The Journal of Nutrition
Nutrition and Disease

Increased Tissue Arachidonic Acid and Reduced Linoleic Acid in a Mouse Model of Cystic Fibrosis Are Reversed by Supplemental Glycerophospholipids Enriched in Docosahexaenoic Acid[1–3]

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Abstract

An imbalance in (n-6)/(n-3) PUFA has been reported in cystic fibrosis (CF) patients. Glycerophospholipids enriched in docosahexaenoic acid (GPL-DHA) have been shown to regulate the (n-6)/(n-3) fatty acid ratio in the elderly. Here, we tested the effect of GPL-DHA supplementation on PUFA status in F508del homozygous CF mice. GPL-DHA liposomes were administrated by gavage (60 mg DHA/kg daily, i.e. at maximum 1.4 mg DHA/d) to 1.5-mo-old CF mice (CF+DHA) and their corresponding wild-type (WT) homozygous littermates (WT+DHA) for 6 wk. The PUFA status of different tissues was determined by GC and compared with control groups (CF and WT). There was an alteration in the (n-6) PUFA pathway in several CF-target organs in CF compared with WT mice, as evidenced by a higher level of arachidonic acid (AA) in membrane phospholipids or whole tissue (21 and 39% in duodenum-jejunum, 32 and 38% in ileum, and 19 and 43% in pancreas). Elevated AA levels were associated with lower linoleic acid (LA) and higher dihomo-γ-linolenic acid levels. No DHA deficiency was observed. GPL-DHA treatment resulted in different PUFA composition changes depending on the tissue (increase in LA, decrease in elevated AA, DHA increase, increase in (n-6)/(n-3) fatty acid ratio). However, the DHA/AA ratio consistently increased in all tissues in CF+DHA and WT+DHA mice. Our study demonstrates the effectiveness of an original oral DHA formulation in counter-balancing the abnormal (n-6) fatty acid metabolism in organs of CF mice when administrated at a low dose and highlights the potential of the use of GPL-DHA as nutritherapy for CF patients. J. Nutr. 139: 2358–2364, 2009.

Introduction

Cystic fibrosis (CF)[11] is the most common autosomal recessive disorder in Caucasians and results from a mutation in the CF transmembrane conductance regulator (CFTR) protein, which leads to altered regulation of the epithelial cell chloride channel (1). At least 70% of patients carry a deletion of the phenylalanine at position 508 (F508del) (2). Abnormalities in the metabolism of essential fatty acid (EFA) or/and their long-chain polyunsaturated metabolites are often reported in CF patients (3–6), as recently reviewed (7,8), resulting in linoleic acid (LA) and docosahexaenoic acid (DHA) deficiency, and less consistently in arachidonic acid (AA) increase (3), in plasma or erythrocyte phospholipids, and organs (4,9). Mechanisms involved might be linked to fat malabsorption (9), increased PUFA β-oxidation (10), higher production of eicosanoids favoring a proinflammatory status (11), higher PUFA peroxidation (12), or a possible intrinsic defect in the PUFA utilization in F508del-CF cells (13). Alterations in fatty acid metabolism have also been reported in CF mice including total null mice and models with specific *cftr* mutations such as F508del (14–16), but

[1] Supported by Vaincre la Mucoviscidose (Cystic Fibrosis French Association) and the Fonds Spécial de Recherche (Université Catholique de Louvain).
[2] Author disclosures: M. Mimoun, T. C. Coste, J. Lebacq, P. Lebecque, P. Wallemacq, T. Leal, and M. Armand, no conflicts of interest.
[3] Supplemental Figure 1 and Supplemental Table 1 are available with the online posting of this paper at jn.nutrition.org.
[10] M. Mimoun and T. C. Coste contributed equally as first authors and T. Leal and M. Armand contributed equally as last authors.
[11] Abbreviations used: AA, arachidonic acid; ALA, α-linolenic acid; CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; DGLA, dihomo-γ-linolenic acid; DHA, docosahexaenoic acid; EFA, essential fatty acid; F508del, deletion of the phenylalanine at position 508; GPL-DHA, glycerophospholipids enriched in docosahexaenoic acid; LA, linoleic acid; WT, wild type.
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Manuscript received June 3, 2009. Initial review completed June 30, 2009. Revision accepted October 2, 2009.
First published online October 14, 2009; doi:10.3945/jn.109.110999.

data are often inconsistent, possibly due to differences in mouse genotypes, breeding conditions, and age. For instance, a PUFA imbalance has been reported in 1-mo-old *cftr*$^{-/-}$ mice, characterized by higher levels of AA associated with lower levels of DHA in CF target organs such as pancreas, lungs, and intestine (ileum) (17). However, this imbalance was not observed by others in tissues from 1-, 2-, or 3-mo-old *cftr*$^{-/-}$ mice and 3-mo-old F508del-CF mice, which had similar levels of LA, α-linolenic acid (ALA), AA, and DHA compared with classic controls or wild-type (WT) littermates (18,19). Moreover, 2-mo-old *cftr*$^{-/-}$ mice did not display any deficiency in DHA and had lower AA levels in pancreas and lungs (20) or a lower LA level and a higher level in the terminal fatty acids of the (n-6) pathway in pancreas (21).

DHA is an important component of cell membrane phospholipids, plays a key role in maintaining optimal cell functions (22), and exerts antiinflammatory properties via retro-conversion into eicosapentaenoic acid or direct transformation to resolvin D (23). Therefore, a great deal of interest in DHA-based oral nutritherapy in CF patients using fish or DHA-enriched algae oils has emerged (7,8,24,25). In CF mice, oral supplementation with a high DHA dose (40 mg/d) corrected the lipid imbalance and reversed some morphological features of the disease, improving pancreatic, ileal, or liver function and decreasing lung inflammation related to *Pseudomonas aeruginosa* infection (17,19). But the DHA doses used in these studies were very high compared with recommendations for healthy populations (120–220 mg/d) and might increase peroxidation (26). Further, the form in which DHA is administered is relevant, as phospholipids transfer DHA more efficiently into different tissues or biological fluid compared with ethyl ester or triglycerides (27,28). Interestingly, DHA carried by glycerophospholipids increased the DHA level in erythrocyte membranes and also regulated AA levels, resulting in a balanced (n-6)/(n-3) fatty acid ratio in elderly patients (27). We thus aimed to study the PUFA status of different organs from F508del-CF mice compared with normal homozygous WT littermates and to evaluate whether supplementation of DHA provided as glycerophospholipids could correct the PUFA imbalance.

Materials and Methods

Mouse models. CF mice homozygous for the F508del mutation in the 129/FVB outbred background (F508del-cftr ^{tm1EUR}, with the F508del exon 10 insertional mutation) (14) and their normal homozygous WT sex-matched littermates were studied after weaning starting at 1.5 until 3 mo old. The mice were genotyped at 21 d of age using Taqman quantitative PCR, as previously described (29). The mice were housed at the Animal Care Facility of the University of Louvain and the study and procedures were approved by the local Ethics Committee for Animal Welfare and conformed to the European Community regulations for animal use in research (CEE 86/609) (30).

Diet and DHA supplementation. Mice were divided into 4 groups (n = 6 or 7): WT and F508del-CF (CF) as controls and 2 experimental groups (WT+DHA and CF+DHA). All mice consumed a liquid diet, Peptamen-Junior formula (40 g lipid/L, providing triglycerides with medium-chain and EFA; 30 g whey peptides/L; and 132 g maltodextrin/L; Nestlé Clinical Nutrition), ad libitum to minimize intestinal obstruction and optimize long-term viability in our CF-mice. The experimental groups were supplemented daily by oral gavage between 1700 and 1900 h with 60 mg DHA/kg (~1.4 mg DHA/d maximum) for 6 wk using glycerophospholipid-liposomes obtained from DHA-enriched eggs (GPL-DHA). Controls were administered saline. This dose, which is very low compared with those used in previous studies (17,19), was selected based on a prior study in rats (31). Fatty acid intake was calculated using Peptamen-Junior and GPL-DHA fatty acid profiles (**Supplemental Table 1**) assuming a daily consumption of 0.2 kg solids/kg body weight and knowing the amount of GPL-DHA given (**Table 1**). Body weights were measured biweekly.

Tissue collection and preparation. After anesthesia, CF target [proximal (duodenum-jejunum) and distal (ileum) intestine, pancreas, lungs] and nontarget (heart, liver, kidney) organs were isolated, rinsed in ice-cold saline, frozen in liquid nitrogen after removal of adherent tissues, and kept at −80°C until use. For homogenate preparation, tissues were weighed, rinsed in ice-cold saline, chopped into small pieces, and homogenized at 4°C in 2 mL of ice-cold buffer (11 mmol/L Tris, pH 7.4) with a homogenizer (Potter model 94348, Heildoph) using 3 15-s bursts. Cell membrane phospholipids were purified from homogenates using 3 differential centrifugation steps at 4°C according to a described procedure (32) with modifications (1500 × g for 5 min; 7500 × g for 15 min; 100,000 × g for 60 min; Sorvall WX Ultra Series, Thermo ultracentrifuge, rotor TLX 100 Beckman). The final pellet (purified membrane) was collected in 1–2 mL ice-cold buffer and stored at −80°C.

Fatty acid composition determination. Lipids were extracted as previously described from homogenates and purified membranes (33) and from formula and GPL-DHA (34) with methanol and chloroform. Fatty acid composition was analyzed after methylation with BF3-methanol (Sigma) (35) by GC (Perkin Elmer Autosystem XL, flame ionization detector, Turbochrom software) using a fused silica capillary

TABLE 1 Body weight, volume of formula, and amount of fatty acids consumed daily in CF and WT mice that were or were not supplemented with GPL-DHA for 6 wk[1]

		Formula (estimated)					GPL-DHA (administrated)				
Groups	Mouse weight	Volume	SFA	MUFA	LA	ALA	SFA	MUFA	LA	AA	DHA
	g	*mL*					*mg/d*				
Start of study											
WT	19.4 ± 1.0	19 ± 1	436 ± 19	107 ± 5	182 ± 8	24 ± 1	—	—		—	
CF	18.4 ± 1.2	18 ± 1	414 ± 27	102 ± 7	173 ± 11	23 ± 1	—		—	—	
WT+DHA	20.3 ± 0.7	20 ± 1	456 ± 16	112 ± 4	190 ± 7	25 ± 1	8.8 ± 0.3	5.5 ± 0.2	2.8 ± 0.1	0.50 ± 0.02	1.2 ± 0.4
CF+DHA	17.9 ± 1.0	18 ± 1	402 ± 22	99 ± 5	168 ± 9	22 ± 1	7.8 ± 0.4	4.8 ± 0.3	2.4 ± 0.1	0.44 ± 0.02	1.1 ± 0.6
End of study											
WT	22.7 ± 1.4*	22 ± 1*	511 ± 32*	125 ± 8*	213 ± 13*	28 ± 2*	—		—	—	
CF	22.2 ± 1.3*	22 ± 1*	499 ± 29*	122 ± 7*	208 ± 12*	27 ± 2*	—		—	—	
WT+DHA	23.2 ± 1.0*	23 ± 1*	521 ± 23*	128 ± 6*	217 ± 9*	29 ± 1*	10.1 ± 0.4*	6.2 ± 0.3*	3.2 ± 0.1*	0.57 ± 0.02*	1.4 ± 0.6*
CF+DHA	23.0 ± 1.4*	23 ± 1*	517 ± 31*	127 ± 8*	216 ± 13*	28 ± 2*	10.0 ± 0.6*	6.2 ± 0.4*	3.1 ± 0.2*	0.57 ± 0.03*	1.4 ± 0.8*

[1] Values are means ± SEM, n = 6–7. *Different from the start of the study within a group, $P < 0.05$. Groups did not differ from one another at either time.

column (Omegawax 250, 30-m × 0.25-mm i.d., 0.25-μm film thickness) (Sigma-Supelco) and hydrogen as the carrier gas. The oven temperature program ranged from 60°C to 215°C with a temperature rise of 45°C/min. Fatty acids were identified by their retention times on the column using appropriate standards (PUFA 2, Sigma-Supelco).

Statistical analysis. Data are presented as means ± SEM. A Kolmogorov-Smirnov test for normality and a Bartlett test for homogeneous variance were performed for each variable. Changes within a group were analyzed using the Wilcoxon matched pairs test and differences among groups at the end of the study were analyzed using the Mann-Whitney U test with differences of $P < 0.05$ considered significant. All analyses were conducted using STATVIEW software (Abacus Concepts).

Results

Fatty acid composition of CF-target organs. We compared the PUFA composition of intestine, pancreas, and lung homogenates of CF and WT mice fed the basal formula (**Fig. 1**). LA tended to be lower in the ileum (11%, $P = 0.10$) and was significantly lower in the pancreas (17%) of CF mice (Fig. 1*B*,*C*). ALA levels were very low and did not differ between groups (data not shown). AA levels were significantly greater in duodenum-jejunum (39%), ileum (32%), and pancreas (43%), but not in lungs, of CF mice compared with WT mice (Fig. 1). Dihomo-γ-linolenic acid (DGLA) levels were significantly higher in the proximal intestine and pancreas but not in the distal intestine of CF mice (Fig. 1*A*–*C*). The CF mice did not have reduced levels of DHA in ileum, pancreas, or lungs and had a significantly higher concentration in the duodenum-jejunum than in the WT mice (Fig. 1*A*).

Fatty acid composition of non-CF target organs. Because PUFA status is not very well documented and results have been inconsistent in CF mouse models, we investigated PUFA levels in a variety of tissues not expected to be affected by CF such as liver, heart, and kidney. There were no differences between CF and WT mice (data not shown).

Fatty acid profiles of cell membrane phospholipids. Phospholipid LA levels were significantly lower in organs phenotypically affected by CF, especially in pancreas (25%) (**Fig. 2***A*). Phospholipid AA levels of CF mice were significantly higher in the duodenum-jejunum (21%), in the ileum (39%), and in the pancreas (19%) than in WT mice (Fig. 2*B*). As in homogenates (Fig. 1), DGLA levels were significantly higher in the duodenum-jejunum and pancreas but not the ileum of CF compared with WT mice (data not shown). DHA levels in CF mice did not differ from those in WT mice, except in the proximal intestine, where it was 31% higher ($P < 0.05$) and in the ileum, where it tended to be 22% higher ($P = 0.1$) (Fig. 2*C*). The (n-6)/(n-3) fatty acid ratio was significantly lower in the proximal intestine of the CF mice (Fig. 2*D*), mainly due to a relatively higher DHA level compared with AA and a 60% greater level of eicosapentaenoic acid compared with WT mice (data not shown). The (n-6)/(n-3) fatty acid ratio did not differ between the groups in ileum (the trend for a higher DHA level counter-balanced the higher AA level), in pancreas (the higher level of AA was counter-balanced by a 25% lower level in LA), or in lungs.

In contrast with organs typically affected by CF, the phospholipid fatty acid profiles of organs not phenotypically affected by the disease did not differ between the groups (**Supplemental Fig. 1**).

GPL-DHA effects on tissue PUFA composition. Several marked changes in PUFA levels were found after GPL-DHA treatment, depending on the tissue and the mouse type (CF +DHA vs. CF and WT+DHA vs. WT). Indeed, in organ homogenates from CF but not WT mice, GPL-DHA significantly restored LA levels in both ileum and lungs but not in pancreas (Fig. 1*B*–*D*). Furthermore, GPL-DHA treatment tended to decrease the AA level in the duodenum-jejunum of CF mice (12.5%, $P = 0.1$) and decreased it significantly in ileum (34%) and pancreas (23%) to levels similar to those of unsupplemented WT mice. In parallel, DGLA levels tended to decrease in intestine ($P = 0.1$). Curiously, DHA levels were not increased by DHA supplementation in organs of CF or WT mice except in the proximal intestine of the latter. Regarding cell membrane phospholipids, PL-bound LA levels did not change with DHA supplementation in either organs affected (Fig. 2*A*) or not affected by the disease (Supplemental Fig. 1*A*), whereas PL-bound AA levels tended to decrease compared with unsupplemented CF mice in pancreas (8%) and lung (18%) ($P = 0.1$) (Fig.

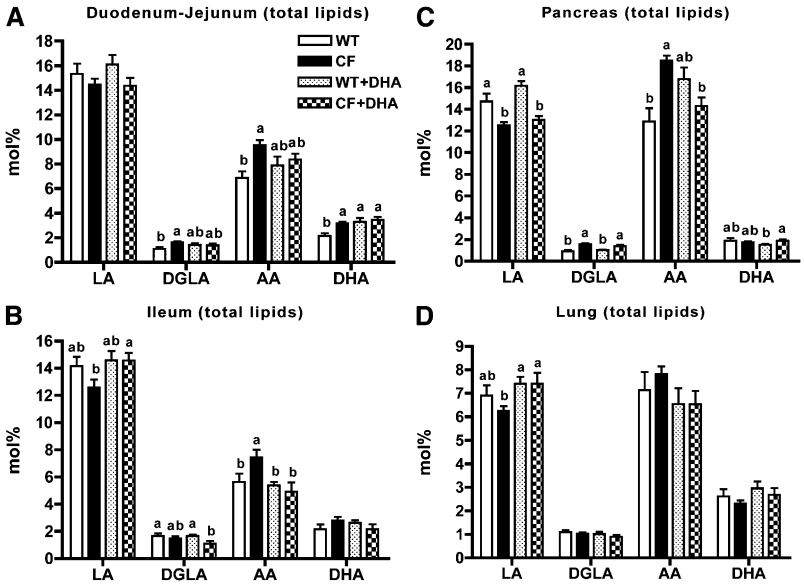

FIGURE 1 Relative concentrations of LA, DGLA, AA, and DHA in lipid extracts from homogenates of duodenum-jejunum (*A*), ileum (*B*), pancreas (*C*), and lung (*D*) of CF and WT mice that were or were not administered GPL-DHA for 6 wk. Individual fatty acid concentrations are expressed as mol% of total fatty acids. Values are means ± SEM, $n = 6$ or 7. Labeled means without a common letter differ, $P < 0.05$.

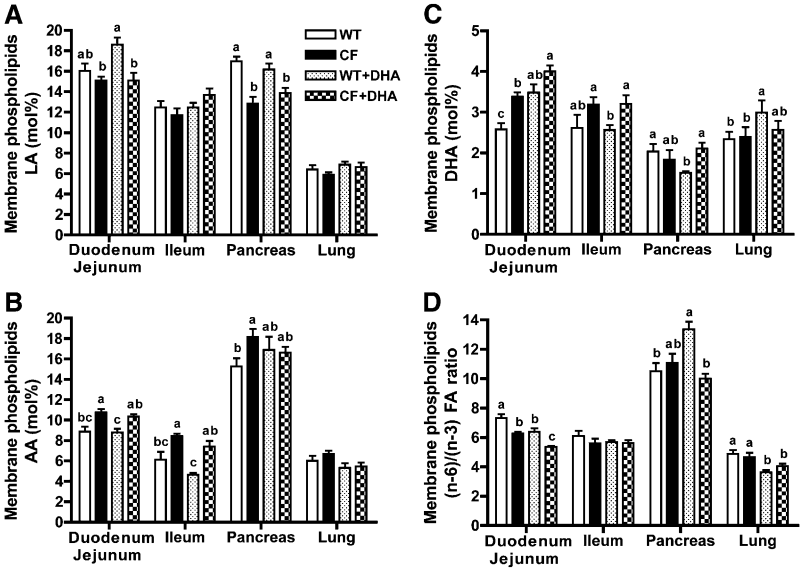

FIGURE 2 Relative concentrations of LA (*A*), AA (*B*), DHA (*C*), and total (n-6)/(n-3) fatty acid ratio (*D*) in purified membrane phospholipids extracts from organs currently affected by CF of CF and WT mice that were or were not administered GPL-DHA for 6 wk. Individual fatty acid concentrations are expressed as mol% of total fatty acids. Values are means ± SEM, $n = 6$ or 7. Labeled means without a common letter differ, $P < 0.05$.

2*B*). This last finding was more pronounced in organs not phenotypically affected by CF, with significant decreases in PL-AA levels in heart from CF and WT mice (21 and 25%) and a trend for decreased PL-AA in liver (11%, $P = 0.06$) and kidney (10%, $P = 0.07$) of CF mice (Supplemental Fig. 1*B*). PL-bound DHA levels were significantly increased in the proximal intestine of CF (18%) and WT (35%) mice and in lung of WT mice (28%), did not change in distal intestine, and was decreased (26%) in pancreas of WT but not CF mice (Fig. 2*C*). Although PL-DHA levels seemed to be increased in heart and kidney (Supplemental Fig. 1*C*), supplemented and unsupplemented mice did not differ significantly because of large variances.

The (n-6)/(n-3) fatty acid ratio in membrane phospholipids was decreased significantly by DHA supplementation in both WT and CF mice in proximal intestine (13 and 14%) and lungs (26 and 13%) (Fig. 2*D*) and in the hearts of CF mice (14%) (Supplemental Fig. 1*D*). The decrease in the (n-6)/(n-3) fatty acid ratio resulted mainly from the combination of a decrease in AA and an increase in DHA levels in lung and heart or a large increase in DHA levels (proximal intestine).

The DHA/AA ratio, which is of great importance for evaluating long chain PUFA imbalance, was calculated in the phospholipid fraction for affected (**Fig. 3***A*) and nonaffected organs (Fig. 3*B*). Without GPL-DHA treatment, the DHA/AA ratio was similar in WT and CF groups in almost all tissues except pancreas, where DHA/AA was significantly lower in CF mice due to a high level of AA. After GPL-DHA supplementation, and despite the low DHA dose given, the DHA/AA ratio significantly increased in most tissues from both genotypes; it tended to increase in pancreas ($P = 0.06$), liver ($P = 0.06$), and kidney ($P = 0.1$) of CF mice. The extent of this increase was organ- and genotype-dependent, because there were significant increases of 26 and 38% in proximal intestine, 13 and 28% in distal intestine, 31 and 44% in lungs, 11 and 19% in liver, and 41 and 46% in heart in CF and WT mice, respectively.

Overall, the results suggest an interesting dichotomy in the effects of our low-dose DHA vector: an effect common to all organs, namely an increase in the DHA/AA ratio, and more organ-specific effects such as an increase in LA, a decrease in AA, an increase in DHA, and a decrease in the (n-6)/(n-3) fatty acid ratio.

Discussion

The mechanisms by which fatty acid alterations in CF occur have not yet been elucidated. A first step toward this goal is to identify the fatty acid changes in target organs such as intestine, pancreas, and lung. We therefore studied the PUFA status of the F508del CF mouse model, which displays the most common genotype of the human disease and thus might be susceptible to developing AA and DHA imbalance (4). Our second goal was to test the hypothesis that a specific original vector of DHA

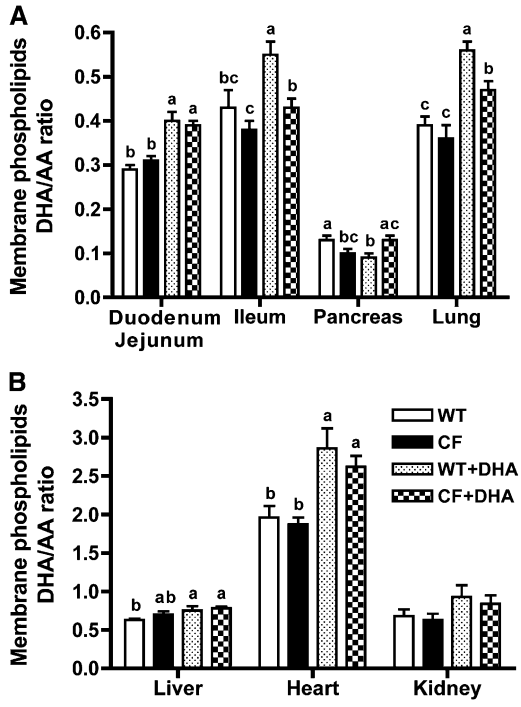

FIGURE 3 The DHA/AA ratio in purified membrane phospholipid extracts from organs currently affected (*A*) or not affected (*B*) by CF of CF and WT mice that were or were not administered GPL-DHA for 6 wk. Values are means ± SEM, $n = 6$ or 7. Labeled means without a common letter differ, $P < 0.05$.

(glycerophospholipids) would be able to correct the altered (n-6)/(n-3) fatty acid ratio (27).

One of the interesting findings reported herein is that, although our CF mice received a sufficient dietary supply of EFA, levels of LA were markedly decreased in tissues affected by the disease (intestine, pancreas, and lung). Concomitantly, high levels of DGLA and AA were found in those CF tissues. The major changes in phospholipids reflected those found in the total lipid extract, which mainly showed increased DGLA (especially in proximal intestine and pancreas) and AA. This observation suggests an increased flux through the (n-6) fatty acid pathway in this CF model, confirming an unbalanced intracellular utilization of LA in several disease-specific organs. Similar metabolic features were reported in phospholipids from CF human pancreatic epithelial cells (13), from pancreas of *cftr* knockout mice (21), and, more recently, in human bronchial epithelial cells not expressing CFTR (36). Possible mechanisms that might explain decreased LA and increased AA in CF are activation of desaturases and elongases, enzymes that convert LA to downstream (n-6) fatty acid metabolites (21), and/or changes in AA transport from liver and adipose tissues to other organs, which can favor eicosanoid production. Indeed, AA is the substrate for biosynthesis of inflammatory mediators and therefore its increased production from LA may have a great impact on the excessive inflammatory response reported in this CF model (37), a response which is typically found in CF patients even in the absence of infection (4,38).

The results of this study also demonstrate that supplementing the CF mice for 6 wk with a low dose of DHA provided by phospholipids, i.e. at maximum 1.4 mg DHA/d, corrected the (n-6) fatty acid metabolism dysregulation in CF-affected organs. Indeed, GPL-DHA treatment allowed the restoration of LA levels in intestine and lungs, but not in pancreas, and diminished the biosynthesis of AA in all 3 organs. Because LA levels did not change in WT mice supplemented with DHA and DHA was the main fatty acid that was increased in the diet, we suggest that DHA per se corrects a specific defect that results in the loss of CFTR function, but only in disease target-tissues. DHA inhibited the LA-derived increase in AA levels in CF, normalizing them to WT values, possibly via an increase in lipocortin expression that inhibits the liberation of AA via phospholipase A2 inhibition (39) and via a downregulatory effect on $\Delta 5$ desaturase enzyme (40), which mediates the formation of AA. It was previously shown that feeding *cftr* knockout mice DHA either as FFA or esterified in triglycerides normalizes the disease-related changes in AA that occur in pancreatic and lung cells, but only at the dose of 10 or 40 mg/d DHA (lower doses of 0.5 and 2 mg/d were not effective) (17). As shown in the present study, correction of the (n-6) fatty acid pathway defect by a low dose of DHA esterified in GPL makes it a potential adjuvant therapy to decrease the chronic inflammatory response in CF-patients via downregulation of the production of AA-derived inflammatory eicosanoids. This is supported by the increase of the DHA/AA ratio in membrane phospholipids.

Another important observation is that the F508del mouse model does not reproduce the DHA deficiency state reported in patients (3–8) and in the *cftr* knockout mice used by Freedman et al. (17) in 1999. However, our data showing stable or somewhat increased DHA levels are consistent with several studies in *cftr* knockout (18,19,21) and F508del mice (18). The discrepancies in DHA status in several CF mouse models compared with CF patients are likely related to several confounding factors, including diet (18,41), genetic background (15–17,20), and age. Considering diets, it is possible that substantial amounts of medium-chain fatty acids from the formula used for longer than 7 d in our study exerted a protective effect against β-oxidation of EFA, thus resulting in a more efficient use of ALA for DHA biosynthesis. Our experience with the F508del-mouse model also differed from results reported by Werner et al. (18); in this study, mice had a low incidence of intestinal obstruction when fed standard non-purified diet, obviating the use of special formulated liquid diets or osmotic laxatives, contrary to our model. A possible explanation is that the nonpurified diet contained DHA due to the presence of fish flour, accounting for $1.62 \pm 0.32\%$ of total fatty acids ($n = 3$ analyses) compared with DHA-free standard nonpurified diet ($0 \pm 0\%$; $n = 3$ analyses). The presence of DHA in nonpurified diet might favor tissue accretion of DHA during fetal and lactation periods, protecting pups against intestinal obstruction. This point would also explain why Werner et al. (18) found high levels of DHA and no change in AA in their F508del mice, as DHA supplementation would normalize AA biosynthesis (17). A more suitable diet should be designed to simulate in F508del mice the (n-3) fatty acid metabolism defect found in humans. Moreover, the genetic background of mice is recognized as very important for PUFA status (15,16). Indeed, a decrease in the DHA concentration of lungs, pancreas, and intestine seems to be related to the use of *cftr* $^{-/-}$ mice bred into a congenic background (17,21) compared with *cftr* $^{-/-}$ mice bred into a mixed genetic background (20,21) as used in our study. This factor has to be taken into account to develop a more suitable model to elucidate PUFA metabolism abnormalities. In addition, CF mouse models are often studied at 1–3 mo of age (17–20,41) and no influence of age on PUFA status has been reported. If, as in humans, PUFA deficiency occurs later in life, we postulate that this phenomenon might occur in older mice, suggesting that PUFA metabolism should be studied in mice that are at least 6 mo old.

Despite discrepancies in PUFA composition in the different CF mouse models, even from the same genetic background (17,19), oral DHA supplementation has convincingly shown beneficial effects by correcting the lipid imbalance and reversing the morphological changes in pancreas and ileum (17) or by ameliorating the severity of liver disease (19). However, very high DHA doses (40 mg/d) were used in these studies (17,19), making their use in CF patients less satisfactory because of a potential risk of increasing oxidative stress (26). Thus, the results of the present study highlight a great potential benefit of supplemental GPL-DHA for CF patients. Numerous studies have shown that important changes in PUFA composition occur in patients with CF as reviewed recently by Al-Turkmani et al. (8) and our group (7). Freedman et al. (4) found an imbalance between DHA and AA in nasal and rectal biopsy specimens from CF patients and a decrease in their plasma DHA levels. The latter finding was also observed in a large cohort of CF patients with or without pancreatic insufficiency from a CF reference center in Brussels (6). Because DHA levels are decreased in CF patients, supplementation with DHA becomes advisable. GPL-DHA is very promising for nutritherapy in CF patients for several reasons: a phospholipidic vector allows for better membrane accretion of DHA compared with triglycerides or ethyl esters (28), GPL-DHA has positive effects in F508del CF-mice at low doses that are unlikely to mediate oxidative stress, and GPL-DHA has already been used safely and effectively in the elderly, resulting in balanced AA levels (27). This may be particularly beneficial in CF patients with pancreatic insufficiency who have shown alterations in AA levels concomitant with decreased levels of DHA (6).

In conclusion, this article presents novel findings on PUFA status changes in F508del-CF fed an EFA-sufficient diet. The F508del-CF mouse fed the specified formula is a suitable model that reproduces (n-6) fatty acid metabolism anomalies found in F508del CF patients. However, further research is warranted to understand why this model is not DHA deficient. Nevertheless, this study highlights that DHA delivered via a glycerophospholipid vector at a low dose has beneficial effects in a relevant animal model of CF by correcting the (n-6) fatty acid status and increasing the DHA/AA ratio in most of the analyzed organs and merits further study as nutritherapy for CF.

Acknowledgments

The authors thank Dr. G. Pieroni for the generous gift of GPL-DHA (Application Santé des Lipides, Bioparc Vichy, Hauterive, France), Dr. B. Scholte for the generous gift of Cftrtm1EUR [F508del (FVB129)] breeding pairs (Erasmus MC Rotterdam, Netherlands), and Dr. J. Philpott (Oklahoma University, OK) and Professor J. Storch (Rutgers University, New Brunswick, NJ) for English reading. T.C.C., P.W., T.L. and M.A. designed research, M.M. and T.C.C. conducted research and analyzed data, J.L. and P.L. gave advice, T.C.C., T.L., J.L. and M.A. wrote the paper, and M.A. had primary responsibility for final content. All authors read and approved the final manuscript.

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