Molecular Species Compositions of Lung and Pancreas Phospholipids in the cftr$^{\text{tm1HGU/tm1HGU}}$ Cystic Fibrosis Mouse

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ABSTRACT

Fatty acid analysis of phospholipid compositions of lung and pancreas cells from a cystic fibrosis transmembrane regulator (CFTR) negative mouse (cftr$^{-/-}$) suggested that a decreased concentration of docosahexaenoate ($22:6_{n-3}$) and increased arachidonate ($20:4_{n-6}$) may be related to the disease process in cystic fibrosis (CF). Consequently, we have determined compositions of the major phospholipids of lung, pancreas, liver, and plasma from a different mouse model of CF, the *cftr*$^{\text{tm1HGU/tm1HGU}}$ mouse, compared with ZTM:MF-1 control mice. Electrospray ionization mass spectrometry permitted the quantification of all of the individual molecular species of phosphatidylcholine (PtdCho), phosphatidylethanolamine (PtdEtn), phosphatidylglycerol (PtdGly), phosphatidylserine (PtdSer), and phosphatidylinositol (PtdIns). There was no deficiency of $22:6_{n-3}$ in any phospholipid class from lung, pancreas, or liver from mice with the *cftr*$^{\text{tm1HGU/tm1HGU}}$. Instead, the concentration of $20:4_{n-6}$ was significantly decreased in plasma PtdCho species and in pancreas and lung species of PtdEtn, PtdSer, and PtdIns. These results demonstrate the variability of membrane phospholipid compositions in different mouse models of CF and suggest that in *cftr*$^{\text{tm1HGU/tm1HGU}}$ mice, the apparent deficiency was of 20:4n-6– rather than of 22:6n-3–containing phospholipid spe-

cies. They highlight a need for detailed phospholipid molecular species analysis of cells expressing mutant CFTR from children with CF before the therapeutic effects of administering high doses of $22:6_{n-3}$–containing oils to children with CF can be fully evaluated. (*Pediatr Res* **53: 447–454, 2003**)

Abbreviations

20:4$_{n-6}$, arachidonate
22:6$_{n-3}$, docosahexaenoate
18:2$_{n-6}$, linoleate
18:0, stearate
16:0, palmitate
CF, cystic fibrosis
CFTR, cystic fibrosis transmembrane regulator
ESI-MS, electrospray ionization mass spectrometry
EFA, essential fatty acid
PtdCho, phosphatidylcholine
PtdEtn, phosphatidylethanolamine
PtdGly, phosphatidylglycerol
PtdIns, phosphatidylinositol
PtdSer, phosphatidylserine

The possibility that membrane phospholipid compositions might be altered in patients with cystic fibrosis (CF) has been suggested over many years. For instance, low concentrations of the essential fatty acid (EFA) linoleate ($18:2_{n-6}$) have been reported in phospholipid, triacylglycerol, esterified, and non-esterified cholesterol fractions of plasma (1, 2), in red blood cell membrane phospholipid (3, 4), and in total lipid extracts of

liver, lungs, and heart muscle from patients with CF (1). Although disease-related impairments of lipid digestion and absorption have largely been corrected by introduction of diets with high energy and fat contents combined with pancreatic lipase supplementation, most evidence suggests that a degree of EFA deficiency remains in CF. In this context, the decreased concentration of $18:2_{n-6}$ not only in patients with CF and pancreatic insufficiency but also in those who had pancreatic sufficiency (5) and in people who were heterozygotic for the CF transmembrane regulator (CFTR) mutation (2) is particularly relevant.

A potential role for CFTR function to regulate membrane phospholipid composition is suggested by a number of studies. Inhibition of chloride conductance channels in cultured human

epithelial cells decreased fatty acid incorporation into membrane phospholipid (6), with the greatest effect observed for $18:2_{n-6}$. Recent evidence suggests that this response may be mediated by CFTR function, as $18:2_{n-6}$ incorporation into phosphatidylcholine (PtdCho) was increased in epithelial cells expressing the normal CFTR gene and decreased in cells expressing ΔF_{508} CFTR (7). Clinically, uptake of $18:2_{n-6}$ was decreased in red blood cells from patients with CF (8), and the rate of PtdCho synthesis was increased in platelets and fibroblasts from patients with CF with no overall alteration to PtdCho concentration (9). In animal models of CF, lung Ptd-Cho metabolism is altered in the *cftr*[tm1HGU/tm1HGU] CF mouse, which displays increased alveolar fractions of newly synthesized PtdCho (10).

In this context, the report that the *cftr*^{−/−} null mouse was unable to maintain normal concentrations of the polyunsaturated fatty acid docosahexaenoate ($22:6_{n-3}$) in membrane phospholipids of cells from lung and pancreas was particularly intriguing (11). This observation raised the possibility that dietary supplementation of patients with CF and $22:6_{n-3}$ might both redress a putative imbalance between $22:6_{n-3}$ and the proinflammatory fatty acid arachidonate ($20:4_{n-6}$) in cftr-expressing cells and lead to clinical benefit. Phospholipid fatty acid analysis of plasma and erythrocytes from children with CF have varied widely, possible as a result of nutritional factors, but have generally reported decreased concentrations of all polyunsaturated fatty acids rather than a specific deficiency of $22:6_{n-3}$ (1–5).

Consequently, we evaluated the role of aberrant CFTR function on membrane phospholipid composition using a different mouse model of CF. The *cftr*[tm1HGU/tm1HGU] mouse has a low residual penetration of CFTR protein into epithelial cell membranes comparable to that reported for $\Delta F508$ CFTR, the most common mutation in the white population with CF (12–14). Consequently, the *cftr*[tm1HGU/tm1HGU] mouse generally has less severe lung pathology but still exhibits defective CFTR function. We reasoned that any membrane phospholipid abnormality that was a primary consequence of dysfunctional CFTR should be apparent in the *cftr*[tm1HGU/tm1HGU] mouse, but not any that were secondary to either impaired ion transport or to pathologic responses.

In addition most previous compositional studies have relied on measurement of total phospholipid fatty acids, ignoring the complexity of the structure of intact phospholipid molecules. The biologic functions of phospholipids are determined by their composition in terms of individual molecular species, determined by polar head groups and the combinations of fatty acyl groups attached at the *sn*-1 and *sn*-2 positions of the glycerol backbone (15). Consequently, interpretation of the physiologic implications of any modulation to overall phospholipid fatty acid composition requires more detailed analysis as individual molecular species. In addition, such detailed analysis is capable of identifying more easily the relatively subtle changes to membrane composition that may result from dysfunctional CFTR protein, especially if these are restricted to a subset of phospholipid classes. Recent developments in instrument design have established electrospray ionization mass spectrometry (ESI-MS) as a sensitive and versatile technique for the detailed analysis of molecular species compositions of the phospholipids commonly found in biologic membranes (16–18). ESI-MS enables phospholipid species to be analyzed with unrivaled detail, sensitivity, and rapidity. In this study, we used ESI-MS to quantify the tissue compositions of phospholipid molecular species in lipid extracts of lung, pancreas, liver, and plasma from *cftr*[tm1HGU/tm1HGU] and control mice.

METHODS

Specific pathogen-free *cftr*[tm1HGU/tm1HGU] mice supplied from Edinburgh and ZTM:MF-1 control mice were bred at the animal house of Hanover Medical School. Animals were kept in a flexible film isolator under specific pathogen-free conditions as described previously (19) and were free from murine pathogens. They were fed with an irradiated (5 Mrad) standard diet (Altromin 1314) and given autoclaved water (134°C, 50 min) to drink. At 8–9 wk old, four animals (two males, two females) of each strain were anesthetized, and heparinized blood was obtained from the vena cava inferior. Liver, lung, and pancreas were removed and frozen immediately in liquid nitrogen. Blood was kept on ice until centrifuged to separate blood cells from plasma, which was then frozen at −20°C. One hundred to 200 mg of liver, lung, and pancreas and 200 μL of plasma were extracted according to Bligh and Dyer (20) after PC14:0/14:0 and PE14:0/14:0 were added as internal standards. Samples were dried down under a stream of nitrogen and stored at −20°C until analyzed.

All ESI-MS analysis was performed on a triple-quadruple tandem mass spectrometer (Quattro *Ultima*; Micromass, Manchester, England) equipped with an ESI interface (16–18). Total phospholipid extracts were dissolved in methanol:chloroform:water (7:2:1, v:v) for single-stage and tandem MS analysis of PtdCho, PtdIns, PtdSer, PtdGro, and PtdEtn. Plasma samples were dissolved in 5 μL of solvent and analyzed by nanospray ESI-MS, whereas tissue samples were dissolved in 200 μL of solvent and infused directly into the mass spectrometer *via* a syringe pump (Model 11; Harvard Apparatus, Holliston, MA, U.S.A.) at a flow rate of 5 μL/min. Dry heated nitrogen was used as both the cone and desolvation gas (70 and 450/h, respectively), and dry argon was used as the collision gas (3.5×10^{-3} mbar). All data were recorded at mass resolution, as a signal average of 10–20 scans/collection, with a scan time of 2.5–12 s.

PtdCho species were detected by positive ionization, whereas PtdIns and other acidic phospholipids were preferentially detected using negative ionization. After fragmentation with argon gas, PtdCho molecules produced a fragment with $m/z = 184$, corresponding to the protonated phosphocholine head group, and precursor scans of the m/z 184 moiety provided diagnostic determination of PtdCho. Collision gas-induced fragmentation of PtdIns species generated a common dehydrated inositol phosphate fragment with $m/z = -241$, and precursor scans of this m/z 241 moiety provided diagnostic determination of PtsIns. Identities of PtdSer species were confirmed by constant neutral loss scans of serine ($m/z = -87$), PtdEtn species by neutral loss scans of phosphoethano-
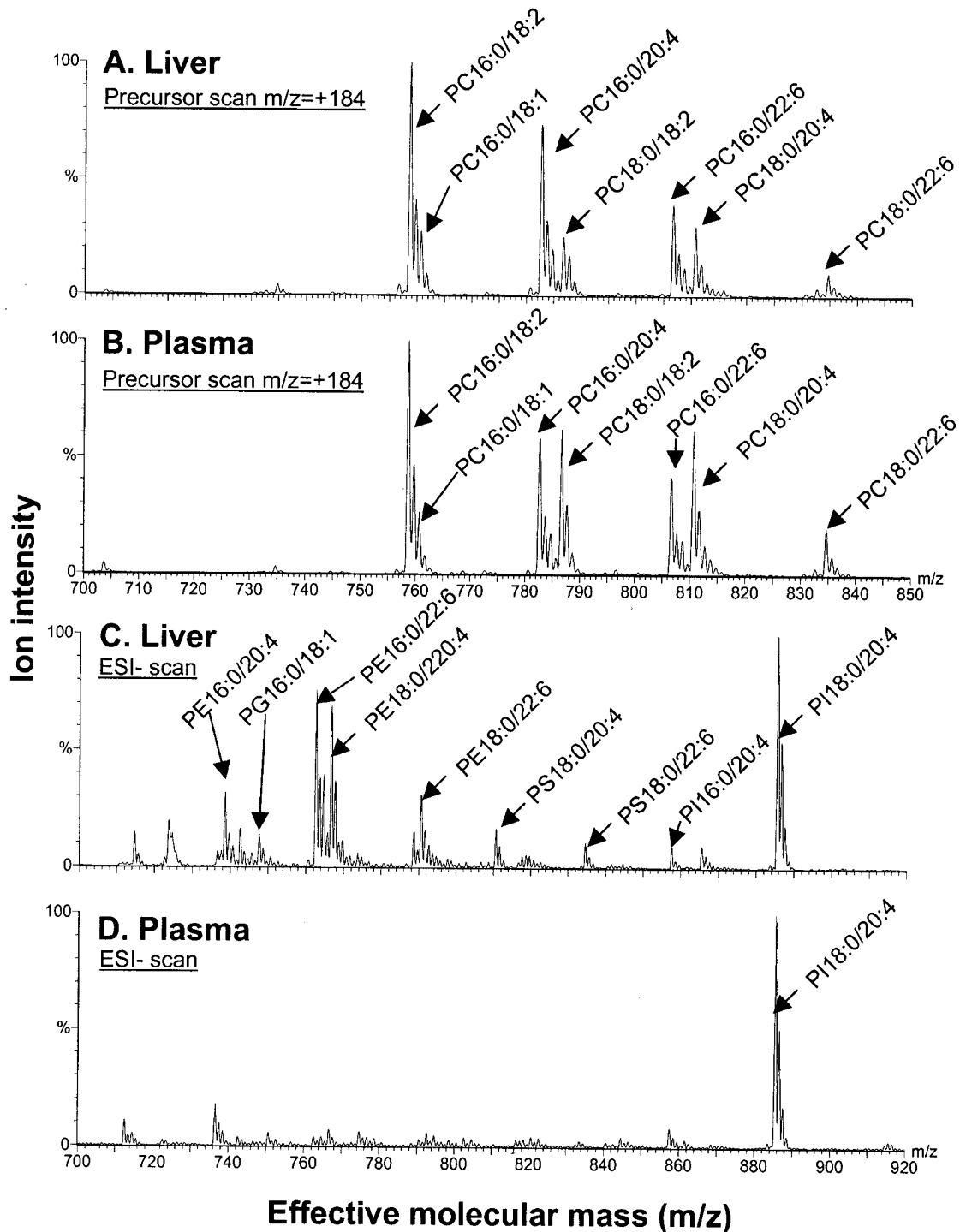
Figure 1. Typical mass spectrometry analysis of phospholipid compositions of liver and plasma from the ZTM:MF-1 mouse. Total lipids were extracted from 100 mg of liver, or 200 μL of plasma and aliquots were analyzed by a variety of ESI-MS methodologies. (*A* and *B*) PtdCho compositions from liver and plasma, respectively, detected as tandem MS/MS precursor scans of $m/z = 184$ in positive ionization. (*C* and *D*) ESI$^-$ scans of liver and plasma, respectively, in negative ionization, which detects a combination of PtdEtn, PtdSer, PtdGly, and PtdIns.

lamine ($m/z = 141$), and PtdGro species by precursor scans of glycerophosphate ($m/z = -153$). Data were acquired and processed using MassLynx NT software. After conversion to centroid format according to area, correction for ^{13}C isotope effects and for reduced response with increasing m/z values, the phospholipid species in each class of phospholipid were expressed as percentages of their respective totals present in the sample. The predominant molecular species present for each ion peak resolved was determined by analysis of fatty acyl fragments generated by collision gas-induced fragmentation under negative ionization. PtdCho was quantified from the parents scan of $m/z = 184$ under positive ionization mode, whereas all other phospholipid classes were quantified from the negative ionization scan.

All data were analyzed by the Mann-Whitney U test. $P < 0.05$ was regarded as significant.

RESULTS

The $cftr^{tm1HGU/tm1HGU}$ mutation caused a marked decrease in the growth of transgenic CF mouse (body wt 24.1 ± 2.0 g) compared with the ZTM:MF-1 control mouse (34.2 ± 2.5 g, $p > 0.05$). To obtain an overall view of nutritional status, we first analyzed hepatic phospholipid compositions, as the liver has a key position in the overall integration of lipid metabolism, and synthesis and secretion of lipoprotein PtdCho is a major contributor to the directed supply of essential fatty acids to extrahepatic tissues (21). Hepatocytes do not express CFTR, and consequently we argued that any alterations to liver phospholipid composition in CF mice were probably secondary to impaired lipid digestion and absorption from the intestine rather than directly to the gene defect. In control mice, PtdCho was composed almost completely of species containing the essential fatty acids $18:2_{n-6}$, $20:4_{n-6}$, and $22:6_{n-3}$ at the *sn-2* position of the molecule (Fig. 1*A*). Palmitate (16:0) acid was predominant at the *sn-1* position, with PC16:0/18:2 (31.3 ± 4.9 mol%) being the main component. Liver PtdEtn (Fig. 1*C*) was equally enriched in polyunsaturated fatty acids but with a very different distribution from that of PtdCho. PE16:0/22:6 (26.0 ± 2.0 mol%) and PE18:0/20:4 (23.9 ± 1.6 mol%) were predominant, and together with PE16:0/20:4 and 18:0/22:6 these species contributed 73.1 ± 0.4 mol% of total PtdEtn. In contrast, PtdGly, although present only at low concentration compared with lung (Fig. 1*C*), was enriched in PG16:0/18:1 (32.1 ± 2.4 mol%) and the three $18:2_{n-6}$-containing species PG16:0/18:2 (13.6 ± 1.8 mol%), PG18:0/18:2 (16.3 ± 2.7 mol%), and PG18:1/18:2 (8.9 ± 1.1 mol%). PtdSer comprised predominantly PS18:0/18:1 (27.3 ± 1.9 mol%), PS18:0/20:4 (28.3 ± 5.5 mol %), and PS18:0/22:6 (18.9 ± 2.7 mol%), whereas PtdIns was composed nearly completely of PI18:0/20:4 (87.6 ± 2.5 mol%; Fig. 1*C*).

The $cftr^{tm1HGU/tm1HGU}$ mutation was associated with relatively few changes to the molecular species composition of liver phospholipid. Concentrations of a selection of $20:4_{n-6}$-containing PtdCho and PtdEtn species were decreased in CF mice compared with control mice (PC16:0/20:4 15.9 ± 1.2 *versus* 23.2 ± 1.4 mol%, PE16:0/20:4 10.2 ± 0.5 *versus* 12.2 ± 0.9 mol%, PE18:1/20:4 6.8 ± 0.4 *versus* 9.3 ± 0.8 mol%; $p < 0.05$), whereas concentrations of PG18:0/18:2 (23.4 ± 2.8 *versus* 16.1 ± 2.7 mol%; $p < 0.05$) and PE18:0/22:6 (14.8 ± 0.3 *versus* 11.1 ± 0.9 mol%; $p < 0.05$) were increased.

The pattern of plasma phospholipid is determined largely by the specificity of hepatic lipoprotein secretion. Consequently, the compositions in control mice of plasma PtdCho (Fig. 1*B*) and PtdIns (Fig. 1*D*), which are the main circulating phospholipids, was very similar to those measured in liver, with PC16:0/18:2 and PI18:0/20:4 again being the predominant species. For plasma, however, there were considerable differences of plasma phospholipid compositions between CF and control mice. The concentration of total plasma PtdCho was decreased in CF mice (890 ± 173 nmol/mL) compared with control mice (1243 ± 139; $p < 0.05$; Table 1), with signifi-

Table 1. *Plasma concentrations (nmol/mL) of the major molecular species of PtdCho in ZTM:MF-1 and* cftr$^{tm1HGU/tm1HGU}$ *mice*

Molecular species	ZTM:MF-1 ($n = 4$)	cftr$^{tm1HGU/tm1HGU}$ ($n = 4$)
PC16:0/18:2	395 ± 104	305 ± 73
PC16:0/18:1	54 ± 19	59 ± 13
PC16:0/20:4	207 ± 27	104 ± 26*
PC18:0/18:2	172 ± 28	141 ± 27
PC16:0/22:6	138 ± 10	95 ± 25*
PC18:0/20:4	144 ± 27	85 ± 24*
PC18:0/22:6	47 ± 12	41 ± 12
Total PtdCho	1243 ± 139	890 ± 173*

The total lipid extract of 200 μL of plasma was analysed for PtdCho by ESI-MS. Data are mean values and SD. Total PtdCho includes all other molecular species in addition to those reported individually. * $p < 0.05$.

cantly decreased individual contributions from PC16:0/20:4, PC18:0/20:4, and PC16:0/22:6. The restricted nature of these changes was emphasized by the observation that the plasma concentration of the other PtdCho species containing docosahexaenoate, PC18:0/22:6, was the same in both CF and control mice.

As lung and pancreas both express CFTR and are severely affected by the defect of this gene in CF, we reasoned that the phospholipid composition of these tissues might exhibit the greatest alteration in the CF mice. First, however, ESI-MS analysis showed clearly that lung and pancreas maintained very different phospholipid compositions *in vivo* (Fig. 2). As expected, lung phospholipids reflected strongly the contribution of pulmonary surfactant-associated lipids, with high concentrations of PC16:0/16:0 (35.2 ± 3.1 mol%; Fig. 2*A*) and the PtdGly species PG16:0/18:2 (29.7 ± 0.9 mol%) and PG16:0/18:1 (19.6 ± 0.3 mol%; Fig. 2*C*). The major components of lung PtdSer and PtdIns were similar to liver and were PS18:0/18:1 (19.4 ± 0.2 mol%), PS18:0/20:4 (21.3 ± 0.6 mol%), and PI18:0/20:4 (61.8 ± 2.5 mol%) (Fig. 2*C*). Finally, lung PtdEtn comprised largely alkenylacyl (plasmalogen) species with PE16:0alk/20:4 (15.8 ± 0.4 mol%) and PE18:0alk/20:4 (13.6 ± 0.8 mol%) being most abundant (Fig. 2*C*).

In contrast to lung, pancreas PtdCho contained negligible amounts of disaturated species, elevated concentrations of arachidonoyl-containing species (35.9 ± 2.5 mol%), and virtually no docosahexaenoyl-containing species (Fig. 2*B*). Pancreatic PtdSer consisted mainly of PS18:0/20:4 (35.8 ± 3.5 mol%) and PS18:0/18:2 (36.2 ± 1.5 mol%). PtdIns and PtdEtn were the only classes of pancreatic phospholipid whose composition closely resembled that of lung, both being dominated by arachidonoyl-containing species (Fig. 2*D*).

Whereas CF mice displayed altered compositions of some lung and pancreatic phospholipids containing $20:4_{n-6}$ and $22:6_{n-3}$, the differences compared with control mice were relatively slight. Consequently, for distinguishing any consistent effect of the gene defect on membrane phospholipids, the fractional concentrations of all $20:4_{n-6}$ and $22:6_{n-3}$-containing phospholipid species in lung and pancreas are detailed in Figs. 3 and 4, respectively. Results are expressed as a percentage of the molar concentration of all of the molecular species resolved in that phospholipid class. These comparisons show clearly that there was no generalized deficiency of molecular species containing $22:6_{n-3}$ for any phospholipid class in either lung or
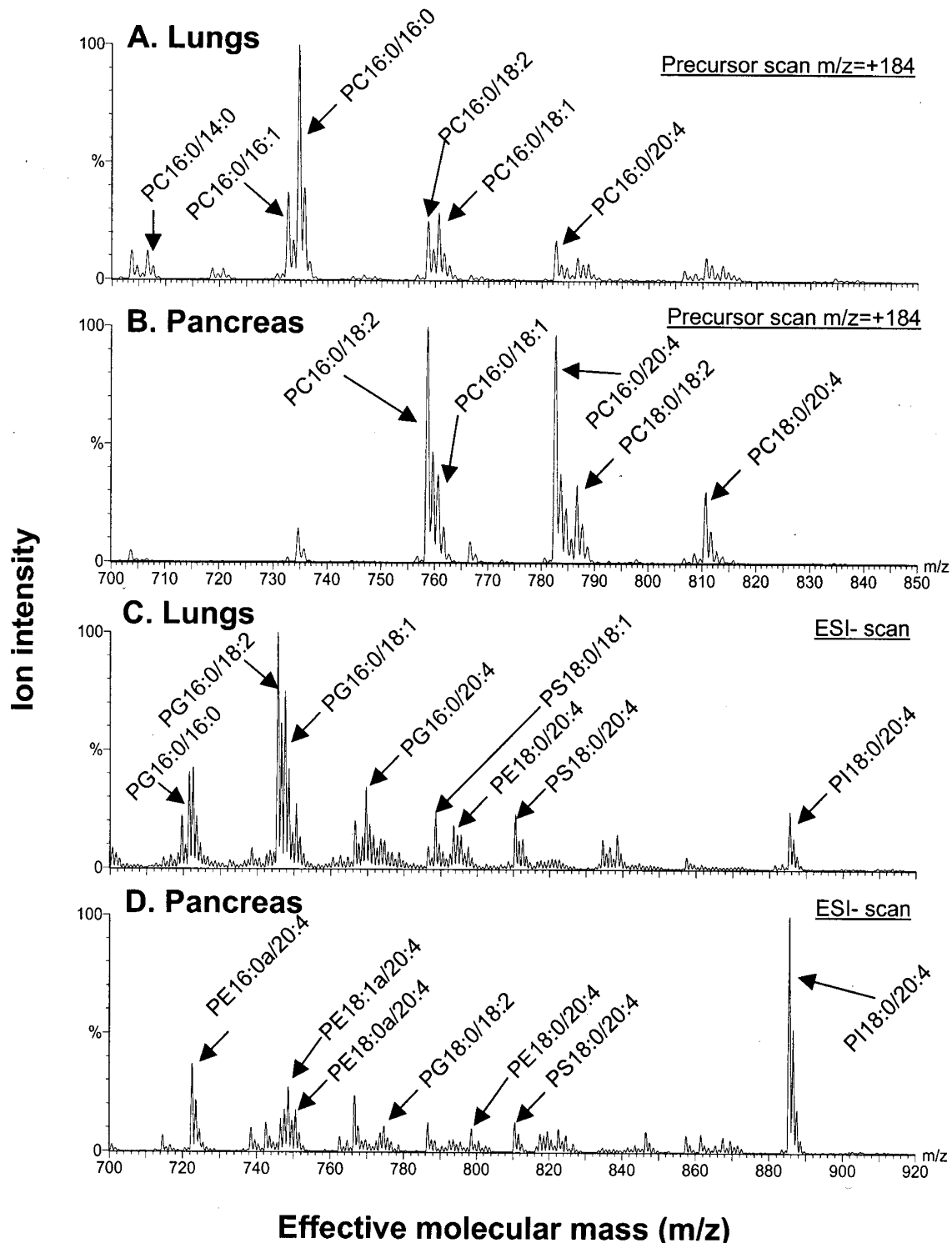
Figure 2. Typical mass spectrometry analysis of phospholipid compositions of lung and pancreas in ZTM:MF-1 mice. Total lipids were extracted from 100–150 mg of lungs and pancreas, and aliquots were analyzed by a variety of ESI-MS methods. (*A* and *B*) PtdCho compositions from lungs and pancreas, respectively, detected as tandem MS/MS precursor scans of $m/z = 184$ in positive ionization. (*C* and *D*) ESI$^-$ scans of lungs and pancreas, respectively, in negative ionization, which detects a combination of PtdEtn, PtdSer, PtdGly, and PtdIns.

pancreas from CF mice. For lung, although there were significant differences between CF and control mice in the fractional concentrations of phospholipids containing either $20:4_{n-6}$ (Fig. 3*A*) or $22:6_{n-3}$ (Fig. 3*B*), these were of small magnitude and were inconsistent between phospholipid classes. For instance, concentrations

of PE 16:0/20:4, PS18:0/20:4, and PI16:0/20:4 all were increased, but values for PG16:0/20:4 (not shown) and PI18:0/20:4 were lower in lungs from CF compared with control mice.

In contrast to lungs, CF mice had alterations of greater magnitude to pancreatic phospholipids, and these changes were
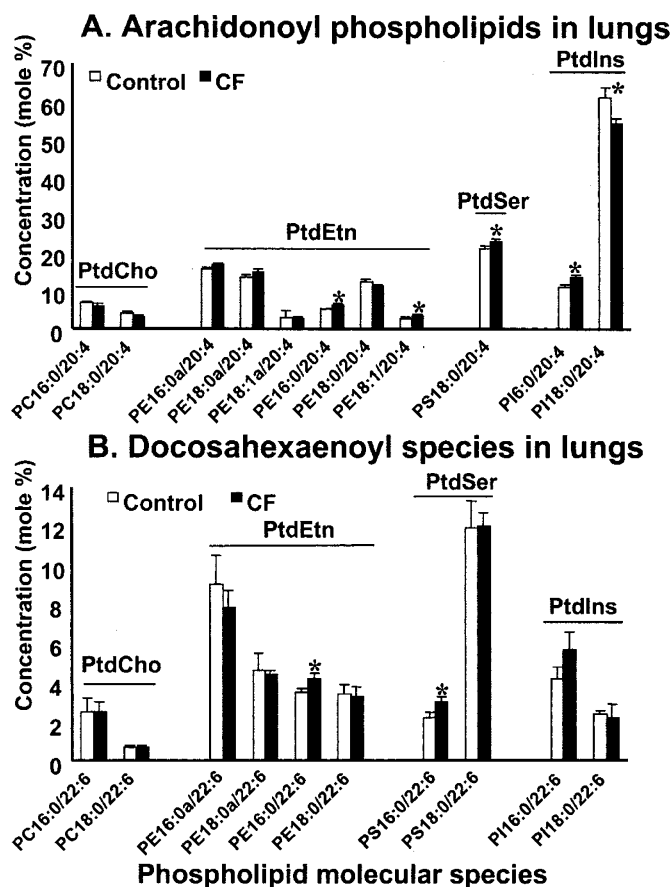
Figure 3. Polyunsaturated phospholipid molecular species in mouse lungs. (*A*) Arachidonoyl-containing phospholipid species. (*B*) Docosahexaenoyl-containing phospholipid species. PtdCho was quantified from precursor scans of $m/z = 184$ in positive ionization mode, whereas all other phospholipid classes were quantified from the ESI$^-$ scan. Arachidonoyl- and docosahexaenoyl-containing species of each class were expressed as a percentage of the total amount of that phospholipid class present in the sample. Data are mean values and SD, $n = 4$.

Figure 4. Polyunsaturated phospholipid molecular species in mouse pancreas. (*A*) Arachidonoyl-containing phospholipid species (*B*) Docosahexaenoyl-containing phospholipid species. PtdCho was quantified from precursor scans of $m/z = 184$ in positive ionization mode, whereas all other phospholipid classes were quantified from the ESI$^-$ scan. Arachidonoyl- and docosahexaenoyl-containing species of each class were expressed as a percentage of the total amount of that phospholipid class present in the sample. Data are mean values and SD, $n = 4$.

much more consistent between the range of phospholipid classes. Essentially, the significant compositional differences found reflected decreased concentrations of molecular species containing $20:4_{n-6}$ (Fig. 4*A*) and increased concentrations of $22:6_{n-3}$-containing species (Fig. 4*B*). These differences to pancreatic compositions were most striking for acidic phospholipids containing stearate (18:0) at the *sn*-1 position. In CF compared with control mice, concentrations of PS18:0/20:4 and PI18:0/20:4 were respectively 17.8 ± 3.6 *versus* 35.9 ± 3.4 mol% and 79.0 ± 1.9 *versus* 85.7 ± 0.8 mol% (Fig. 4*A*). Corresponding values for PS18:0/22:6 and PI18:022:6 were 8.3 ± 1.3 *versus* 3.4 ± 0.9 mol% and 0.7 ± 0.0 and 0.2 ± 0.2 mol% for CF and control mice, respectively.

Finally, concentrations of total PtdCho and PtdEtn were calculated for both lungs and pancreas as the aggregates of concentrations of individual molecular species, expressed as μmol/g wet wt of tissue. Neither PtdCho nor PtdEtn concentrations of either lungs or pancreas were significantly altered in the CF mice (Table 2).
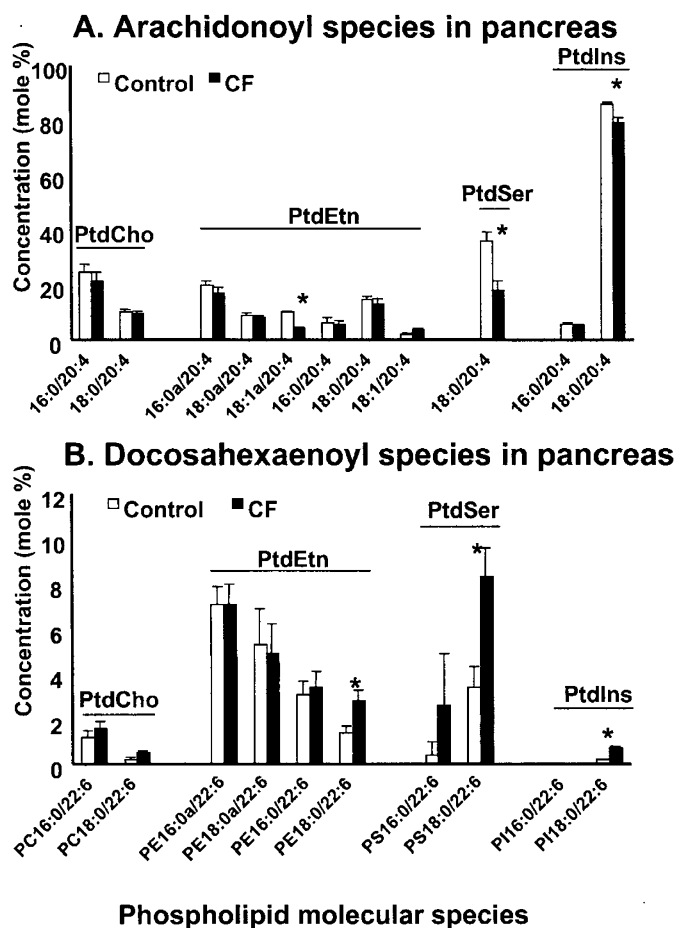
DISCUSSION

The results presented in this article compare tissue phospholipid compositions between the *cftr*[tm1HGU1/tmHGU] mouse and a ZTM:MF-1 control mouse, which is an outbred line and the background strain for our CF mouse model. There was no systematic decreased concentration of $22:6_{n-3}$-containing phospholipid species in either the lungs or the pancreas as a consequence of the gene defect in CF. Rather, instead of being decreased, concentrations of acidic phospholipids PtdSer and PtdIns containing $22:6_{n-3}$ were increased in the CF mouse, especially in the pancreas. These changes were at the expense of corresponding decreased concentrations of phospholipid species containing $20:4_{n-6}$. These results contrast with the reported decreased concentration of $22:6_{n-3}$ and increased concentration of $20:4_{n-6}$ in phospholipid from lung and pancreas cells isolated from the cftr$^{-/-}$ null mouse (11). Some of the discrepancy between our results and those of Freedman *et al.* (19) may be a consequence of the different extents of the gene defect but may also be due to variations inherent in different

Table 2. *Tissue concentrations of PtdCho and PtdEtn from lungs and pancreas of ZTM:MF-1 and* cftr*[tm1HGU/tm1HGU] mice*

	Lung		Pancreas	
	ZTM:MF-1 (n = 3/4)	cftr[tm1HGU/tm1HGU] (n = 4)	ZTM:MF-1 (n = 4)	cftr[tm1HGU/tm1HGU] (n = 3)
PtdCho concentration (nmol/100 mg tissue)	887 ± 151	1055 ± 143	761 ± 88	987 ± 98
PtdEtn concentration (nmol/100 mg tissue)	396 ± 23	441 ± 30	744 ± 99	818 ± 108

The total lipid extract of 100–150 mg of tissue was analyzed for PtdCho and PtdEtn by ESI-MS. Data are mean values ± SD.

strains of mice. However, although we have previously demonstrated that the $22:6_{n-3}$ content of PtdCho from lungs of different strains of control mice can vary considerably, the magnitude of such variation is inadequate to explain the discrepancy reported here. One other factor is also the difference in analytical methods used by the two studies. Measurement of fatty acid compositions of total phospholipid extracts provides an estimate of the total amount of $22:6_{n-3}$ in the sample, whereas ESI-MS measures compositions of intact individual molecular species of each phospholipid class present in tissue extracts. This distinction is important, as biologic functions of membranes are determined by the combination of the molecular species present, rather than simply by fatty acid composition. In practical terms for this study, any putative defect of $22:6_{n-3}$ would have had to be readily apparent by molecular species analysis to have any functional consequence, and this was certainly not the case for the cftr[tm1HGU1/tmHGU] mouse.

The Edinburgh cftr[tm1HGU/tm1HGU] mouse (12) develops a mild form of CF because of a low level of residual wild-type CFTR expression (13) but nevertheless has striking similarities to humans with CF. Mice with this mutation have an impaired capacity to clear *Staphylococcus aureus* and *Burkholderia cepacia* from their lungs (22) and have a low level of meconium ileus (13) and an increased alveolar surfactant phospholipid pool size in their lungs (19). However, although patients with CF have generally been reported to have lower levels of $18:2_{n-6}$ (23, 24), we found more pronounced alterations in the metabolic downstream product $20:4_{n-6}$. Any comparison of membrane phospholipid compositions between mice and people must recognize the lower $\Delta 5$ desaturase activity in human liver (25) leading generally to phospholipids from mouse compared with human tissues having higher relative concentrations of $20:4_{n-6}$-containing species. Consequently, the decreased concentrations of PC16:0/20:4 and PC18:0/20:4 in plasma from the cftr[tm1HGU/tm1HGU] mouse may reflect similar metabolic processes to those that cause the decreased concentration of $18:2_{n-6}$ in plasma PtdCho both from patients with CF even after pancreatic lipase supplementation (5) and from CFTR heterozygotic subjects (2). In effect, the contribution of n-6 fatty acids to plasma PtdCho is similarly decreased for both CF mice and CF patients, albeit the $20:4_{n-6}$ chain elongation product for the mice and the $18:2_{n-6}$ dietary precursor for patients.

This decreased concentration of $20:4_{n-6}$-containing PtdCho species in plasma from the cftr[tm1HGU/tm1HGU] mouse may also be related to overall inadequate nutrition, predominantly impaired lipid digestion and absorption, and may reflect de-creased hepatic synthesis and secretion of lipoprotein PtdCho for the directed transport of EFA from liver to peripheral tissues (21). The relatively unchanged compositions of lung and pancreas phospholipids suggest that these tissues are to some extent protected from the extent of the any decreased hepatic EFA supply, possibly as a result of lower overall requirements in cftr[tm1HGU/tm1HGU] mice of lower body weight. However, there were relatively subtle but significant abnormalities of lung and pancreas phospholipid in the CF mice, and these all reflected decreased concentrations of $20:4_{n-6}$-containing species, especially in the acidic phospholipid fractions from pancreas.

Although defective CFTR function may directly alter membrane phospholipid composition, it is also possible that altered phospholipid structures may modulate the membrane localization and functional activity of CFTR protein. The most common mutation of the CFTR gene, Δ_{F508}, is transcribed normally, but there is ineffective insertion of the abnormal gene product into the plasma membrane. Treatment of Δ_{F508}-expressing cells in culture with membrane perturbing agents, including glycerol (26) and sodium butyrate (27), leads to increased localization and activity of the Δ_{F508} CFTR protein in the plasma membrane. It is possible that appropriate manipulation of epithelial cell membrane phospholipid compositions in patients with CF may exert a comparable increased expression of Δ_{F508} CFTR activity in the plasma membrane, but no direct evidence exists to support this possibility.

The very different changes to membrane phospholipid compositions of the two different mouse models of CF suggest that further clarification of any role of membrane alterations to the disease process will require studies of phospholipid composition, synthesis, and turnover in a comprehensive range of animals with different CFTR mutations and in appropriate defined strains of control mice. In addition, because of the significant differences in phospholipid compositions and metabolism between mice and people, detailed analysis of compositions of phospholipid molecular species from human cells expressing mutant CFTR is essential to evaluate the potential clinical relevance of any of the results found in mouse models of CF. Administration of $22:6_{n-3}$ to children with CF has great potential benefit as an anti-inflammatory therapy (28), but the possibility that this intervention may correct a basic defect of CFTR function requires more careful study both in mice and in patients with CF.

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