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Persistence of Essential Fatty Acid Deficiency in Cystic Fibrosis Despite Nutritional Therapy

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ABSTRACT: To study the evolution of plasma fatty acid composition of patients with cystic fibrosis (CF) in relation to nutritional status, pancreatic function, and development of CF-related liver disease (CFRLD) and diabetes mellitus, 24 CF pediatric patients with stable pulmonary disease were studied before and after an approximate period of 8 y. Nutritional status, pulmonary function, pancreatic function, and presence of CFRLD or diabetes mellitus were recorded. Results were compared with data obtained in 83 healthy children. Patients with CF have significantly lower linoleic acid (LA), docosahexaenoic acid (DHA), lignoceric acid, and LA × DHA product and higher oleic acid, mead acid, dihomo-γ-linoleic acid, and docosapentaenoic acid (DPA). Comparison of samples taken at first and second studies revealed a significant decrease in LA levels and lignoceric acid associated with a significant increase in dihomo-γ-linoleic acid levels. Patients with CFRLD showed significantly higher mead acid/arachidonic acid ratio and lower total ω6 polyunsaturated fatty acids content. There was no relation of plasma fatty acids composition with pancreatic function, pulmonary function, or diabetes mellitus. Follow-up of patients with CF shows that essential fatty acids deficiency, particularly in LA and DHA content, persisted unmodified along time despite an adequate nutritional therapy. Future studies after supplementation with ω3 polyunsaturated fatty acids should be undertaken. **(*Pediatr Res* 66: 585–589, 2009)**

Cystic fibrosis (CF) is the most common autosomal recessive disease in the white population. CF is caused by mutations in the gene encoding for the CF transmembrane conductance regulator (CFTR). More than 1300 mutations have been identified, but the most frequent mutation is a deletion of phenylalanine in position 508 ([Delta]F508), which results in defective synthesis and folding of the mutant protein with failure to reach the apical membrane of many epithelial cell types. The search for a genotype-phenotype correlation has not been very successful, with modifier genes and environmental factors playing a part in determining severity of the disease (1).

The prognosis of CF has improved steadily over the past five decades, mainly because of aggressive treatment before the onset of irreversible pulmonary changes. Adequate nutritional therapy has been a basic element for the improvement in the quality of life of the patients (2). However, increased survival rates have revealed the development of varied metabolic alterations, and different reports have pointed out that essential fatty acid (EFA) deficiency may play an important role in symptoms and disease progression (3–5). The pattern found is a decrease in plasma content of both linoleic (LA) and docosahexaenoic acids (DHA), without gross alteration in the content of the precursor of the ω3 series, α-linolenic acid (6–8). This profile is different from that usually present in typical nutritional EFA deficiency (9). Fatty acid composition may be influenced by genetic factors as shown in murine models (10,11) or in humans (12). The role played by pancreatic insufficiency may also be especially relevant because fat malabsorption persists in many patients despite pancreatic enzyme replacement (13).

We report herein plasma fatty acid composition in 24 patients with CF followed up as a cohort for a period of ~8 y to establish its relationship with nutritional status, pancreatic function, and development of CF-related liver disease (CFRLD) and diabetes mellitus.

PATIENTS AND METHODS

We have studied 24 patients with CF (15 men, 9 women) meeting the consensus-statement requirement for the diagnosis of this disease (14) and regularly followed up in our center. Twenty-two of the 24 patients presented the mutation [Delta]F508 in at least one of the alleles and 9 were homozygous for the mutation. Patients were studied twice: before and after an approximate period of 8 y. Age ranged from 7.8 ± 3.9 y at the first study to 14.0 ± 3.6 y at the second study.

Patients ingested a diet providing a calorie intake of about 120% of RDA with 35–40% of calories coming from fat. No supplementation with ω3 polyunsaturated fatty acids (PUFA) was given. All patients who were pancreatic insufficient received supplements of fat soluble vitamins containing cholecalciferol (1,000 IU), α-tocopherol acetate (100 mg), phytomenadion (1 mg), retinol acetate (10,000 IU), and pancreatic enzyme replacement therapy (15).

Control group was formed by 83 healthy children (age: 6.2 ± 3.6 y) who were undergoing minimal surgical procedures. None of them had history of chronic or recent illness, and they were not taking any medication.

The study was reviewed and approved by the ethics committee of the hospital. Informed consent from the children and/or parents was obtained.

Clinical and anthropometric studies. Genotypes were determined with the INNO-LiPA CFTR19 and INNO-LiPA CFTR17+Tn Update kit (Innogenetics N.V.) or sequencing of the CFTR genome. Nutritional status was assessed by BMI [weight (gram)/height^2 (square meter)]. Anthropometric values were expressed as z scores using Spanish reference data (16). Respiratory involvement was assessed by lung function testing. Forced expiratory volume in 1 s

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Abbreviations: AA, arachidonic acid; **CF,** cystic fibrosis; **CFRLD,** cystic fibrosis related liver disease; **CFTR,** cystic fibrosis transmembrane conductance regulator; **DHA,** docosahexaenoic acid; **DPA,** docosapentaenoic acid; **EFA,** essential fatty acid; **LA,** linoleic acid; **MUFA,** monounsaturated fatty acids; **PUFA,** polyunsaturated fatty acids; **SFA,** saturated fatty acids

was expressed as percentage of the predicted value for age, sex, and height (17). Exocrine pancreatic sufficiency were defined as a fecal elastase-1 concentration >200 IU/g or a fat content in stool samples <7% (wt/wt) of the amount of dietary fat (18). The diagnosis of CFRLD was made by ultrasonography of the liver. According to Williams *et al.* (19), CFRLD is present when an ultrasound score above 6 is associated with a splenomegaly. None of our patients with CFRLD had ascites, coagulation disorders, or hypoalbuminemia. CF-related diabetes was defined by requirement for insulin therapy.

Laboratory procedures. Biochemical determinations were carried out in blood drawn in the morning after an overnight fast of 12 h. Blood samples were cooled in an ice-water bath and immediately centrifuged at 1000 × g for 45 min at 4°C. The platelet-poor plasma was aliquoted and stored at −20°C until the assay was performed, usually within a few days.

Fecal elastase-1 was determined with a commercial ELISA kit (She-Bo.Tech, Giessen, Germany). Plasma total fatty acids were transmethylated according to Lepage and Roy (20) using tridecanoic acid as the internal standard. The fatty acid methyl esters were separated and quantified on a Hewlett Packard GC 5890 gas chromatograph using a flame-ionization detector on a capillary column SP 2330 (30 m × 0.25 mm, 0.20 mm) (Supelco, Bellefonte, PA). The oven temperature was 80°C at injection, and this was maintained for 1 min, then raised by 50°C/min to 140°C, 5°C/min to 190°C and maintained for 5 min, and finally raised by 5°C/min to 215°C and isothermally maintained for 15 min. Injector and detector temperatures were 250°C. Helium was used as the carrier gas under a pressure of 0.5 bars. Identification of fatty acids was performed by comparison with commercial standards (Nu Chek, Elysian, MN).

Quantification of each fatty acid was done by electronic integration. Results for fatty acids are expressed as percent weight. The fatty acids were grouped in saturated fatty acids (SFA) (14:0, 16:0, 17:0, 18:0, 22:0, and 24:0), monounsaturated fatty acids (MUFA) (14:1 ω5, 16:1 ω7, 18:1 ω9, and 24:1 ω9), ω6 PUFA (18:2 ω6, 18:3 ω6, 20:3 ω6, 20:4 ω6, 22:4 ω6, and 22:5 ω6), and ω3 PUFA (18:3 ω3, 20:5 ω3, 22:5 ω3, and 22:6 ω3).

The product/precursor ratios were used to calculate the activities of the enzymes involved in fatty acids metabolism: elongase (18:0/16:0), delta 6 desaturase (18:3 ω6/18:2 ω6), and delta 5 desaturase (20:4 ω6/20:3 ω6). We determined the EFA deficiency by the measurement 20:3 ω9 (mead acid)/20:4 ω6 [arachidonic acid (AA)] ratio and sufficiency of DHA index (22:6 ω3/22:5 ω6).

Statistical analysis. We performed analysis using SPSS Version 16.0 statistical software. Descriptive statistic is presented as median and interquartile range. Spearman's [*rho*] test was used to evaluate relationship between continuous variable. Statistically significant differences between groups were analyzed using the Kruskall-Wallis test. The Kendall-Tau test was used to measure correlations between ordinal-level variables and the strength of their relationships. All p values are two tailed, and the level of significance was $p < 0.05$.

RESULTS

Anthropometric and clinical findings. Table 1 summarizes anthropometric and clinical findings in patients with CF at first and second studies. No significant changes took place in SDS values for height, weight, and BMI. All, except one, evidenced signs of exocrine pancreatic insufficiency at both studies. At the second study, nine patients had ultrasonic signs of hepatopathy and six manifested clinical diabetes mellitus. As shown in Table 2, at the second study, plasma values for glucose and insulin had increased significantly.

Table 1. *Anthropometric and clinical studies (mean ± SD)*

	First study (n = 24)	Second study (n = 24)	p
Age (y)	7.8 ± 3.9	14.0 ± 3.6	<0.001
Height (cm)	121.1 ± 23.4	151.3. ± 14.9	<0.001
SDS	−0.73 ± 0.74	−0.98 ± 0.82	NS
Weight (kg)	26.5 ± 11.9	45.6 ± 10.6	<0.001
SDS	−0.61 ± 0.50	−0.73 ± 0.58	NS
BMI (kg/m^2)	17.1 ± 2.1	19.4 ± 3.1	<0.001
SDS	−0.33 ± 0.75	−0.39 ± 0.69	NS
FEV1 (% of predicted)	97.0 (63.7–129.0)	105.5 (95.6–115.9)	NS
Exocrine pancreatic insufficiency (n)	23/24	23/24	NS
CF-related liver disease (n)	5/24	9/24	NS
CF-related diabetes mellitus (n)	4/24	6/24	NS

Table 2. *Metabolic studies (mean, range)*

	First study (n = 24)	Second study (n = 24)	p
Glucose (mg/dL)	82 (66–99)	90 (76–150)	0.002
Glucose 30 (mg/dL)*	181.5 (111.0–227.0)	132.0 (99.0–239.0)	NS
Insulin (μU/mL)	9.7 (5.2–19.0)	19.0 (12.0–52.0)	0.012
Insulin 30 (μU/mL)*	34.5 (16.5–70.5)	28.0 (24.0–76.0)	NS
Glycosylated Hb (%)	5.6 (4.2–7.7)	5.5 (4.9–7.8)	NS

* Values at 30 min after an oral glucose load of 1.75 g/kg (maximum, 75 g).

Plasma fatty acid composition. Plasma fatty acid composition in patients with CF differed significantly from that in healthy controls (Table 3). Overall, in patients with CF, contents of SFA and MUFA were increased, whereas content of PUFA was globally decreased. Among PUFA, mean concentrations of LA, DHA, lignoceric acid, and LA × DHA product were decreased, whereas those of dihomo-γ-linoleic acid and docosapentaenoic acid (DPA) were increased. Comparison of samples taken at first and second studies revealed a significant decrease in LA levels and lignoceric acid associated with a significant increase in dihomo-γ-linoleic acid levels.

Patients who evidenced CFRLD showed significantly higher mead acid/AA ratio at the first study (0.032 ± 0.012 *versus* 0.022 ± 0.009, $p = 0.035$) and lower total ω6 PUFA content at the second study (33.51 ± 3.79 *versus* 37.32 ± 4.49, $p = 0.041$) in comparison with patients without CFRLD. No differences were found between patients with or without diabetes mellitus.

Correlations with anthropometric values. Forced expiratory volume in 1 s correlated significantly with BMI at both studies ($r = 0.47$, $p = 0.031$ and $r = 0.53$, $p = 0.009$, respectively) (Table 4).

At the first study, BMI correlated positively with DHA and negatively with α-linolenic acid. At the second study, BMI correlated positively with LA and negatively with oleic acid, total MUFA, and mead acid/AA ratio.

Correlations between fatty acid values. At both studies, LA related negatively with oleic acid, total MUFA, mead acid/AA ratio, and δ6 desaturase. Lignoceric acid related positively with total PUFA and ω6 PUFA and negatively with oleic acid, total MUFA, and mead/AA ratio, as can be seen in Table 4.

Comparison between initial and follow-up fatty acid values showed that an increase in γ-LA predicted a decrease in LA. Initial values for LA correlated positively with follow-up values for AA and δ6 desaturase.

DISCUSSION

Our results confirm the known presence of profound alterations in plasma fatty acid profile in patients with CF with decreased content of both LA and DHA and increased content of DPA (6–8,12). It should be remarked that DHA and DPA levels remained steady over time, whereas LA levels de-

Table 3. *Plasma fatty acid composition (% wt/wt) (median, quartiles Q1–Q3)*

	First study (n = 24)	Second study (n = 24)	Control subjects (n = 83)
Palmitic acid (18:0)	7.6 (7.0–8.1)	7.6 (7.0–8.3)	7.9 (7.5–8.2)
Lignoceric acid (24:0)*†	0.67 (0.59–0.72)	0.50 (0.44–0.60)	0.78 (0.68–0.88)
Oleic acid (18:1 ω9c)*	21.9 (19.5–22.7)	22.8 (20.8–24.1)	18.9 (16.9–21.1)
Mead acid (20:3 ω9)*	0.15 (0.11–0.26)	0.16 (0.14–0.16)	0.12 (0.10–0.17)
LA (18:2 ω6)*†	27.0 (24.3–29.2)	25.2 (22.6–27.7)	30.7 (27.2–32.8)
γ-Linoleic acid (18:3 ω6)*	0.6 (0.5–0.7)	0.6 (0.5–0.8)	0.3 (0.3–0.4)
Dihomo-γ-linoleic acid (20:3 ω6)*†	1.9 (1.6–2.1)	2.1 (1.9–2.4)	1.7 (1.5–1.8)
AA (20:4 ω6)	6.8 (6.2–7.7)	7.1 (6.4–7.8)	7.2 (6.6–7.9)
DPA (22:5 ω6)*	0.24 (0.20–0.29)	0.25 (0.19–0.29)	0.22 (0.17–0.26)
α-Linolenic acid (18:3 ω3)	0.3 (0.2–0.5)	0.3 (0.2–0.4)	0.3 (0.2–0.4)
EPA (20:5 ω3)	0.45 (0.3–0.6)	0.5 (0.3–0.7)	0.5 (0.4–0.6)
DHA (22:6 ω3)*	1.7 (1.2–2.0)	1.9 (1.2–2.3)	2.5 (2.0–2.8)
SFA*	32.7 (32.0–33.7)	33.6 (30.6–34.6)	31.9 (31.1–32.7)
MUFA*	26.8 (24.3–28.3)	27.4 (26.1–29.5)	23.6 (21.3–26.0)
PUFA*	40.7 (38.2–43.1)	39.3 (34.9–43.1)	44.0 (41.5–46.7)
ω6 PUFA*	37.6 (35.0–39.6)	36.9 (31.3–37.9)	40.6 (37.3–43.7)
ω3 PUFA*	3.1 (2.6–3.6)	3.2 (2.4–4.0)	3.7 (2.2–4.2)
18:0/16:0*	0.35 (0.32–0.39)	0.35 (0.29–0.38)	0.40 (0.36–0.42
AA (20:4 ω6)/LA (18:2 ω6)*	0.27 (0.19–0-31)	0.28 (0.22–0-33)	0.24 (0.20–0.28)
Mead acid (20:3 ω9)/AA (20:4 ω6)*	0.02 (0.02–0.03)	0.02 (0.02–0.04)	0.02 (0.01–0.02)
AA (20:4 ω6)/DHA (22:6 ω3)*	5.0 (3.2–5.8)	4.8 (3.8–5.6)	3.0 (2.5–3.7)
LA (18:2 ω6) × DHA (22:6 ω3)*	46.0 (29.9–55.7)	44.5 (28.1–69.9)	72.9 (59.4–86.9)

Significance ($p < 0.05$): * patients *vs* control subjects; † first study *vs* second studies.

LA, linoleic acid; AA, arachidonic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoicoic acid; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Table 4. *Significant correlations between parameters*

Correlations between anthropometric and fatty acid values		
First study		
BMI *vs* DHA	$r = 0.54$ ($p = 0.006$)	
BMI *vs* α-linolenic acid	$r = -0.53$ ($p = 0.008$)	
Second study		
BMI *vs* LA	$r = 0.54$ ($p = 0.006$)	
BMI *vs* oleic acid	$r = -0.42$ ($p = 0.045$)	
BMI *vs* total MUFA	$r = -0.45$ ($p = 0.030$)	
BMI *vs* mead acid/AA ratio	$r = -0.48$ ($p = 0.020$)	
Correlations between fatty acid values		
First and second studies, respectively		
LA *vs* oleic acid	$r = -0.71$ ($p = 0.001$)	$r = -6.67$ ($p = 0.001$)
LA *vs* total MUFA	$r = -0.81$ ($p = 0.001$)	$r = -0.78$ ($p = 0.001$)
LA *vs* mead acid/AA ratio	$r = -0.77$ ($p = 0.001$)	$r = -0.67$ ($p = 0.001$)
LA *vs* δ6 desaturase	$r = -0.66$ ($p = 0.001$)	$r = -0.65$ ($p = 0.001$)
Lignoceric acid *vs* total PUFA	$r = 0.48$ ($p = 0.020$)	$r = 0.67$ ($p = 0.001$)
Lignoceric acid *vs* total ω6 PUFA	$r = 0.54$ ($p = 0.010$)	$r = 0.65$ ($p = 0.001$)
Lignoceric acid *vs* oleic acid	$r = -0.49$ ($p = 0.003$)	$r = -0.49$ ($p = 0.013$)
Lignoceric acid *vs* total MUFA	$r = -0.46$ ($p = 0.005$)	$r = -0.60$ ($p = 0.002$)
Lignoceric acid *vs* mead acid/AA ratio	$r = -0.42$ ($p = 0.003$)	$r = -0.40$ ($p = 0.050$)
First *vs* second studies		
γ-linoleic *vs* LA	$r = -0.52$ ($p < 0.001$)	
LA *vs* AA	$r = 0.40$ ($p = 0.050$)	
LA *vs* δ6 desaturase	$r = 0.65$ ($p = 0.001$)	

MUFA, monounsaturated fatty acids; LA, linoleic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoicoic acid; sufficiency of DHA index, 22:6 ω3/22:5 ω6.

creased progressively. The LA × DHA product has been recently proposed as a predictive diagnostic marker to differentiate patients with CF from non-CF controls: a value ≤40 could be used as a clinical cutoff for CF (21). Our findings support such conclusion because many patients had a LA × DHA product below 40. Although the patients did not evidence clinical signs of EFA deficiency, the elevation of DPA and mead acid levels suggests that subclinical deficiency was indeed present (22).

It is generally believed that alterations in plasma fatty acid profile in CF are directly dependent on poor nutrition and fat malabsorption (23). In fact, these factors could play a role in our patients, who presented some degree of exocrine pancreatic insufficiency despite adequate nutritional therapy.

Significant correlations were found between BMI and several fatty acids, such as LA, α-linolenic acid, MUFA, and mead acid/AA ratio. In addition, BMI correlated positively with DHA at the first study, but this correlation was lost posteriorly.

Explanation for the low levels of DHA present throughout the study is less evident if we take into account that plasma levels of this fatty acid are not exclusively dependent on exocrine pancreatic function (24). Competitive metabolism with fatty acids ω6 or accentuated catabolism could be in cause (25). The behavior of lignoceric acid was especially interesting. Plasma concentration of this fatty acid related better with PUFA than with other SFA. The interpretation of this finding is not apparent but, probably, reflects an increment of its β-oxidation both at mitochondrial and peroxisomal levels (26). Plasma lignoceric acid could, therefore, be considered as a possible biomarker to follow PUFA supplementation.

Genetic factors may be also implicated in the genesis of EFA deficiency. CFTR is expressed in the apical membranes of various epithelial cells and acts as an important regulator of phospholipid composition (27). It has been shown that in CF cells the incorporation of LA into phospholipids is impaired resulting in increased formation and turnover of AA due to the action of phospholipase A_2 (28). Both the experimental correction of CFTR mutation and the inhibition of phospholipase A_2 permit the liberation of AA from membrane phospholipids suggesting that there is a close association between the genetic defect and plasma fatty acid abnormalities (29,30). Our findings agree with this hypothesis. The significant elevation of the AA/LA ratio indicates that the pathway from LA to AA was enhanced. Furthermore, the association of low levels of LA with normal or high levels of α-linolenic acid, oleic acid, mead acid/AA ratio and δ6 detasurase would suggest that fat malabsorption was not the only causative agent. The different plasma fatty acid profile found in patients with or without CFRLD will support such conclusion.

Over the last decades, many studies have pointed out the fundamental role that ω6 and ω3 long chain fatty acids play in the healthy status of human beings (31). The ω6 long chain fatty acids are precursors of eicosanoids and prostanoids, which are important messengers for endothelial integrity, acid secretion, and inflammatory processes. Liberation of AA from cell membranes, by the action of phospholipase A_2, is a limitant step in the synthesis of prostanoids (32). Freedman *et al.* (33) have observed an increased AA/DHA ratio in tissues of cftr (−/−) mice. The authors cannot conclude whether the simultaneous elevation of prostanoids in such tissues represents the cause or the consequence of the inflammatory process observed in CF. Inflammation may also contribute to EFA deficiency through an increased oxidation of fatty acids (34).

The ω3 long chain fatty acids, and particularly DHA, are structural components of cell membrane phospholipids and are necessary for normal neurologic development (35,36). DHA is also a precursor of several mediators such as docosatrienes, resolvins, and neuroprotein D (37,38). These mediators are involved in the resolution phase of the inflammatory process, which, as mentioned, is enhanced in patients with CF. A decreased bioavailability of DHA may, therefore, have a deleterious effect on tissue functionality and, particularly, on pulmonary and pancreatic cells (24).

As expected, development of CFRLD resulted in aggravation of the EFA deficiency status (12). It should be paid attention to the progressive deterioration of endocrine pancreatic function as revealed by changes in fasting plasma glucose and insulin levels and by the high proportion of patients (25%) developing clinical diabetes mellitus over a follow-up period of 8 y. In this sense, regular control of blood glycosylated Hb seemed to be of little value for early detection of this complication. The lack of influence of overt diabetes on plasma fatty acid profile would indicate that the role played by insulin secretion is not relevant.

Nutrition therapy stands as an obligatory tool in the management of patients with CF. There is a consensus that a fat-rich, high-calorie diet should be provided given the persistence of some degree of exocrine pancreatic insufficiency despite oral administration of pancreatic enzymes (39,40). In our patients, nutrition status seemed to be adequate as judged by values of BMI and its close relation with improvement of pulmonary function. However, plasma values of LA and DHA remained persistently diminished along time. Long chain fatty acids of ω3 series, in the form of fish oil capsules, are easily absorbed by the intestine and become incorporated into plasma and cell membrane phospholipids (41). Daily administration of 150 mg of DHA during 12 wk was followed by a 50% increase in DHA-containing phosphatidyl choline both in plasma and in red cell membrane phospholipids, associated with a proportional decrease in AA content (42). Therefore, regular administration of ω3 log chain fatty acids could bring an important clinical benefit with lack of adverse effects (43).

In conclusion, follow-up of patients with CF shows that EFA deficiency, particularly in LA and DHA content, persisted unmodified along time despite an adequate nutritional therapy, which included supplements of fat soluble vitamins and pancreatic enzyme replacement. The persistence of EFA deficiency supports the conclusion that fat malabsorption was not the unique cause of dyslipidemia and that genetic-related endogenous factors may also play an important role on its development. In this sense, it remains to be demonstrated whether oral administration of DHA supplements, as it has been recently recommended, improves ω3 PUFA deficiency and brings a clear clinical benefit to patients with CF. The potential value of new biomarkers, such as LA × DHA product or lignoceric acid, to survey such therapeutic intervention should be also investigated.

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