Surfactant Lecithin Fatty Acid Composition and Its Relationship to the Infantile Respiratory Distress Syndrome

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Summary

In order to evaluate the role of surfactant lecithin composition in the development of the infantile respiratory distress syndrome (IRDS) we have examined lecithin fatty acid composition from gastric aspirates obtained at the time of delivery from 14 full term healthy infants, 9 control premature infants without respiratory distress, and 16 premature infants who developed IRDS. The latter had significantly reduced concentrations of palmitic acid ($48.4 \pm 1.8\%$ vs. $65.3 \pm 1.5\%$ in fullterm and $59.5 \pm 0.8\%$ in control premature infants, $P < 0.001$). None of the infants in whom palmitic acid was more than 60% of total lecithin fatty acids developed IRDS, whereas 10 of 11 infants with values below 50% did develop this complication. Lecithin from gastric aspirate was shown to be similar in fatty acid composition to lecithins from tracheal washings and amniotic fluid. Palmitic acid concentrations in lecithins of serial tracheal washings paralleled the clinical course of eight infants with IRDS.

Speculation

The enzyme systems involved in the biosynthesis of pulmonary surfactant are complex. Failure of maturation of these systems can result in inadequate synthesis of total lecithins or in a selective deficiency of dipalmitoyl lecithin, an essential component of surfactant. Therefore, studies directed to determining the composition of surfactant lecithins may result in better diagnosis, understanding, and management of IRDS.

The respiratory distress syndrome is a major cause of neonatal mortality particularly in the premature infant. Decreased amount of pulmonary surfactant is considered to be the major contributing factor to the development of this syndrome (1, 3, 18, 26). Although some investigators have questioned the pulmonary origin of the phospholipids in amniotic fluid (28), others have indicated that they are largely derived from that source (27, 29). The composition of these phospholipids has been examined by many investigators as a means of predicting fetal lung maturity (17). Lecithin in amniotic fluid has been quantified in a number of different ways, including lecithin/sphingomyelin ratios (7, 17), determination of amniotic fluid lecithin concentration based on lipid phosphorus measurement (24), as well as measurement of total palmitic acid (21, 31). Similar studies have also been carried out on tracheal, pharyngeal (8), and gastric aspirates (9, 13). These tests measure quantities of lecithin either relative to sphingomyelin or in terms of concentration per given volume of fluid studies, or by their ability to maintain foam stability.

When these tests indicate a "high" level of lecithin, their success in predicting freedom from IRDS is close to 100% (14). However, when the lecithin levels are "low" or "intermediate" there has been considerable controversy concerning the probability of developing IRDS (14). The incidence of IRDS at these "low" levels

has varied from 10% to 100%, with a mean incidence of 45% (14). Based on a number of studies showing that lecithin, as measured by palmitic acid content (a measure of the proportion of dipalmitoyl lecithin) is reduced in immaturity and IRDS, Brumley *et al.* (11) and Avery (4) suggested that the quality of surfactant lecithin may be as important as the quantity.

Previous studies from our laboratory (19, 20) showed that when the palmitic acid content of surfactant lecithin in rats was reduced by dietary means (essential fatty acid deficiency), this surfactant lecithin was less effective in lowering minimal surface tension on compression in a surface balance. The reduction of palmitate content was shown to correlate predictably with a diminished amount of dipalmitoyl lecithin present in lung lavage fluid (19). These data indicated that indeed the quality of the lecithins in surfactant, that is their fatty acid composition, is an important determinant of their effectiveness as a surface active material as previously suggested by Brown (10) and Morgan and colleagues (22). This concept was confirmed by detailed *in vitro* studies by Watkins (32) and correlated with IRDS by Farrell and Avery (14).

Gastric aspirates are obtained routinely from infants at the time of delivery in our institution, as in some others (23). It has been shown that these aspirates contain swallowed surfactant material (13, 23). We have examined the fatty acid composition of the lecithins of gastric aspirates obtained at birth from normal full term and premature infants and, when possible, have also studied the composition of the lecithins from tracheal washings and amniotic fluid. We hoped to answer the following questions: (1) Is gastric aspirate lecithin fatty acid composition similar to that of tracheal wash and amniotic fluid? (2) Can gastric aspirate lecithin fatty acid composition be used to predict whether an infant is likely to develop IRDS? (3) Can serial examination of tracheal wash lecithins in newborns with IRDS be useful in following the course of the patient?

While these studies were in progress Motoyama *et al.* (23) reported that the fatty acid composition of lecithins from tracheal and gastric liquids was similar in the newborn. They showed further that the content of palmitate in gastric aspirate lecithins was lower in premature than in full term infants and that it was even lower in one infant who developed IRDS.

MATERIALS AND METHODS

The percentage of lecithin and its fatty acid composition was determined in lipid extracts from gastric aspirates obtained from 14 healthy full term infants, 9 premature infants who did not have IRDS (control premature infants), and 16 infants with IRDS who were also premature. Of the control premature infants, 1 was delivered by cesarian section as were 6 of 16 of the IRDS group. None of the mothers of infants in the study were on heroin or other addictive drugs. In infants with IRDS who were already intubated, serial tracheal washings were obtained for periods of 4 days to 3 weeks and analyzed. These studies were performed with

the approval of the Committee on Research Involving Human Subjects, and with the informed consent of the parents.

Gastric aspirates were obtained through a polyvinyl catheter passed into the stomach at the time of birth as part of routine neonatal care. Tracheal aspirates, using 5 ml sterile physiologic saline, were obtained only in infants who already had endotracheal tubes in place for the treatment of IRDS or neonatal asphyxia. Tracheal aspirate volumes varied from 3–5 ml. No complications were associated with this lavage procedure. Amniotic fluid was obtained during routine amniocentesis carried out for accepted clinical indications. Samples of amniotic fluid, gastric aspirates and tracheal washings were obtained within a few hours (less than 24) of each other. Severity of IRDS was assessed using the usual clinical, radiologic, and biochemical criteria.

Samples contaminated with blood or meconium were discarded. All other samples were extracted without prior centrifugation in chloroform-methanol (15). Samples of gastric and tracheal aspirates were not centrifuged before extraction since preliminary studies showed, as previously reported by others (23), that this was not necessary. Phospholipids were separated on thin layer plates of silica gel (5) and quantified by determination of lipid phosphorus (6). Lecithin as a percentage of total phospholipid was calculated from these data. The fatty acid composition of lecithin was determined by gas-liquid chromatography of their methyl esters (5) using a Hewlett-Packard 5830 gas chromatograph. Results were expressed as moles % (mean $\pm$ SEM). Statistical analysis where appropriate was carried out using Student's *t*-test.

RESULTS

The fatty acid composition of the lecithins in newborn infants with IRDS was similar in gastric aspirates and tracheal washings (Table 1, part A). In 13 patients both materials were available for analysis. Palmitic acid accounted for 48.4% of total fatty acids in the lecithins of gastric aspirates and 48.0% in tracheal washings. Total saturated fatty acids were 68.9% and 69.7% respectively. Comparisons with amniotic fluid were possible in three infants delivered by cesarian section. No significant differences were demonstrated. Table 1, part B, shows the similarity in the fatty acid composition of gastric aspirate and amniotic fluid lecithins from control full term infants.

Table 2 presents the gestational ages, weight at birth, total lipid phosphorus extracted, percentage of lecithin, and fatty acid composition of lecithins from gastric aspirates of control full term infants, control premature infants, and infants with IRDS. The degree of severity of IRDS is also shown. The birth weights of the control full term infants at 3295 ± 100 g (mean $\pm$ SEM) were within the normal range. The gestational ages of the control premature infants and those with IRDS were similar (31.6 ± 1.1 and 32.9 ± 0.7 weeks, respectively). However, the birthweights of the control premature infants were lower (1493 ± 188 g vs. 1889 ± 179 g) than those with IRDS, but this difference was not statistically significant ($P > 0.05$).

Lecithins from gastric aspirates of infants with IRDS contained significantly less palmitic acid ($48.4\% \pm 1.8$ vs. 65.3 ± 2.1 , $P < 0.001$) and significantly more stearic acid (12.9 ± 1.0 vs. 6.0 ± 0.6 , $P < 0.001$), oleic acid (16.2 ± 1.2 vs. 11.1 ± 0.7 , $P < 0.005$), and arachidonic acid (4.8 ± 0.9 vs. 1.7 ± 0.3 , $P < 0.01$) than those

from full term control infants (Table 2). When compared to the control premature infants, the differences in oleic and arachidonic acids were not significant, but the differences in palmitic and stearic acids content were again highly significant (Table 2). There was no correlation between the severity of IRDS and the percentage of palmitic acid in lecithins when examined as a group (Table 2).

In eight infants with IRDS receiving ventilation by endotracheal tube daily tracheal washings were analyzed for the fatty acid composition of the lecithins. Palmitic acid, as a percentage of total fatty acids in the lecithins of these sequential samples, was compared with the daily clinical grading. In all seven patients showing clinical improvement following appropriate respiratory support, the percentage of palmitate rose to normal levels in parallel with the clinical improvement (Table 3). Thus, the percentage of palmitate in individual patients does appear to be related to their clinical course. A typical example is presented in Figure 1 which shows the course of patient 1. On day 11 the patient had a sudden deterioration in pulmonary function; however, the palmitic acid content of tracheal wash lecithin was normal, suggesting that the clinical deterioration was not due to abnormal surfactant. The cause of the problem proved to be atelectasis due to a mucous plug. In the eighth patient failure to improve clinically was associated with persistently low concentrations of palmitate in tracheal wash lecithins (Table 3).

DISCUSSION

The values obtained in this study for palmitic acid concentrations in amniotic fluid lecithins of full term pregnancies is in fair agreement with those reported by Singh *et al.* (30), Frantz and coworkers (16), Arvidson *et al.* (2), and Ogawa *et al.* (25). As shown in Table 1, stearic acid concentration in these lecithins was increased in premature infants with IRDS as indicated by Frantz *et al.* (16). Table 1 also shows that the fatty acid composition of lecithins from gastric and tracheal aspirates and amniotic fluid were similar. This finding confirms the report of Motoyama *et al.* (23) in respect to the similarity of lecithins from gastric and tracheal liquids. Our results differ from those of these latter authors (23), in that the concentration of palmitic acid in the lecithins of gastric aspirates from healthy premature infants was not significantly lower, and in that stearate levels were not higher in these subjects than in full term control subjects (Table 2). Frantz *et al.* (16) stressed the importance of increased concentrations of stearic acid in lecithins of amniotic fluid in premature infants as a predictor of RDS. As shown in Table 2, we also observed significantly higher stearate concentrations in gastric aspirate lecithins in infants with IRDS, than in either of the two control groups. However, 4 of 16 infants with IRDS had normal values for stearate, and one of these died.

The results of our studies as shown in Table 2 and Figure 2 demonstrate good separation between normal full term infants, healthy premature infants, and infants with IRDS using the percentage of palmitate in lecithins of gastric aspirate as the criterion. These data show that irrespective of gestational age or birthweight, if palmitic acid accounts for more than 60% of total fatty acids of gastric lavage lecithins, none of the infants developed IRDS. By contrast, 10 of 11 infants with palmitic acid values

Table 1. Fatty acid composition of lecithins from gastric aspirates (GA) tracheal washings (TW), and amniotic fluid (AF) (moles % mean $\pm$ SEM)

	<i>n</i>	14:0	16:0	16:1	18:0	18:1	18:2	20:4
A								
GA IRDS	16	7.6 $\pm$ 1.6	48.4 $\pm$ 1.8	5.6 $\pm$ 0.7	12.9 $\pm$ 1.0	16.2 $\pm$ 1.2	4.5 $\pm$ 0.6	4.9 $\pm$ 0.9
TW IRDS	13	10.0 $\pm$ 2.0	48.0 $\pm$ 2.6	6.9 $\pm$ 1.0	11.0 $\pm$ 1.1	15.7 $\pm$ 1.1	4.8 $\pm$ 1.3	3.6 $\pm$ 0.7
AF IRDS	3	8.2 $\pm$ 0.9	46.7 $\pm$ 4.5	4.7 $\pm$ 1.3	16.4 $\pm$ 1.4	17.2 $\pm$ 1.0	4.2 $\pm$ 0.5	2.7 $\pm$ 0.8
B								
GA Control	14	7.1 $\pm$ 0.6	65.3 $\pm$ 1.5	5.2 $\pm$ 0.5	6.0 $\pm$ 0.6	11.1 $\pm$ 0.7	3.7 $\pm$ 0.8	1.7 $\pm$ 0.3
AF Control	8	5.9 $\pm$ 1.1	67.9 $\pm$ 1.9	4.9 $\pm$ 0.6	6.3 $\pm$ 1.6	9.9 $\pm$ 1.1	3.3 $\pm$ 0.4	1.7 $\pm$ 0.5

¹ Fatty acid given as carbon chain length/number of double bonds.

Table 2. Fatty acid composition of lecithins (Lec) from gastric aspirates (moles %, mean $\pm$ SEM)

Samples	14:0	16:0	16:1	18:0	18:1	18:2	20:4	Total lipid P (%)	% Lec	Birth wt. g	Gestational age, weeks	
IRDS												
1	5.6	45.4	7.5	20.0	21.4	0.1	Trace	18.8	73.3	2470	36	Severe; survived
2	4.2	42.5	3.4	14.1	18.4	8.2	9.2	3.7		2130	35	Severe; survived
3	9.3	37.7	8.8	15.5	23.3	2.2	3.2	3.3		1190	30	Severe; died
4	4.0	59.1	8.4	8.0	14.3	3.4	2.7	130.7	93.7	1085	33	Severe; died
5	2.4	59.7	6.6	8.2	13.3	5.1	4.7	157.6	93.3	2850	34	Severe; survived
6	13.7	37.0	8.2	14.4	21.7	3.0	2.1	2.8		1800	34	Severe; died
7	3.7	47.0	3.0	11.0	16.7	6.8	11.8	10.1		800	26	Severe; died
8	7.2	54.4	5.3	7.4	14.3	4.9	6.6	11.0		910	28	Mod. Sev.; survived
9	10.9	46.5	10.3	8.2	11.8	5.5	6.7	9.8		3020	36	Mod. Sev.; survived
10	2.8	58.1	3.8	22.2	9.1	2.2	1.7	¹		2300	35	Mod. Sev.; survived
11	2.9	46.1	4.8	12.3	24.6	5.0	4.2	29.8	88.4	1300	32	Mod. Sev.; survived
12	24.4	45.4	7.5	10.6	12.1	Trace	Trace	¹		2090	33	Mod. Sev.; survived
13	5.6	53.5	3.4	13.0	11.5	5.9	7.2	18.2		1730	34	Mod. Sev.; survived
14	17.8	49.5	Trace	14.9	12.2	5.5	Trace	2.8		2740	36	Mild; survived
15	4.7	40.5	3.7	13.4	19.4	7.0	11.2	26.5	93.8	1920	33	Mild; survived
16	2.8	52.5	5.3	12.8	14.3	6.6	5.6	118.1	93.3	²	32	Mild; survived
Total (16 subjects)												
Mean	7.6	48.4	5.6	12.9	16.2	4.5	4.8	38.7	89.3	1889	32.9	
SD	6.3	7.2	2.7	4.2	4.7	2.4	3.8	53.7	8.1	717	2.9	
SEM	1.6	1.8	0.7	1.0	1.2	0.6	0.9	14.4	3.3	179	0.7	
Premature control												
1	5.4	46.6	4.0	8.9	20.4	8.3	6.5	16.3		1420	32	
2	2.4	60.9	7.3	5.3	16.8	2.7	4.6	44.1	84.2	1110	29	
3	3.1	65.1	4.8	9.7	11.5	3.0	2.9	154.5	91.3	1418	34	
4	5.7	55.2	8.2	7.7	16.8	2.7	3.5	12.9	89.5	1800	32	
5	6.1	65.9	7.3	4.0	11.8	3.1	1.8	154.9	89.9	2450	36	
6	4.9	61.4	8.8	4.3	16.1	2.6	1.9	72.2	82.1	750	27	
7	5.8	66.0	7.9	4.5	12.5	2.9	0.5	141.2	86.5	910	27	
8	5.5	57.1	6.3	7.7	14.2	6.2	2.9	82.2	93.3	2180	35	
9	3.8	57.0	1.1	15.9	11.8	9.8	0.7	3.5		1400	32	
Total (9 subjects)												
Mean	4.7	59.5	6.2	7.6	14.7	4.6	2.8	75.6	88.1	1493	31.6	
SD	1.3	6.3	2.5	3.8	3.1	2.8	1.9	61.9	4.0	563	3.3	
SEM	0.4	0.4	0.8	1.3	1.0	0.9	0.6	20.6	1.3	188	1.1	
Full term (FT) controls (14)												
Mean	7.1	65.3	5.2	6.0	11.1	3.7	1.7	43.7	87.6	3295	FT	
SD	2.4	5.5	1.9	2.2	2.5	3.1	1.3	47.0	8.2	407	FT	
SEM	0.6	1.5	0.5	0.6	0.7	0.8	0.3	12.6	2.2	109	FT	
P values												
IRDS vs. FT control	NS	<0.001	NS	<0.001	<0.005	NS	<0.01		NS			
IRDS vs. premature control	NS	<0.001	NS	<0.005	NS	NS	NS		NS	NS	NS	

¹ Lipid extracts applied directly to thin layer chromatography without prior phosphorus measurements.² Patient transferred from another hospital; birth weight unobtainable.

below 50% did develop IRDS, whereas only 3 of 16 infants whose lecithins contained more than 55% palmitate showed evidence of IRDS. The findings are consistent with those of Frantz *et al.* (16) based on studies of amniotic fluid, but extend them to gastric aspirates. Motoyama *et al.* (23) reported that one infant who developed IRDS had only 30% palmitate in the lecithin of the gastric fluid. Thus, our observations validate this preliminary report.

The percentage of palmitate in gastric lavage lecithin, obtained at birth, was a good predictor of the risk of development of IRDS, but the absolute values for palmitate did not correlate significantly with the severity of IRDS in this group of children. However, in individual infants the change in percentage of palmitate in tracheal lavage lecithin did parallel the clinical course. Therefore, another area where estimation of palmitate concentration in presumed surfactant lecithin may prove valuable is as an aid in the clinical management of the infant with IRDS. Failure to respond to

treatment in such infants may be due to continued inadequacy of the surfactant synthesizing system, in which case the percentage of palmitic acid in the lecithins of tracheal washings will continue to be low. On the other hand, if failure to show clinical improvement is due to other causes, for example, as shown in the infant illustrated in Figure 1, to atelectasis, palmitate levels will return to normal. Thus, serial determinations of palmitate concentrations may prove to be a useful guide to the management of these infants, and an indication as to the timing of extubation. We are currently conducting a critical prospective study to evaluate the use of this determination in the clinical management of IRDS.

Data in the literature has validated the usefulness of the L/S ratio in amniotic fluid in predicting the possibility, or probability, of development of IRDS in an infant (14). Others have shown the value of this ratio for this purpose in tracheal, pharyngeal, and gastric aspirates (8, 9). In the present studies lecithin represented more than 87% of total phospholipids in gastric aspirates when

Table 3. Percentage of palmitic acid in lecithins of tracheal aspirates of infants with RDS; Relationship to clinical course

Patient	% of total fatty acids		Outcome
	Initial conc	Final conc ¹	
1 ²	47.4	61.5	Recovered
2 ²	30.0	71.4	Recovered
8 ²	51.3	59.5	Recovered
L ³	51.9	59.0	Recovered
G ³	30.2	45.0 ⁴	Died
P ³	55.4	66.4	Recovered
R ³	48.4	66.3	Recovered
D ³	52.5	64.4	Recovered

¹ Final value based on sample obtained on day of extubation.

² Same number as in Table 2.

³ Not shown in Table 2 as no gastric aspirates obtained.

⁴ Value on day 8, subsequently, palmitate concentration fell to below 40%.

Fig. 1. Palmitic acid as percentage of lecithin fatty acids in tracheal washings compared to the clinical grade of an infant with IRDS (case 1, Table 2) receiving ventilatory support during the first 14 days of life. In order to determine the degree of improvement of the patient, a Clinical Grading System was developed (on a scale of 1-5) based primarily on the level of respiratory support required. Percentage of palmitic acid (x), clinical grading (o), atelectasis due to a mucous plug (↑), extubation (↓↓).

Fig. 2. Comparison of the birthweight with the palmitic acid as % of lecithin fatty acids in gastric aspirates of newborns. ○: control full term infants, ●: control premature infants, ■: infants with IRDS. The means $\pm$ SD for each group are indicated.

determined by direct assay of lipid phosphorus. The determination of the palmitic acid content of the lecithins in gastric aspirates of newborns may, therefore, prove valuable in those cases where the L/S ratio is borderline. Furthermore, it can be used in infants delivered prematurely, without prior amniocentesis, to predict the

risk of IRDS. This method can thus supplement established diagnostic tests, and is not intended to replace them.

The results reported here indicate that the quality as well as the quantity of surfactant lecithin may be an important factor in the etiology of IRDS. The percentage of palmitic acid in lecithin from gastric aspirate or tracheal wash is a good predictor of the future course of the infant with IRDS. It may prove useful, when combined with the determination of the L/S ratio, in improving its predictive value. Furthermore, serial measurement of the palmitic acid content of tracheal wash lecithins may prove valuable in the planning of the respiratory support measures in the infant with IRDS.

CONCLUSION

The fatty acid composition of lecithins from gastric aspirates, tracheal washings, or amniotic fluid of infants with IRDS is similar. Compared to either full term or premature control subjects, palmitic acid content is decreased, and stearic acid content is increased. The percentage of palmitic acid in these lecithins correlates well with the development or absence of IRDS, and can be independent of gestational age or birthweight. When the molar percentage of palmitic acid in lecithin was above 60% none of the infants developed IRDS, whereas 10 of 11 with palmitic acid concentrations of 50% or less did. Therefore, we believe this determination can be helpful in predicting the risk of development of IRDS in an infant seen after delivery, and can also supplement the L/S ratio test when the ratio is in the low range. Furthermore, serial determinations of palmitic acid content of tracheal wash lecithins may be useful in the management of infants with IRDS. Finally, the observation that palmitic acid concentration in such lecithins is low in patients with IRDS, is consistent with the view that synthesis of dipalmitoyl lecithin is selectively impaired in such infants.

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