Eustachian tube surfactant is different from alveolar surfactant: determination of phospholipid composition of porcine eustachian tube lavage fluid

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Abstract Phosphatidylcholine (PC) is the main phospholipid in lung surfactant and, more specifically, dipalmitoyl PC (PC16:0/16:0) is the major surface-active component. Several studies have tentatively shown that eustachian tube lavage fluid (ETLF) contains surface-active material. The aim of the present study was to determine, using electrospray ionization mass spectrometry, whether the phospholipid molecular species composition of ETLF is similar to that of lung surfactant. PC was the main component of both ETLF and bronchoalveolar lavage fluid (BALF). The concentration of phosphatidylethanolamine was higher and phosphatidylglycerol was undetectable in ETLF compared with BALF. The molecular species composition of PC in ETLF was notably different from that of BALF, palmitoyloleoyl PC being the major component. Importantly, given its predominance in BALF PC, the concentration of PC16:0/16:0 was low in ETLF. As expected on the basis of this molecular species composition of PC, ETLF did not generate low surface tension values under dynamic compression in a pulsating bubble surfactometer. We conclude that the surfactant in ET is different from lung surfactant, and that low surface tension is not a major determinant of ETLF function.—Paananen, R., A. D. Postle, G. Clark, V. Glumoff, and M. Hallman. **Eustachian tube surfactant is different from alveolar surfactant: determination of phospholipid composition of porcine eustachian tube lavage fluid.** ***J. Lipid Res.*** **2002.** 43: **99–106.**

Supplementary key words mass spectrometry • middle ear infection • phospholipid molecular species • surface tension

Pulmonary surfactant is a complex of lipids and proteins that lines the interior of the lung and prevents atelectasis by the opposing surface tension forces at the air/liquid interface of the alveolus (1, 2). The phospholipid component is crucial for the physiological function of lung surfactant and comprises about 80% of the total lipid fraction, with cholesterol as the predominant neutral lipid. Phosphatidylcholine (PC) is the main phospholipid in lung surfactant, comprising approximately 70–80% of phospholipids, about half of which consist of the dipalmitoyl species (PC16:0/16:0). The disaturated nature of PC16:0/16:0 enables the surfactant to withstand high surface pressure when compressed at the air/liquid interface, and this property is thought to prevent the collapse of the small alveoli and conducting airways (3). The acidic phospholipids phosphatidylglycerol (PG) and phosphatidylinositol (PI) are the next most abundant phospholipids (8–15%) in lung surfactant (4). Phosphatidylethanolamine (PE) and phosphatidylserine (PS), phospholipids characteristic of cell membranes, are present in only trace amounts in lung surfactant.

The eustachian tube (ET) connects the upper respiratory tract to the middle ear. ET epithelium is morphologically similar to lower airway epithelium, and ET dysfunction is considered to be the principal pathogenic factor in susceptibility to recurrent otitis media (5). There are close anatomical and physiological similarities between human and porcine ET and middle ear (6). For this reason, the pig ET has been proposed as a potential model system for human middle ear infections and otitis media. Several studies have purported to show that ET lavage fluid (ETLF) contains surface-active material that is analogous to lung surfactant (7–12). Lamellar structures containing phospholipid have been detected within epithelial cells and extracellular spaces of rabbit and mouse ET (13, 14), and ET has been reported to synthesize disaturated PC (10, 13). In our more recent studies (15, 16), we found a few

Abbreviations: BALF, bronchoalveolar lavage fluid; ET, eustachian tube; ETLF, eustachian tube lavage fluid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine. Phospholipid molecular species are designated by head group followed by fatty acyl moieties at the *sn*-1 and *sn*-2 positions; for instance, PC16:0/16:0 is dipalmitoyl phosphatidylcholine, and PI18:0/20:4 is *sn*-1 stearoyl *sn*-2 arachidonoyl phosphatidylinositol.

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concentric lamellar structures in the apical cytoplasm of ET epithelial cells by transmission electron microscopy, but no lamellar bodies typical of the type II alveolar epithelial cells that secrete lung surfactant could be detected in cryosections of porcine ET epithelium.

The aim of the present study was to evaluate whether a detailed analysis of phospholipid molecular species compositions would support the proposed similarity of ETLF and bronchoalveolar lavage fluid (BALF) surfactant. Whereas the predominant phospholipid in ETLF has been shown to be PC (11, 12), little is known about the molecular species composition of this material. In this study, we used electrospray ionization mass spectrometry (ESI-MS), which is an extremely sensitive technique and provides detailed structural information about the molecular composition of phospholipids.

MATERIALS AND METHODS

Preparation of ETLF and BALF for phospholipid and surface activity analyses

ETLF and BALF fractions were obtained as described previously (15, 16). For a batch of ETLF, approximately 300 pigs supplied by a local slaughterhouse were used. Lavages containing visible blood contamination were discarded. Cells were removed by centrifugation at 500 *g* for 10 min, and the samples were recentrifuged at 100,000 *g* to collect the sedimentable lipid-protein complexes. Both ETLF and BALF samples were resolved by sucrose density gradient centrifugation as previously described (15). Three fractions with sucrose concentrations of 0.2–0.45, 0.5–0.65, and 0.7–1.0 M were collected, diluted with 0.15 M NaCl, and recentrifuged at 50,000 *g* for 16 h. The pellets were collected and diluted in PBS. The total PC concentration was determined by an enzymatic method based on phospholipase D and choline oxidase (Phospholipids B kit; Wako Chemicals, Richmond, VA). For an analysis of the phospholipid composition of the underlying ET epithelial cells, the ET lining material was first removed by lavage, the cartilaginous part of the ET was dissected, and the cells lining the remaining surface were harvested by scraping with a scalpel.

Analysis of phospholipid molecular species by ESI-MS

Total lipid was extracted according to Bligh and Dyer (17) from 800 μl of cell-free crude ETLF and BALF preparations and from the three fractions of both ETLF and BALF obtained from the sucrose density gradient. Similar lipid extraction was performed on the ET epithelial cells scraped from the surface of the tube after lavage. Before extraction, 5 nmol of dimyristoylphosphatidylcholine (PC14:0/14:0), 1 nmol of dimyristoylphosphatidylethanolamine (PE14:0/14:0), and 1 nmol of dimyristoylphosphatidylglycerol (PG14:0/14:0) were added to each sample as internal recovery standards. Total lipid extracts were partially purified before analysis, using solid-phase aminopropyl extraction cartridges (Bond Elut$_{NH2}$; Varian, Palo Alto, CA). Dried lipid extracts were dissolved in chloroform (1 ml) and applied to a 100-mg cartridge. After removing neutral lipids with two 1-ml washes of chloroform, PC and PE species were selectively eluted with, respectively, 1 ml each of chloroform-methanol 3:2 (v/v) and methanol. The acidic species PI, PS, and PG were then eluted with three sequential aliquots of methanol-water 19:1 (v/v) containing 40 mM choline chloride and 1% (w/v) orthophosphoric acid, and the combined eluate was back-extracted with chloroform to remove salts. ESI-MS of phospholipid species was performed on a Micromass Quatro Ultima triple quadrupole mass spectrometer (Micromass, Wythenshaw, UK) equipped with an electrospray ionization interface. Purified fractions of samples were dried under nitrogen gas, dissolved in methanol-chloroform-water 7:2:1 (v/v/v), and introduced into the mass spectrometer by nanoflow infusion. Each molecular species of phospholipid was identified by a selection of tandem MS/MS methodologies.

PC species were detected under conditions of positive ionization. Collision-induced fragmentation of PC molecular ions with argon gas produced an intense fragment at m/z 184 and separate analysis of authentic phosphorylcholine standard under positive ionization conditions verified that formation of this ion fragment at m/z 184 likely originated from the protonated phosphorylcholine head group of PC. All PC species were subsequently selectively detected and quantified as $[M+H]^+$ ions generated by parent scans of the ion fragment at m/z 184. This approach simplified spectral analysis by avoiding detection of $[M+Na]^+$ adducts of PC species. The same analysis conditions also identified sphingomyelin species, but these were readily distinguished from PC species by the numerical value of their molecular mass being consistently odd instead of even. For instance, the $[M+H]^+$ of palmitoyl sphingomyelin is 703.7 whereas that of PC16:0/14:0 is 706.7. Collision gas-induced fragmentation of PE species under positive ionization conditions generated an ion fragment 141 Da lower than the $[M+H]^+$ molecular ion, corresponding to the neutral loss of the phosphoethanolamine head group. Consequently, tandem MS/MS scans for the neutral loss of m/z 141 were used to identify PE species. However, as neutral loss scans preferentially detected diacyl rather than alkenyl-acyl PE species, PE compositions were quantified from the corresponding electrospray scan under negative ionization conditions.

All acidic phospholipids were identified and quantified under conditions of negative ionization. Collision gas-induced fragmentation of PI molecular ions generated a common ion fragment of m/z 241, characteristic of the hydrated inositol phosphate moiety. Parent ion scans of this m/z 241 fragment were used to identify PI species as their $[M-H]^-$ ions. Comparable fragmentation of PS species resulted in the expulsion of serine (loss of 87 Da) and consequently PS species were identified by constant neutral loss scans of m/z 87. Whereas no specific diagnostic fragment ion was formed on fragmentation of PG species, all acidic phospholipids generated a common fragment of m/z 153, corresponding to the glycerophosphate backbone of the molecule. PG species were provisionally identified from parent scans of this m/z 153 fragment ion, and compositions of individual PG species were confirmed by determination of the fatty acyl moieties in the molecule. Assignment of acyl substitutes was based on the generation of characteristic fatty acyl ion fragments of $[M-H]^-$ (PE, PI, PS, and PG) or $[M-15]^-$ (PC) ions on collision gas-induced fragmentation under conditions of negative ionization. The data were acquired and processed with MassLynx NT software (Waters, Milford, MA). After conversion into centroid format according to area and correction for ^{13}C isotope effects and for the reduced response with increasing m/z values, molecular species compositions of individual phospholipid classes were expressed as molar percentages of their respective totals present in the sample.

Measurement of the surface activity of ETLF

The surface tensions of cell-free crude lipid-protein complex preparations of ETLF and BALF as well as the surfactant fraction recovered from BALF and the comparable fraction from ETLF (sucrose concentration, 0.2–0.45 M) were measured with a pulsating bubble surfactometer (Electronetics, Amhurst, NY) as described (18). The concentrations of BALF and ETLF were ad-

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justed on the basis of the PC concentration at 0.25 or 1.0 mM in PBS containing Ca^{2+}. The radius of the bubble was varied between 0.4 and 0.55 mm at a frequency of 0.33 Hz for 5 min at 37°C for 3 min. The surface tensions during the pulsation were recorded. For the estimation of surface adsorption and the equilibrium surface tension measurements, a bubble with a radius of 0.4 mm was formed and the radius was kept constant for 5 min. The surface tensions were continuously measured during the first 10 s and thereafter at set time points. Surface tension at 5 min was called the equilibrium surface tension.

RESULTS

In contrast to the phospholipid-rich composition of BALF, relatively low concentrations of PC were measurable in ETLF. For a constant volume of recovered lavage fluid, the total PC concentration of ETLF (3 nmol/ml) was one-twentieth of that of BALF (60 nmol/ml). These differences reflect the smaller surface area of the ET compared with the extensive alveolar surface of the lungs. Purification of the lavage fluids into fractions of differing densities also suggested that the physical structures of ETLF and BALF were different. As expected for BALF, the major phospholipid-rich fraction was isolated at a low buoyant density characteristic of lung surfactant (80% of the total PC). By contrast, when ETLF was centrifuged on a sucrose density gradient, greater amounts of PC were recovered in the fractions representing the higher densities (40% of the total PC at 0.5–0.65 M and 50% at 0.7–1.0 M sucrose). The concentrations of PC recovered from ETLF at densities characteristic of lung surfactant (sucrose density, 0.2–0.45 M) were only 10% of the total PC in ETLF.

The molecular species compositions of all the phospholipid classes of ETLF were different from those of BALF. The dominant PC species from BALF was PC16:0/16:0 ($[M+H]^+$ 734.7, 56 mol%), which is the characteristic species of lung surfactant (**Fig. 1A**), whereas the remainder was a combination of saturated (PC16:0/14:0, $[M+H]^+$ 706.7, 5 mol%) and monounsaturated (PC16:0/18:1, $[M+H]^+$ 760.8, 18 mol%) species. By contrast, PC16:0/16:0 (11 mol%) was a minor component of ETLF, which was composed primarily of monounsaturated and diunsaturated species (Fig. 1B). Whereas PC16:0/18:1 ($[M+H]^+$ 760.8) was common to both spectra, ETLF PC contained many species not present in BALF PC: ETLF contained significant amounts of species with *sn*-1 stearate (PC18:0/18:1, $[M+H]^+$ 788.8, 11 mol%) and *sn*-1 oleate (PC18:1/18:1, $[M+H]^+$ 786.7, 17 mol%), in contrast to the BALF PC species, which were all *sn*-1 palmitate. Importantly, arachidonoyl-containing species contributed appreciably to ETLF PC but not to BALF PC (PC16:0/20:4, $[M+H]^+$ 782.7 and PC18:0/20:4, $[M+H]^+$ 810.7). Finally, ETLF but not BALF contained a measurable contribution from sphingomyelin (SM16:0, $[M+H]^+$ 703.7; Fig. 1B). **Figure 2** shows the typical percentage distribution of the different PC species in ETLF and BALF.

In contrast to the saturated (BALF) or monounsaturated (ETLF) compositions of PC, the PE spectra of BALF and ETLF were both highly enriched in polyunsaturated species containing arachidonate (20:4) (**Fig. 3**). However, the

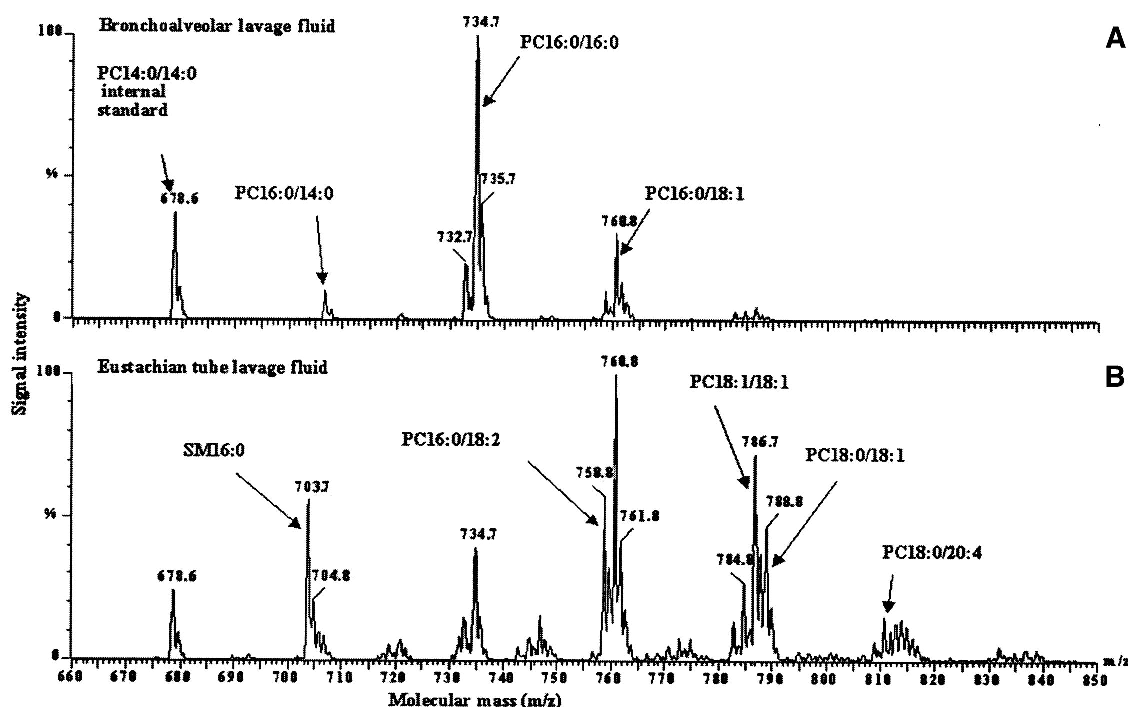

Fig. 1. Typical positive ionization tandem MS/MS spectra of PC molecular species in BALF (A) and ETLF (B). For each spectrum in this and all subsequent figures, results are presented as continuum data, with the ordinate depicting the relative (normalized) ion intensities and the abscissa denoting the *m/z* ratio of the ions. Individual PC species were identified in these spectra as parent ions of the characteristic phosphorylcholine ion at *m/z* 184 obtained by collision gas-induced fragmentation.

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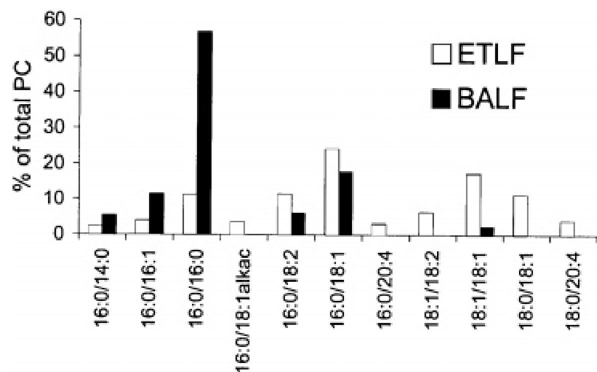

Fig. 2. Distribution of the various PC molecular species in ETLF and BALF. Results are expressed as mol percentage of the total number of PC species identified for each sample type.

molecular structures of these polyunsaturated PE species present in ETLF were different from those in BALF. BALF PE was composed almost exclusively of diacyl species (Fig. 3A), whereas ETLF was highly enriched in ions corresponding to ether-linked species (Fig. 3B). The identities of these species were determined on the basis of the major fatty acyl ions generated by MS/MS daughter scans of molecular ions. Tandem MS/MS of $[M-H]^-$ ions of BALF PE generated two fragment ions reflecting the compositions of the two acyl-linked fatty acid residues, whereas comparable analysis of $[M-H]^-$ ions of ETLF revealed only a single fragment corresponding to the fatty acyl moiety esterified at the *sn*-2 position. The identities of these PE species from ETLF were assigned as vinyl ether alkenyl-acyl species, which are 16 mass units lighter than the corresponding diacyl species. For instance, the plasmenylethanolamine species *sn*-1-*O*-octadec-1′-enyl *sn*-2 arachidonoyl PE (PE18:0a/20:4) has a mass of 750.6 and generated a single product ion at *m/z* 302.3 (arachidonate) (**Fig. 4A**), whereas the corresponding diacyl PE species *sn*-1 stearoyl *sn*-2 arachidonoyl PE has a mass of 766.5 and generated product ions at both *m/z* 283.0 (stearate) and 303.2 (arachidonate) (Fig. 4B). It is possible that some PE species assigned as alkenyl-acyl may also have contained some alkyl-acyl species, but these are generally less abundant in PE.

The most striking differences between BALF and ETLF were observed in the compositions of their acidic phospholipid fractions. This contrast is illustrated in **Fig. 5**, which shows the negative ionization electrospray spectra of acidic phospholipid species in BALF and ETLF. PG species, as expected, were predominant in the BALF spectrum with a monounsaturated (PG16:0/18:1, $[M-H]^-$ 747.8; PG18:1/18:1, $[M-H]^-$ 773.6) and disaturated (PG16:0/16:0, $[M-H]^-$ 721.7) composition comparable to that described for other animal species (19) (Fig. 5A), but were undetectable in ETLF (Fig. 5B). This distinction is emphasized by the spectra obtained by parent scans of *m/z* 153, which confirms that PG species were present in BALF but not in ETLF (**Fig. 6A**). By contrast, whereas PS18:0/18:1 ($[M-H]^-$ 788.8) was a major component in ETLF (Fig. 5B), PS species were barely detectable in BALF (Fig. 5A). Selective detection of PS species as neutral loss scans of *m/z* 87 (Fig. 6B), however, demonstrated that the trace amount of PS in BALF was also predominantly PS18:0/18:1. Finally, although PI species were apparent in both BALF (Fig. 5A) and ETLF (Fig. 5B), their compositions were different. Selective detection of PI species as parent scans of *m/z* 241 (Fig. 6C) shows that, whereas BALF PI

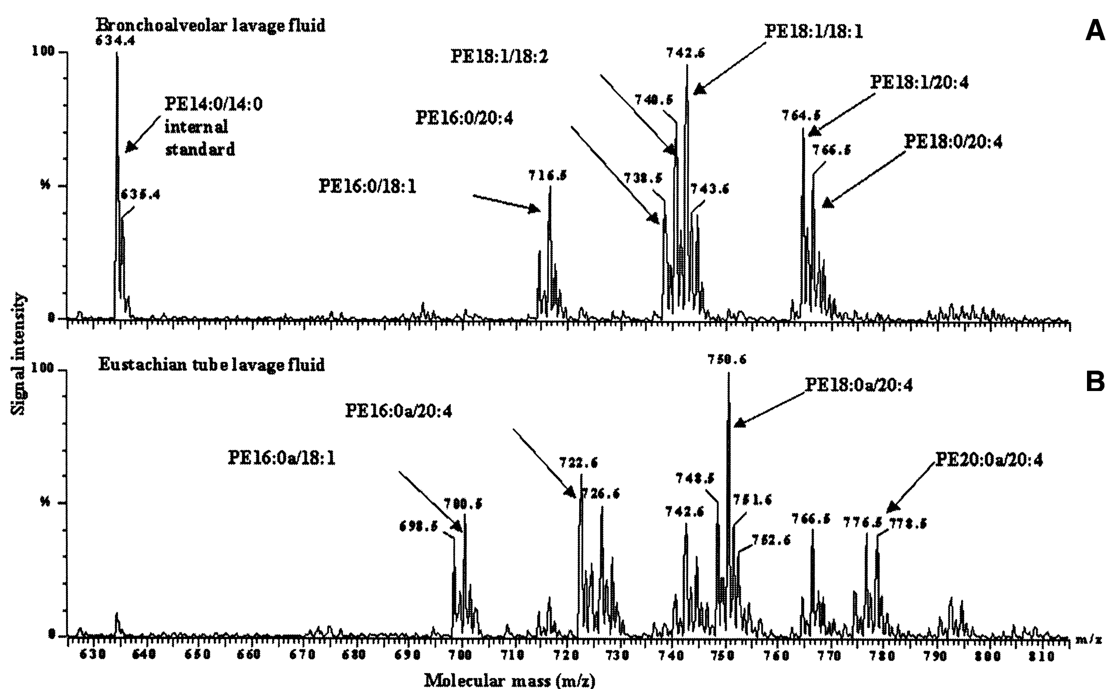

Fig. 3. Negative ionization mass spectra identification of PE molecular species in BALF (A) and ETLF (B).

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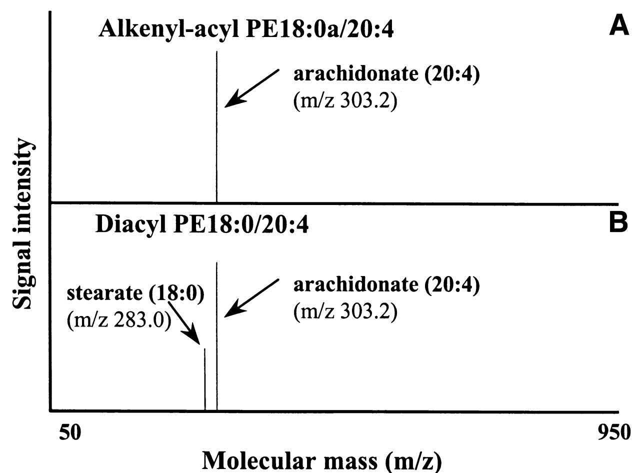

Fig. 4. Comparison of product ions by collision gas-induced dissociation of alkenyl-acyl phosphoethanolamine and diacylphosphoethanolamine species. A: The plasmenylphosphoethanolamine species *sn*-1-*O*-octadec-1′-enyl *sn*-2 arachidonoyl PE (PE18:0a/20:4, *m/z* 750.6) generated a single fragment at *m/z* 303.2, corresponding to the arachidonate anion. B: Fragmentation of the diacylphosphoethanolamine species *sn*-1 stearoyl *sn*-2 arachidonoyl PE (PE18:0/20:4, *m/z* 766.5) generated product ions at *m/z* 283.0 and *m/z* 303.2 corresponding to the stearate and arachidonate anions, respectively.

comprised essentially monounsaturated species such as PI16:0/18:1([M−H]$^-$ 835.9) and PI18:1/18:1 ([M−H]$^-$ 861.6), ETLF was characterized by a high concentration of the polyunsaturated species PI18:0/20:4 ([M−H]$^-$ 885.7).

Further differences between ETLF and BALF were apparent in their fractional distributions of PC, PE, and PG classes as shown in **Fig. 7**, which details their relative concentrations calculated as the sums of their individual component species after correcting for the recovery of the relevant internal standards. Expressed as a proportion of the three lipid classes, PC comprised 92% of BALF but only 66% of ETLF, PE was considerably enriched in ETLF (33%) compared with BALF (2%), and PG, although the second major component in BALF phospholipid (6%), was undetectable in ETLF. The possibility was considered that one of the fractions of ETLF recovered from the sucrose density gradient fractionation might have had a phospholipid composition more similar to that of BALF. However, when all the ETLF and BALF fractions obtained from sucrose density gradient centrifugation were analyzed separately, their PC, PE, PG, PS, and PI compositions were essentially identical to those of the respective total lavage fluids (results not shown). This same result was obtained even for the fraction from ETLF with the same buoyant density as lung surfactant recovered from BALF. Finally, as the phospholipid composition of lung surfactant is enriched in specific components compared with that of the type II alveolar epithelial cell from which it is secreted, we compared the phospholipid composition of ETLF with that of the underlying ET epithelial cells. However, the molecular species compositions of PC, PE, PS, and PI in ETLF were identical with those of ET epithelial cells (results not shown).

Table 1 shows the representative surface tension measurements, using the crude sedimentable lipid-protein complex fraction of BALF and ETLF and the fractions of BALF with the same buoyant density as BALF surfactant, recovered from the sucrose density gradient. There were no detectable differences in the surface tension measured from the different preparations. As expected, BALF re-

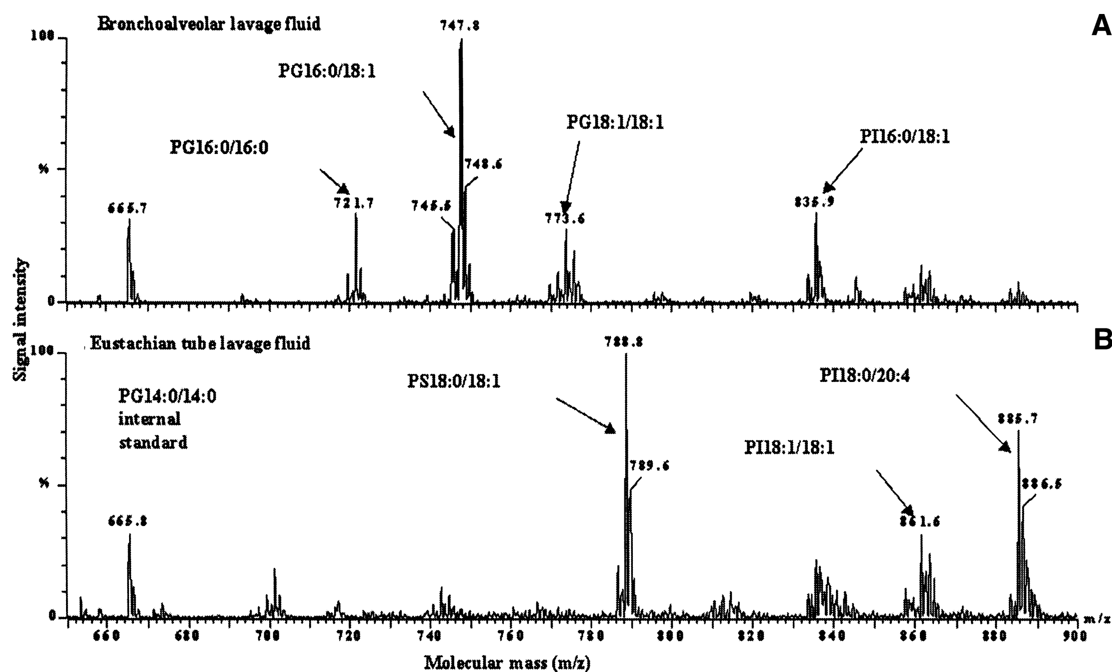

Fig. 5. Negative ionization mass spectra identification of PG, PS, and PI molecular species in BALF (A) and ETLF (B).

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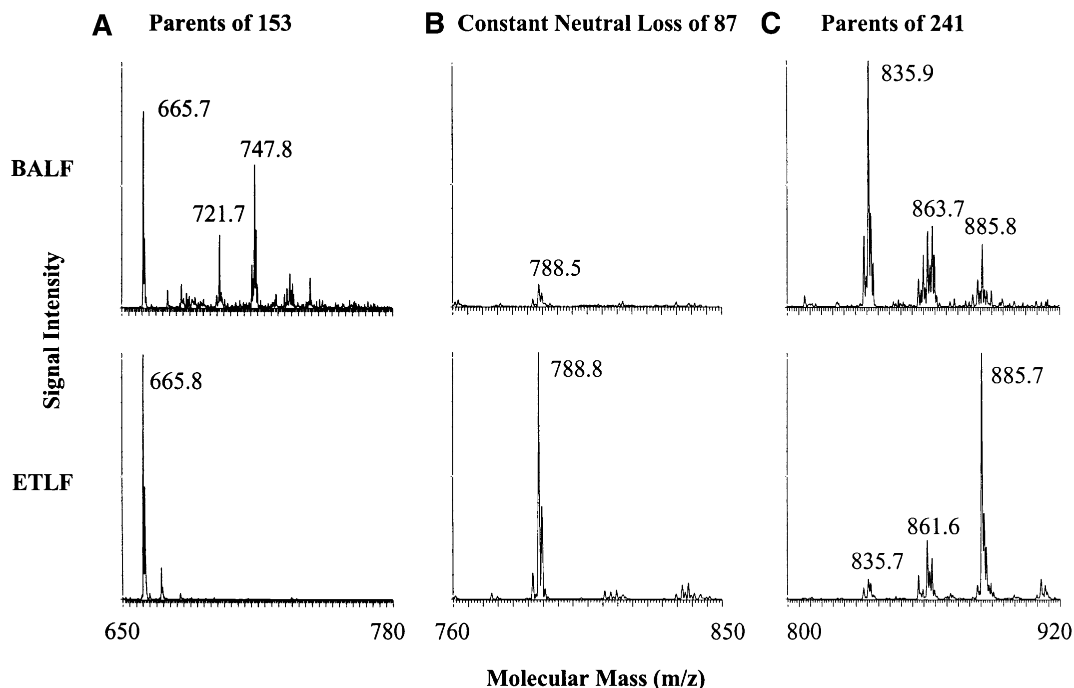

Fig. 6. Diagnostic negative ionization mass spectra of acidic phospholipids in BALF and ETLF. PG species were selectively identified as parent ions of their common glycerophosphate product ion at *m/z* 153 (A), PS species by constant neutral loss of the serine head group (*m/z* 87) (B), and PI species by parent ions of their common hydrated inositol phosphate product ion (*m/z* 241) (C). The molecular masses displayed here are identified in Fig. 5.

vealed typical characteristics of lung surfactant. These included a low surface tension approaching 0 mN/m during compression of the surface, marked hysteresis (during compression surface tensions were much lower than during expansion), rapid surface adsorption from the subphase, and a low equilibrium surface tension of about 23–25 mN/m. In sharp contrast to lung surfactant, the material from ET was not surface active. ETLF had a surface tension below 40 mN/m and low hysteresis during the pulsation of the bubble. There was an instantaneous lowering of surface tension after the formation of the bubble, with an equilibrium surface tension of about 44 mN/m.

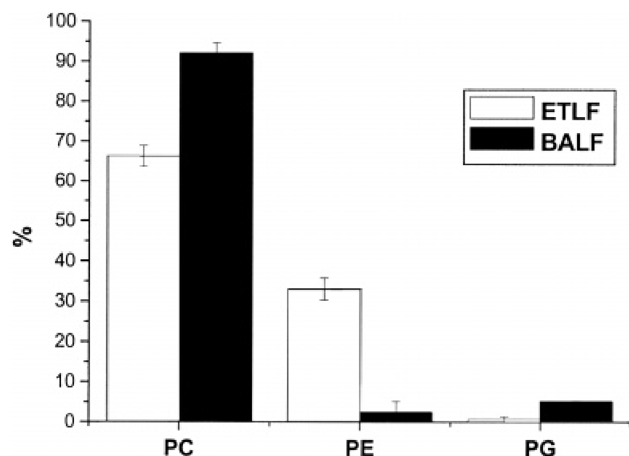

Fig. 7. Composition of phospholipid classes in ETLF and BALF. The determined phospholipids analyzed include PC, PE, and PG. Values represent means ± SD.

DISCUSSION

This is the first detailed report of the molecular species composition of phospholipid species composition of ETLF, and demonstrates that the phospholipid composition of putative ET surfactant is different from that of lung surfactant. The proportion of PC species containing unsaturated fatty acids was distinctly greater in porcine ETLF compared with porcine BALF. Conversely, the fractional concentration of PC16:0/16:0 in ETLF (10 mol%) was much lower than in BALF (55 mol%). There is a considerable discrepancy between these results and previous reports suggesting that the PC compositions of ETLF and BALF are similar (9, 10, 12). It is unlikely that this difference was due to any significant imprecision in the ESI-MS analysis of phospholipid compositions, and we have previously shown that ESI-MS provides an analysis of human BALF PC comparable to that of an established high performance liquid chromatography technique (20). Moreover, the compositions of phospholipid molecular species from porcine BALF reported here are typical of previous analyses of other animal species (19). One possible explanation is that the oxidative technique used previously to measure disaturated PC in ETLF phospholipid is prone to error. This method measures the PC residue resulting

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TABLE 1. Surface tension of the lipid-protein complex preparations and surfactant fractions recovered from ET lavage fluid and bronchoalveolar lavage fluid[a]

	ETLF		BALF	
	0.25	1.0	0.25	1.0
Surface tension during pulsation of the bubble at a given bubble radius				
Pulsation for 15–20 s				
0.40 mm	36 ± 5	33 ± 7	18 ± 4	2 ± 5
0.475 mm expanding	48 ± 6	49 ± 1	43 ± 5	39 ± 6
0.55 mm	53 ± 5	54 ± 2	52 ± 8	54 ± 1
0.475 mm contracting	45 ± 4	45 ± 2	23 ± 12	10 ± 1
Pulsation for 3 min				
0.40 mm	36 ± 3	32 ± 7	5 ± 3	1 ± 0
0.475 mm expanding	50 ± 4	49 ± 2	38 ± 0	42 ± 2
0.55 mm	53 ± 3	54 ± 2	46 ± 2	51 ± 3
0.475 mm contracting	46 ± 3	46 ± 4	14 ± 4	5 ± 1
Surface tension after the formation of a nonpulsating bubble				
1 s	54 ± 6	49 ± 1	53 ± 2	29 ± 0
10 s	47 ± 3	47 ± 3	41 ± 3	24 ± 1
5 min	44 ± 3	44 ± 3	24 ± 0	23 ± 0

[a] Representative surface tension measurements are given as means ± SE (mN/m). The measurements were done for both the crude lipid-protein complex recovered from ETLF and BALF and the fractions recovered from the sucrose density gradient. No detectable differences in surface tensions between the different preparations were observed.

from destructive oxidation of unsaturated species, using OsO_4, has provided comparable analyses of human BALF, and has proved valuable in studies of lung surfactant, which has a high component of PC16:0/16:0. However, as the technique measures all disaturated and some monounsaturated phospholipid species in addition to PC16:0/16:0, its use can result in erroneously high estimates of disaturated PC in samples with a low content of PC16:0/16:0 (21).

Because phospholipids were found in ETLF, it is possible that a specialized organelle or cell type may be involved in the secretion of a surfactant-like material, albeit with a different composition and potential function from lung surfactant. For instance, analogous to alveolar surfactant, it has been proposed that the phospholipid-containing material in the epithelial lining of ET may be derived from the granular organelles of cuboidal ET cells (15). Although our results provide no direct evidence to evaluate such a concept, the identical phospholipid molecular species compositions of ETLF and ET epithelial cells indicate that any such specialized cell for secreted ETLF phospholipid must have a phospholipid composition similar to that of the remainder of the ET epithelial cells. Clearly, further evidence of the origin of ETLF phospholipids is required.

Unlike the tidal gas delivery and gas exchange function of the lung, gas is only intermittently delivered into the middle ear cavity via the ET. Consequently, the ET must be virtually, if not totally, closed much of the time. Intermittent swallowing, rather than regular breathing movements, accomplishes the ventilatory function by separating the opposing mucous surfaces of the cartilaginous ET. An "antiglue" function of ET phospholipid secretion could therefore be more critical for the proper functioning of the tube than any ability to reduce surface tension. As the lumen of the ET tube is wide compared with both alveoli and the respiratory airways, there is no fundamental rationale to argue a role for surface tension reduction in its function. Moreover, in contrast to lung inflation as a consequence of negative pressure generated within the thoracic cavity, ET opening and closing is a direct consequence of muscular action. Such an antiglue function has been proposed as the major function of surfactant in the anuran lung, which lacks alveoli. Interestingly, in the absence of a requirement for generating low surface tension values, surfactant isolated from *Xenopus laevis* lung also had a low content of disaturated PC (22).

As expected on the basis of the molecular species compositions of its phospholipid, ETLF was not surface active. In measurements with a pulsating bubble surfactometer, surface tension revealed only small changes, and surface adsorption was instantaneous. This suggests that the aggregates from ETLF had detergent-like surface properties. In contrast, material from BALF characteristically attained low surface tension on compression of the surface and adsorbed from the subphase to the interface, attaining low equilibrium surface tension.

The relatively high surface tension of material from ET is consistent with the notion that the ET remains closed unless the pharyngeal muscles force it to open. On the other hand, it is anticipated that the detergent-like properties of ETLF maintain the closure of the tube. Furthermore, detergent-like material would decrease the shear forces generated on epithelial surfaces and facilitate the opening of the tube during swallowing. Both continuous patency and persistent closure of ET have been associated with middle ear infections (23). The previous suggestion that material from the ET has surface properties similar to lung surfactant (7, 9) was not confirmed. However, the previous methods used for the measurement of surface activity differed from that presented here, and neither study made any direct comparison with lung surfactant.

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Lung surfactant has been proposed as a treatment of acute middle ear infections. White, Hermansson, and Svinhufvud (24) reported that surfactant reduced the pressure required to force the ET open in rats with ear infection. Venkatayan et al. (25) found a similar effect with intranasal aerosolized synthetic surfactant. The duration of infection was also decreased by the surfactant treatment. Permanent closure of the ET is frequently evident in middle ear infection. Surface-active material is likely to be helpful in opening up the ET. In contrast to peripheral lung, where excess liquid may be removed across the alveolar epithelium, the liquid balance of the middle ear is critically dependent on the function of the ET. To prevent pressure-passive middle ear effusion, equilibration of pressures is necessary and the ciliary function complements the removal of secretions and excess liquid.

We conclude that the phospholipid-rich material in the epithelial lining of the ET is different from the lung surfactant present in the alveolar lining. The minimum surface tension, attained with a pulsating bubble surfactometer, was considerably higher than 10 dyn/cm. The fractional content of PC16:0/16:0, expressed as a molar percentage of total PC, was severalfold lower in ETLF compared with BALF. The phospholipid composition of ETLF is consistent with the observed lack of surface activity properties of ETLF secretions comparable to those described for alveolar surfactant. We propose, therefore, that detergent-like surface properties of ETLF act to support ET function by facilitating the intermittent opening and closure of the ET lumen.

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