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Evidence of increased flux to n-6 docosapentaenoic acid in phospholipids of pancreas from cftr$^{-/-}$ knockout mice

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Abstract

An association has been reported between alterations in fatty acid metabolism and cystic fibrosis (CF). We hypothesized that these alterations are specific for a particular lipid component(s) and are the result of a specific metabolic defect. The different lipid classes were examined for fatty acid changes by using pancreatic homogenates and primary cultures of pancreatic acini from cftr$^{-/-}$ (CF) and wild-type mice. Lipid classes and phospholipids were separated by aminopropyl column chromatography and high-performance liquid chromatography, and fatty acid methyl esters were analyzed. The results indicate that in CF mice (1) linoleate was decreased in phospholipids but not in neutral lipids; (2) there was an increase in dihomo-γ-linolenate and in docosapentaenoate, the terminal fatty acid of the n-6 pathway, in total lipids and total phospholipids, but not in the neutral lipid class; and (3) the docosapentaenoate (n-6)/docosahexaenoate (n-3) ratio was significantly elevated in neutral phospholipids. This suggests an enhanced flux through the n-6 pathway beyond arachidonate. This study provides a more in-depth understanding of the fatty acid alterations found in CF, as reflected by the cftr$^{-/-}$ mouse model.

1. Introduction

Cystic fibrosis (CF) is the most prevalent lethal autosomal recessive disorder among Caucasians [1]. The typical patient experiences pancreatic insufficiency and recurrent pulmonary infections. These infections are accompanied by excessive inflammation in the respiratory system that can ultimately lead to progressive bronchiectasis, respiratory failure, and death [2]. The CF transmembrane regulator (CFTR) is a cyclic adenosine monophosphate–regulated chloride channel [3]. Mutations of the gene for CFTR are integral to the development of CF [3,4]. However, the links between impairment of CFTR function and many of the pathologic manifestations of CF remain unknown.

For several decades, an association has been made between alterations in fatty acid metabolism and the presence of CF. A report was published in 1977 showing abnormal fatty acid composition in the plasma of CF patients with pancreatic insufficiency [5]. It was presumed at that time that a decrease in fatty acid absorption was responsible for fatty acid changes found in the plasma of patients with CF. In 1985, Farrell et al [5] demonstrated that fatty acid composition was abnormal in both plasma and solid tissues in patients with CF, with the major changes being a decrease in linoleate (LA, 18:2 n-6) and an increase in palmitoleate (16:1 n-7) (see Fig. 1 for a

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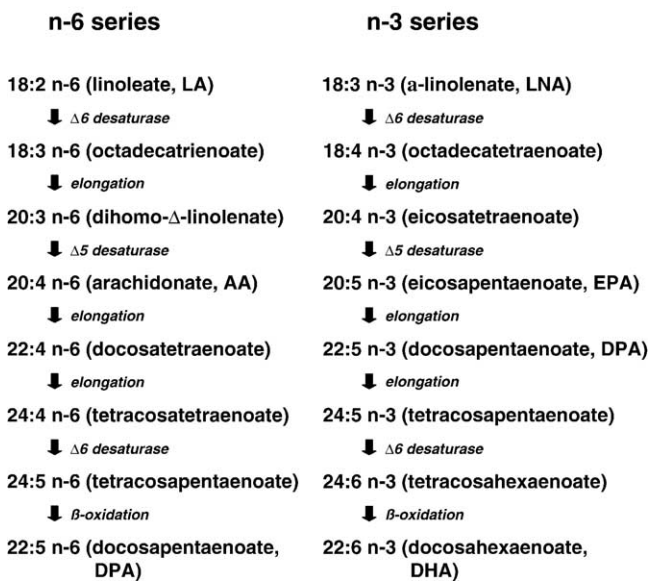

Fig. 1. Schematic representation of the 2 main pathways in the biosynthesis of polyunsaturated fatty acids. The n-6 and n-3 (omega-6 and omega-3, respectively) fatty acid families possess a final double bond 6 or 3 carbons, respectively, from the methyl end of the hydrocarbon chain. Fatty acid notation corresponds to "number of carbons:number of double bonds" followed by the series. The enzymes involved are shown in italic.

schematic description of n-6 and n-3 fatty acid biosynthetic series). Similar findings in solid tissues were demonstrated in fat, psoas muscle, heart muscle, liver, and lungs of 7 deceased patients with CF. Subsequently, a report by Carlstedt-Duke et al [6] showed that there was increased release of arachidonate (AA, 20:4 n-6) from the phospholipids of lymphocytes of patients with CF. This report gave rise to the concept that there may be increased conversion of LA to AA, with increased release of AA from phospholipids for enhanced eicosanoid formation. Christophe et al [7] demonstrated that in the serum of CF patients with pancreatic insufficiency, there was a decrease in LA and an increase in AA in phospholipids and cholesterol esters, and an increase in palmitoleate in cholesterol esters. In addition, there was significant uptake of n-3 fatty acids into the serum phospholipids and cholesterol esters in the patients with CF. This report enhanced the consistency of the finding of decreased AA and increased palmitoleate in patients with CF. Kang et al [8] made a connection between the CFTR gene product and fatty acid changes. In their study, they blocked the chloride channel with an inhibitor and demonstrated that there was decreased incorporation of LA into the phospholipids of membranes from nasal epithelial cells of healthy volunteers. Strandvik et al [9] reported results from a population of 110 patients with CF whose serum phospholipids were analyzed for fatty acid composition and compared with those of healthy controls. Like others, they also found a decrease in LA, but, in addition, observed a decrease in docosahexaenoate (DHA, 22:6 n-3). This decrease in DHA in animals and patients with CF has been subsequently found in a variety of other in vitro and in vivo settings [2,10]. They also demonstrated an increase in the ratio of AA to DHA. Bhura-Bandali and coworkers [11], using cultured cells with or without the CFTR gene product, reported a decrease in LA primarily within the phospholipids (phosphatidylcholine [PC], phosphatidylethanolamine [PE], phosphatidylinositol [PI]) and an increased flux of LA into triglycerides, possibly accounting for the LA decrease in the phospholipids. Present as well was increased conversion of LA to AA, which may have contributed to the consistently observed decrease in LA in the phospholipids. Freedman et al [2] demonstrated with $cftr^{-/-}$ knockout mice that there is a change in fatty acid composition, characterized by a decrease in DHA and an increase in AA, in 3 CFTR-expressing tissues: pancreas, ileum, and lung. The pathologic features of CF were corrected by supplementation with DHA. In a separate report, Freedman et al [10] demonstrated similar fatty acid changes in humans affected by CF.

The pathogenic significance of the fatty acid changes reported to date (some of which are found more often than others, notably the decrease in LA and DHA and the increase in palmitoleate and AA) remains to be explained. In this report, we hypothesized that the fatty acid defects found in CF are specific for a particular lipid component that esterifies fatty acids within the cell and are the result of a relevant pathogenic mechanism. Elucidation of the fatty acid changes within individual complex lipids containing fatty acids would contribute to a better understanding of the fatty acid changes in CF. For this purpose, we analyzed specific lipid components for the fatty acid changes found previously at the organ/tissue level, using pancreatic homogenates and primary cultures of pancreatic acinar cells from $cftr^{-/-}$ mice. This approach uses a CFTR-expressing tissue particularly affected in this disease, the pancreas.

2. Methods

2.1. Mouse colony breeding

An established breeding colony of C57BL/6J exon 10 CFTR ($cftr^{-/-}$) (CF) knockout mice and wild-type (WT) littermates was used for this study. Tail-clip samples of 14-day-old male mice were processed for genotype analysis as previously described [2]. All mice were weaned at 23 days of age and then placed on Peptamen diet (Nestle Clinical Nutrition, Deerfield, IL) and water ad libitum until 40 days of age. In a separate experiment, congenic C57BL/6J mice were used [12]. All experiments were carried out under protocols approved by the Beth Israel Deaconess Medical Center Institutional Animal Care and Use Committee and the Hospital for Sick Children Animal Care Committee.

2.2. Tissue manipulation

Mice were euthanized by carbon dioxide and the pancreas harvested, placed in phosphate-buffered saline,

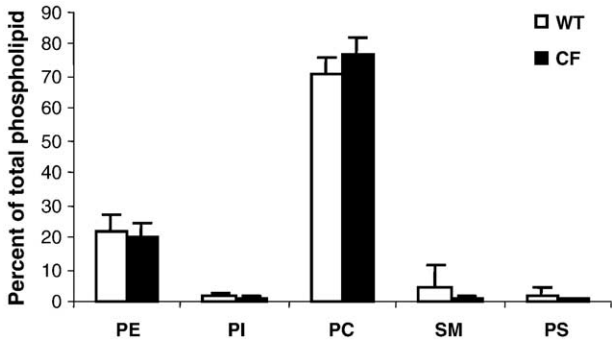

Fig. 2. Phospholipid composition of pancreas homogenates from WT and CF mice. Lipid extracts of pancreas homogenates were subjected to normal-phase HPLC, and detected and quantified by evaporative light-scattering detection. Open bars correspond to WT and dark bars to CF mice. Data are expressed as mean ± SD (n = 5).

and homogenized by brief sonication. Pancreas homogenates were extracted for lipid analysis with 6 volumes of chloroform-methanol (2:1, vol/vol) and stored at −20°C until further analysis.

2.3. Fractionation of lipid classes by solid-phase chromatography

The organic phase was subjected to solid-phase fractionation by using an aminopropyl column (Supelclean, LC-NH2, Supelco, Bellefonte, PA) and following the method of Kaluzny et al [13]. Neutral lipids, including glycerides and cholesterol esters, were eluted with chloroform-isopropanol (2:1, vol/vol). Subsequently, nonesterified fatty acids were eluted from the column with 2% acetic acid in diethyl ether. Phospholipids were then eluted in 2 steps following a variant of the method of Alvarez and Touchstone [14]. Neutral phospholipids were eluted with 100% methanol and acidic phospholipids with chloroform-methanol-0.8 N sodium acetate (60:30:4.5, vol/vol/vol).

2.4. Isolation of phospholipid fractions by high-performance liquid chromatography

The total organic extract was evaporated to dryness under a nitrogen stream, resuspended in chloroform-methanol 2:1 (vol/vol), and microdispersed in a sonication bath (Branson, Danbury, CT). Aliquots (50 μL) were injected into a high-

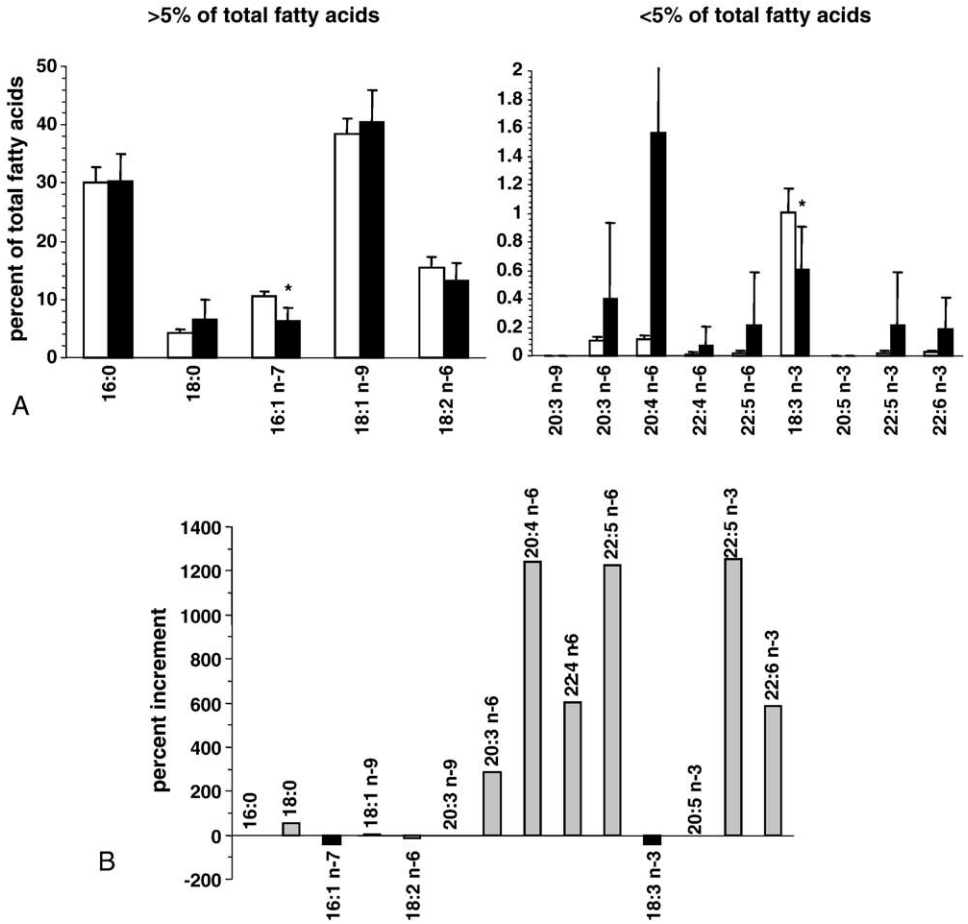

Fig. 3. Fatty acid profile in neutral lipids from pancreas of WT and CF mice. Lipid extracts were subjected to aminopropyl column chromatography. The neutral lipid fraction was eluted with chloroform-isopropanol (2:1, vol/vol), evaporated to dryness, and the fatty acids from the neutral lipids methylated. Fatty acid methyl esters were analyzed by gas chromatography. A, Fatty acid concentration for fatty acids accounting for more than 5% (left) or less than 5% (right) of total fatty acids. Open bars correspond to WT and dark bars to CF mice. *$P < .05$, statistically significant. B, Percent increment of each fatty acid in CF compared with WT. Black bars correspond to statistically significant increments ($P < .05$). Data are expressed as mean ± SD (WT, n = 5; CF, n = 9).

performance liquid chromatography (HPLC) unit (Waters, Milford, MA) with a binary solvent system. Solvent A consisted of 100% chloroform and solvent B was a mixture of chloroform-methanol-water (50:44.5:5.5, vol/vol/vol). The solvent program was 5 minutes of isocratic 100% A, a linear gradient from 100% A to 100% B in 35 minutes, followed by 10 minutes isocratic 100% B, followed by reconstitution of the original conditions. A Sedex-75 evaporative light-scattering detector (Sedere, Cranbury, NJ) was used to identify peaks by comparison of retention times with standards. The flow rate was 1 mL/min, and fractions were collected every 8 minutes. To verify the efficiency of HPLC separation, fractions were analyzed by high-performance thin-layer chromatography on silica plates for phospholipid identification by using 2 consecutive developments in chloroform-ethanol-triethylamine-water (30:35:35:7, vol/vol/vol/vol) and hexane-ether (100:4.5, vol/vol). Bands were identified by immersion in saturated copper sulfate [15].

2.5. Fatty acid analysis of total lipid extracts and subfractions

A 30-μL aliquot of a 1 mg/mL solution of heptadecanoate (17:0, Sigma Chemical, St Louis, MO) in chloroform-methanol (1:1, vol/vol) was added to extracts as an internal standard. Organic extracts were evaporated to dryness under a nitrogen stream and transmethylated with boron trifluoride and methanolic base reagent (BF3-methanol) (Supelco) as previously described [15]. Briefly, dry extracts were resuspended in 0.5 mL of methanolic base, vortexed, and incubated at 100°C for 3 minutes, followed by addition of 14% BF3 in methanol (0.5 mL), vortexing, incubation at 100°C for 1 minute, addition of hexane (0.5 mL), vortexing, incubation at 100°C for 1 minute, and addition of 6.5 mL of saturated NaCl. Samples were vortexed and centrifuged at 800*g* for 2 minutes. The hexane upper layer was transferred to a fresh glass tube. Methyl esters of fatty acids were injected into a Hewlett-Packard 5890A gas chromatograph with a flame ionization detector. A 30-m Supelcowax column (Supelco) (0.5-mm internal diameter) was used. The oven temperature was 150°C for 2 minutes, increased to 200°C for 5 minutes, held at 200°C for 4 minutes, increased to 240°C for 8 minutes, and after 3 minutes increased to 260°C and held at this temperature until the end of the program. The detector temperature was 300°C. Fatty acid methyl ester peaks were identified by comparison of retention times with a standard mixture of fatty acid methyl

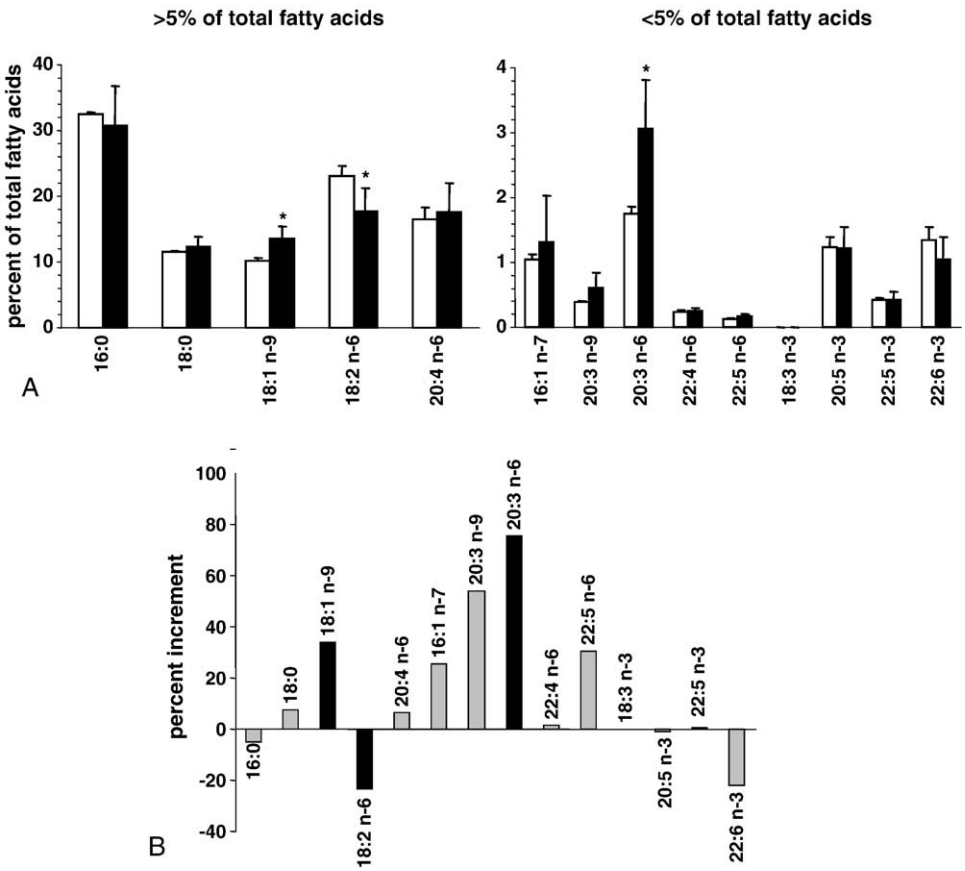

Fig. 4. Fatty acid profile in PC- and PI-containing phospholipid fractions from pancreas of WT and CF mice. Lipid extracts were subjected to normal-phase HPLC. A fraction containing PC and PI was collected, evaporated to dryness, and the fatty acids from PC and PI methylated. Fatty acid methyl esters were analyzed by gas chromatography. A, Fatty acid concentration for fatty acids accounting for more than 5% (left) or less than 5% (right) of total fatty acids. Open bars correspond to WT and dark bars to CF mice. *$P < .05$, statistically significant. B, Percent increment of each fatty acid in CF compared with WT. Black bars correspond to statistically significant increments ($P < .05$). Data are expressed as mean ± SD (WT, n = 5; CF, n = 9).

esters (Nu-Chek Prep, Elysian, MN) and quantified by comparison with the internal standard detector response.

2.6. Statistical analysis

Data are presented as means ± SD. Statistical significance was established by the Student t test, with $P < .05$ denoting significance.

3. Results

In the first series of experiments, we set out to determine the fatty acid changes in the pancreas of the cftr$^{-/-}$ mouse model for CF. Mouse pancreatic homogenates (one pancreas per analysis) were prepared. A total lipid extract was made from each homogenized pancreas to assess by HPLC for differences in the distribution of the major phospholipids between WT and CF mice. There were no statistically significant differences in the percentages of the major phospholipid species (relative to total phospholipid) between WT and CF mice (Fig. 2).

In the total lipid extract of mouse pancreas, we found that CF mice have a lower concentration of LA (data not shown), consistent with many previously published observations [5,7,9,11]. In addition, there was an increase in the amount of n-6 docosapentaenoate (DPA n-6, 22:5 n-6), the terminal fatty acid in the n-6 pathway. A metabolite in the n-6 pathway, dihomo-γ-linolenate (DGLA, 20:3 n-6), was increased in CF mice, indicative of mobilization of LA to DGLA in the pancreas of the CF animals (data not shown). This finding is consistent with a previous report [16] suggesting that the low LA concentration may be the result of increased flux from LA through the n-6 pathway. In addition, there was a significant increase in n-3 docosapentaenoate (DPA n-3, 22:5 n-3) (data not shown). Certain minor fatty acids, such as DGLA, DPA n-6, and DPA n-3, demonstrate the most dramatic differences between CF and WT animals. There was little difference in AA and DHA between CF and WT mice (data not shown).

The mechanisms by which fatty acid alterations in CF are produced have not been elucidated. A first step toward this goal is to isolate each fatty acid–containing lipid in a target organ such as the pancreas and identify the fatty acid changes. Studies were performed with the intention of uncovering mechanisms by which alterations in fatty acid

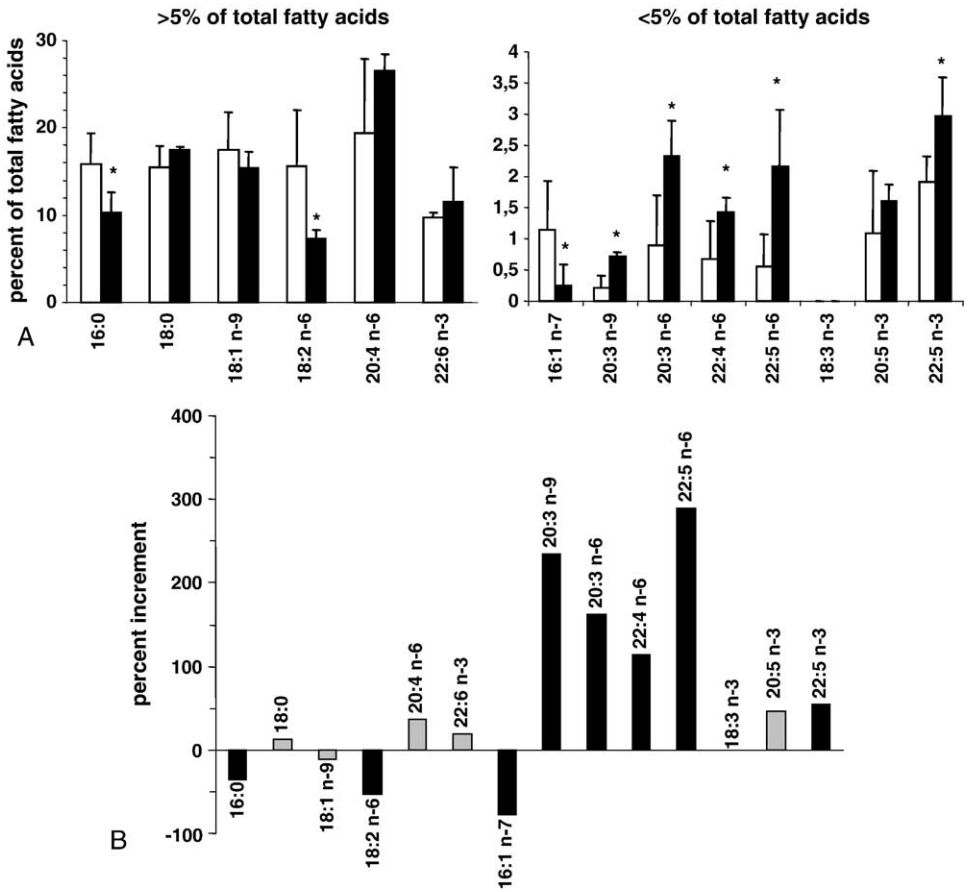

Fig. 5. Fatty acid profile in PE from pancreas of WT and CF mice. Lipid extracts were subjected to normal-phase HPLC. A fraction containing PE was collected, evaporated to dryness, and the fatty acids from PE methylated. Fatty acid methyl esters were analyzed by gas chromatography. A, Fatty acid concentration for fatty acids accounting for more than 5% (left) or less than 5% (right) of total fatty acids. Open bars correspond to WT and dark bars to CF mice. *$P < .05$, statistically significant. B, Percent increment of each fatty acid in CF compared with WT. Black bars correspond to statistically significant increments ($P < .05$). Data are expressed as mean ± SD (WT, n = 5; CF, n = 9).

metabolism in CF are generated. The major changes in phospholipids reflect those found in the total lipid extract, with decreased LA and α-linolenate (LNA, 18:3 n-3), and increased DGLA, DPA n-3, and, more importantly, DPA n-6, with no difference in AA. It must be noted that LNA levels were very low, close to the detection limits. For this reason, this fatty acid could not be detected in the individual phospholipids (data not shown).

The fatty acid profile in the neutral lipids from the lipid extract of mouse pancreas homogenate is shown in Fig. 3. Fig. 3A shows the fatty acid content expressed as mole percent, whereas B indicates the fatty acid change by percent increment or decrement. The top left panel in Fig. 3A shows the predominant fatty acids, with more than 5% of the total fatty acid, and the right panel shows the minor fatty acids, with a different y-axis to allow visualization of the differences in the minor fatty acids. In Fig. 3B, the black bars represent statistically significant differences, and the lighter bars show the changes that are not statistically significant. The same format is used in Figs. 4 to 6. Among the major fatty acids shown in Fig. 3A (left panel), the changes were small, with even LA demonstrating no significant difference between CF and WT animals. For some fatty acids, the differences were large but not statistically significant because there was a high variability in fatty acid composition between animals within CF and within WT groups. Although the hypothesis of mobilization of fatty acids from LA to more elongated and more desaturated n-6 fatty acids cannot be supported by these data, the trends observed are noteworthy. These trends include increases in fatty acids other than LA in the n-6 pathway, accumulation of DPA n-3, and an increase in DHA. Thus, any decrease in DHA is not relevant to the fatty acids in the neutral lipid compartment. These data also indicate that the fatty acid changes in the phospholipid compartment of the CF pancreas are not the same as in the neutral lipid compartment. Therefore, studies to uncover mechanisms of fatty acid alterations in CF must investigate phospholipids and neutral lipids separately.

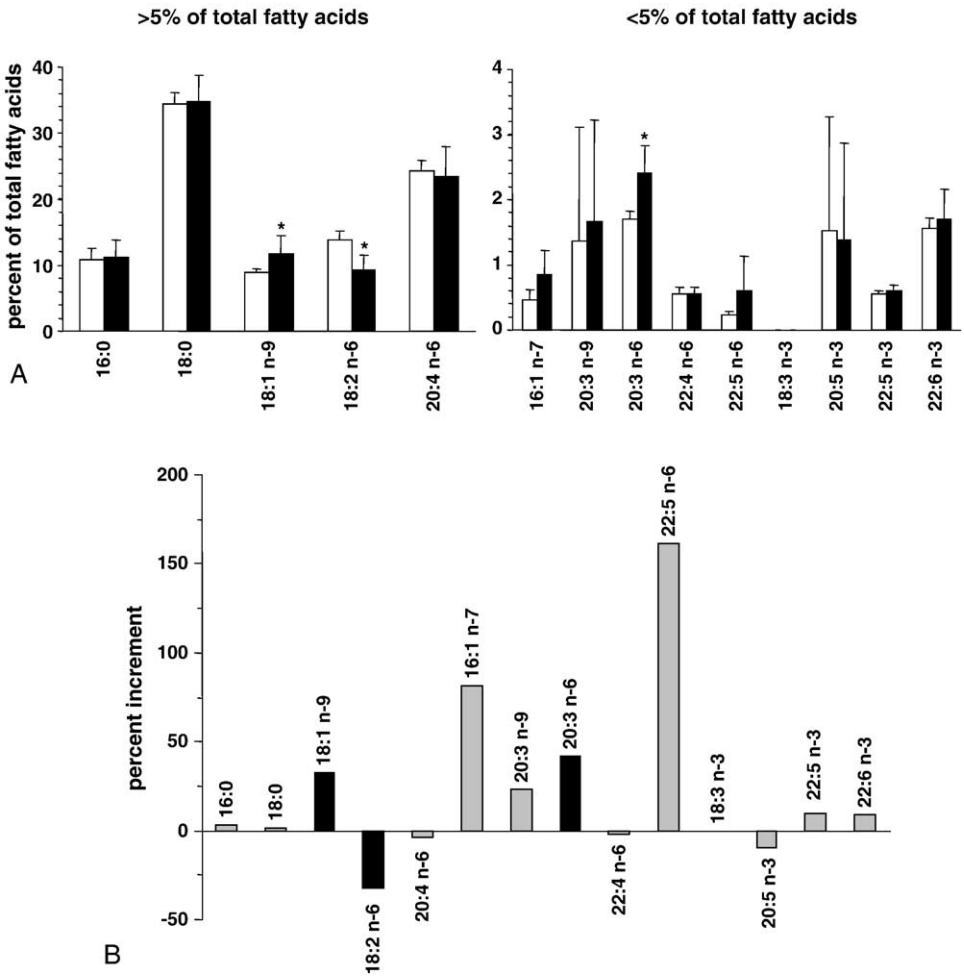

Fig. 6. Fatty acid profile in PS from pancreas of WT and CF mice. Lipid extracts were subjected to normal-phase HPLC. A fraction containing PS was collected, evaporated to dryness, and the fatty acids from PS methylated. Fatty acid methyl esters were analyzed by gas chromatography. A, Fatty acid concentration for fatty acids accounting for more than 5% (left) or less than 5% (right) of total fatty acids. Open bars correspond to WT and dark bars to CF mice. *$P < .05$, statistically significant. B, Percent increment of each fatty acid in CF compared with WT. Black bars correspond to statistically significant increments ($P < .05$). Data are expressed as mean ± SD (WT, n = 5; CF, n = 9).

To further study the fatty acid changes in the phospholipid compartment, phospholipid fractions (PC-PI, PE, phosphatidylserine [PS] and cardiolipin [CL]) were isolated and evaluated. Fatty acid remodeling is phospholipid specific, so fatty acid analysis of isolated phospholipid fractions is an important first step. Fig. 4 shows the fatty acid profile in a fraction of phospholipids that contains both PC and PI. Fig. 4A shows the fatty acid content expressed as mole percent, whereas B indicates the fatty acid change by percent increment or decrement. Consistent with the fatty acid composition of total phospholipids, there was a decrease in the amount of LA and an increase in DGLA. In addition, in this fraction there was an accumulation of DPA n-6, although it was not statistically significant. Fig. 5 shows the data from isolated PE (A shows the fatty acid content expressed as mole percent, whereas B indicates the fatty acid change by percent increment or decrement). The main fatty acid changes were a major decrease in LA and an increase in the downstream metabolites DGLA and, as seen with PC-PI, DPA n-6. There was no increase in AA in the pancreas of CF mice. The fatty acid profile in the isolated PS fraction (Fig. 6) demonstrated low levels of LA and increased levels of oleate (18:1 n-9) and DGLA.

We observed in our previous work on fatty acid alterations in CF in mice [2] and in humans [10] that relatively small changes in AA (increased in CF) and DHA (decreased in CF) might be better expressed as an AA/DHA ratio to enhance the differentiation between mice or humans with CF or without CF. The data in this report suggest that a different ratio may be more useful. AA/DHA and DPA n-6/DHA ratios were determined (Table 1). A

Table 1
Fatty acid ratios in total lipid extracts, lipid classes, and phospholipid species from WT and CF mouse pancreas

	AA/DHA	DPA n-6/DHA
Total extract		
WT	4.9 ± 0.6	0.08 ± 0.01
CF	5.5 ± 0.4	0.14 ± 0.03*
WT (congenic)	7.9 ± 0.3	0.25 ± 0.02
CF (congenic)	19.4 ± 3.1*	2.08 ± 0.19**
Phospholipids		
WT	5.5 ± 1.2	0.10 ± 0.01
CF	6.3 ± 0.7	0.20 ± 0.03**
Neutral lipids		
WT	6.0 ± 4.5	0.96 ± 1.08
CF	7.7 ± 5.6	1.02 ± 1.02
PE		
WT	1.96 ± 0.8	0.09 ± 0.00
CF	2.67 ± 0.9	0.19 ± 0.01**
PC-PI		
WT	12.3 ± 1.9	0.09 ± 0.01
CF	17.2 ± 3.1*	0.16 ± 0.02**
PS		
WT	15.7 ± 2.6	0.14 ± 0.03
CF	14.7 ± 4.6	0.25 ± 0.05*

* $P < .01$, for comparisons between WT and CF animals.
** $P < .001$, for comparisons between WT and CF animals.

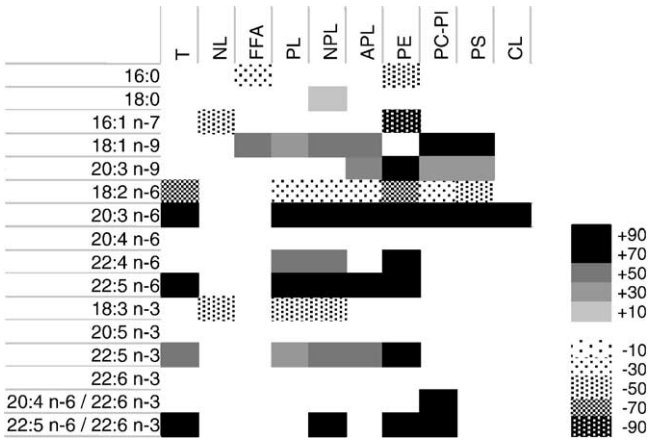

Fig. 7. Heat map of fatty acid increments or decrements in pancreas from CF compared with WT mice. T indicates total extract; NL, neutral lipids; PL, total phospholipids; NPL, neutral phospholipids; APL, acidic phospholipids. Solid squares denote increments, and dotted squares denote decrements, with darker colors indicating greater increments or decrements by 20% intervals. White squares denote nonsignificant changes ($P > .05$).

clear difference between the CF and WT animals is shown in the DPA n-6/DHA ratio for all but the neutral lipids. Although there was an increase in the AA/DHA ratio for CF animals in the PC-PI fraction, differences were not observed in the other fractions. Thus, the DPA n-6/DHA ratio was a more consistent marker of the fatty acid metabolic abnormality in CF. This is supported by the results obtained in the inbred CF congenic mouse model. As shown in Table 1, the DPA n-6/DHA ratio was also consistently more significant in this model.

The "heat map" in Fig. 7 shows the changes in the different fatty acids within the total lipid extract and the lipid fractions including neutral lipids, total phospholipids, and neutral and acidic phospholipids (PE, PC-PI, PS, and CL). Increases are represented by solid rectangles and decreases by dotted rectangles. The darkest rectangles are associated with the greatest increases (>90%) in fatty acids from WT to CF animals, and the lighter rectangles indicate minor decreases. The white rectangles represent essentially no change (<10% increase or decrease). Decreases are represented by dotted rectangles, the darkest indicating the largest decreases (>90%) from WT to CF animals. The most consistent increases, shown by the darkest rectangles, were in DGLA and DPA n-6. The most consistent decreases, shown by the darkest dotted rectangles, were in LA. Changes in AA and DHA were not consistent across the fractions analyzed.

Data on changes in fatty acid composition, such as the ones we found in this report, could help identify what metabolic fatty acid conversions should be preferentially studied. The results of this study support the hypothesis that one mechanism to decrease LA and increase downstream n-6 metabolites is activation of the desaturase and elongase enzymes that mobilize LA to more elongated and desaturated metabolites.

4. Discussion

A complete and functionally significant view of fatty acid modifications in a given tissue requires the analysis of both the total profile of fatty acids and their distribution within the different lipid classes, as demonstrated in other systems [17]. In this report, we present novel observations of the fatty acid changes found in the pancreas of a CF mouse model that shows alterations in specific fatty acid–containing lipids. Specifically, in our experimental system, we observed that (1) a decrease in LA content was found in phospholipids but not in neutral lipids; (2) there was an increase in the DGLA metabolite of LA; (3) there was a consistent increase in all phospholipids in DPA n-6, the terminal fatty acid in the n-6 pathway; and (4) the DPA n-6/DHA ratio was significantly elevated in all phospholipid species analyzed in $cftr^{-/-}$ mice, in the absence of consistent, significant changes in AA and DHA. The observed variability in AA content may be due to its increased conversion either to proinflammatory eicosanoids or to DPA n-6. The cellular levels of free and phospholipid-bound AA are tightly regulated by several enzymes. In particular, phospholipase A2 enzymes catalyze the release of AA from membrane phospholipids, and thereby influence its availability for either eicosanoid production or further metabolism [18]. Such questions represent important topics for further investigation of the mechanisms responsible for fatty acid imbalance in CF.

Our results reflect previous work performed in other tissues from patients and animal models of the disease. The decreased LA present in the current studies, not only in total lipid extracts, but also in total phospholipid extracts, and the individual phospholipids tested is the most established fatty acid change [6-11]. The changes in phospholipids were different from those found in neutral lipids, with the neutral lipids not showing a decrease in LA. This is consistent with a previous observation that a decrease in LA occurs in phospholipids [11].

We have also demonstrated that the increased flux through the n-6 pathway can result in increases in DGLA and DPA n-6, rather than AA. This increased flux is specific for the n-6 pathway, as no equivalent increase in the n-3 terminal fatty acid, DHA, was found. Although DHA levels are not decreased, its precursor DPA n-3 is significantly increased in total lipids, in total phospholipids, and in PE, suggesting a differential regulation of n-6 and n-3 pathways and a potential block in DHA synthesis cannot be ruled out. Interestingly, recent studies performed on rat retina showed that an n-3–deficient diet leads to increased DPA n-6 [19]. This led to impaired G protein–coupled signal transduction prompting the speculation that the increased acyl chain packing with increased amounts of DPA n-6 in the membrane, compared to DHA, alters membrane fluidity and the ability of proteins to move within the lipid bilayer.

The results of this study show several findings consistent with previous reports. However, there were a number of differences in fatty acid composition in this study, which used mice pancreatic homogenates from CFTR knockout mice, from those previously reported. One difference is the absence of a statistically significant decrease in DHA in the pancreas. It should be noted that previous studies with this mouse model [2] showed statistically significant differences in DHA between WT and CF animals, although the major decreases were seen in two other CFTR-expressing tissues, notably ileum and lung. Thus, the findings in this study suggest that there may be differences between CFTR-expressing tissues in the relative decreases in DHA, and that the DHA decrease is lowest in the pancreas. Interestingly, the results obtained in a mouse model presenting a more severe version of the disease, the CF congenic inbreed (Table 1), show more profound differences in both the AA/DHA and the DPA n-6/DHA ratios, which are mainly due to a decrease in DHA content. This decrease was statistically significant in the total pancreas extract (data not shown). This raises the possibility that DHA content and the respective n-6 to n-3 ratios are correlated with the severity of the disease and that fatty acid alterations may not be consistent between different models.

Several reports have also shown an increase in the amount of AA [2,7,11], including one using this mouse model [2]. In the current experiments, the n-6 fatty acid that was consistently increased was DGLA. One possible explanation is that the delta-5 desaturase that converts DGLA to AA was less active in the pancreas and resulted in an accumulation of DGLA instead of AA. The basis for any regulation of the delta-5 desaturase step in this mouse model remains unexplained. A potential explanation for the decreased or unaltered AA when compared with the other n-6 fatty acids would be its increased conversion into prostanoids in CF, as previously reported [20].

The results obtained suggest that the decrease in DHA varies depending on the cell type and the animal model; hence low DHA may not play a primary role in the fatty acid abnormalities related to CFTR dysfunction. However, a remarkable finding in our study is the significant increase in DPA n-6 in the n-3 series, accompanied by nonsignificantly changed DHA, which suggests an impairment at this level of the pathway. The lack of a decrease in DHA in these studies is not inconsistent with this speculation, as the retention of DHA in tissues is greater than for other fatty acids [21]. A previous study using astrocytes incubated with LNA [22] showed an accumulation of fatty acids prior to DPA n-3. In addition, in previous work with CF animals, although DHA supplementation ameliorated the pathologic changes, the CF phenotype could not be reversed by eicosapentaenoate (20:5 n-3) [2]. This would suggest that the metabolism from eicosapentaenoate to DHA is in some way blocked.

Recently, Dombrowsky et al [23] studied the molecular species composition of phospholipids from the lung and pancreas in a CF mouse model with a different mutation from the one studied in this series of experiments.

Quantitative differences in phospholipid composition exist between the 2 studies, probably due to methodological differences. In the Dombrowsky et al study, the authors made the important observation that the decline in DHA was dependent on the molecular species in which DHA was present. It was noted that there was no difference between CF and WT animals in PC with a fatty acid composition of stearate/DHA. However, CF animals had roughly 60% of the DHA as WT animals in the molecular species of PC with a palmitate/DHA composition. Therefore, one possibility is that DHA, delivered as a supplemental fatty acid, should be present not only in a specific phospholipid class, but also in a particular molecular species of this phospholipid class, which must also be found in a specific membrane domain. We postulate that a decrease in DHA levels in this putative membrane domain may play a key role in the pathogenesis of CF. This may also help explain why an overcorrection in DHA levels was required to reverse the pathology observed in these mice [2]. In our previous studies, to correct the defect found in this specific membrane domain, DHA may have had to be incorporated in all cell membranes. To better understand the nature of this lipid defect, it would be interesting to determine DHA incorporation in the different phospholipids of the various membrane compartments after oral administration of DHA to CF and WT mice.

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