to determine the short- and long-term safety of surfactant treatment for these events.

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