Direct transesterification of plasma fatty acids for the diagnosis of essential fatty acid deficiency in cystic fibrosis

Guy Lepage,[1] Emile Levy, Nancy Ronco, Lesley Smith, Narmer Galéano, and Claude C. Roy

Pediatric Gastroenterology Unit, Centre de Recherche Pédiatrique, Hôpital Ste-Justine, and Department of Pediatrics, Université de Montréal, Montréal, Quebec, Canada

Abstract This study was aimed at redefining criteria for essential fatty acid (EFA) deficiency with the use of the direct transesterification procedure (1986. *J. Lipid Res.* **27:** 114–120) and at determining whether a simple assay of total fatty acids (FA) is as predictive of EFA deficiency as the FA pattern from plasma, red cell, and platelet phospholipids. Fasting blood samples were taken from 163 cystic fibrosis (CF) patients who were encouraged to consume 35–40% of their calories as fat. Their mean (± SD) age was 9.6 ± 4.8 yr. The control group consisted of 44 unaffected siblings aged 13.1 ± 3.1 yr. The 20:3(n-9)/20:4(n-6) ratio in 77 (47%) CF children was more than 2 SD above the values (mean ± SD) of 0.021 ± 0.007 obtained in the 44 controls. Groups of EFA-sufficient (n = 10) and EFA-deficient (n = 7) subjects were selected for further studies. The plasma total FA 20:3(n-9)/20:4(n-6) ratios of 0.029 ± 0.003 in EFA-sufficient and of 0.216 ± 0.103 in EFA-deficient was as good a discriminant as FA in phospholipids from plasma, red cell PC, and platelets. Among the 21 individual fatty acids, 20:3(n-9), which was also found in controls, and 16:1(n-7) (palmitoleic) proved to be the most sensitive indices of EFA deficiency. They are equally reliable in plasma, red cells, and platelets, but the inverse linear relationship ($r = -0.91$) between the n-7 family and 18:2(n-6) proved to be more closely associated with EFA deficiency than the one ($r = 0.66$) between 20:3(n-9) and 20:4(n-6). In view of these findings, we recommend that close monitoring of plasma FA be carried out in CF because of the high incidence of EFA deficiency despite efforts to improve and liberalize fat intake. — **Lepage, G., E. Levy, N. Ronco, L. Smith, N. Galeano, and C. C. Roy.** Direct transesterification of plasma fatty acids for the diagnosis of essential fatty acid deficiency in cystic fibrosis. *J. Lipid Res.* 1989. **30:** 1483–1490.

Supplementary key words linoleic acid • palmitoleic acid • gas-liquid chromatography • desaturase

Malnutrition and failure to thrive are two common and severe manifestations of cystic fibrosis (CF) closely related to survival (1). They are due to insufficient energy intake (2–4) despite increased need (5) compounded by infections, chronic lung disease, and malabsorption. Although clinical evidence of essential fatty acid (EFA) deficiency has rarely been reported, a number of studies have documented its high incidence on the basis of plasma lipids (6, 7), erythrocyte and platelet phospholipids (PL) (8), as well as in tissue PL (9).

As constituents of cell membranes, EFA have profound effects on membrane fluidity and function (10). There is evidence that EFA deficiency may have an impact on the clinical status of patients through altered eicosanoid production (11, 12) and immune function both of which could, affect progression of the pulmonary disease (13). Furthermore, progress in analytical procedures (14) and refinements of gas-liquid chromatography technology with the advent of capillary columns have improved the precision, the accuracy of separation, and the threshold of detection.

The aims of this study were twofold: *1*) to redefine criteria of EFA deficiency using state of the art methods, and *2*) to determine whether a simple assay of total plasma FA is as predictive of EFA deficiency as the FA pattern from plasma, red cell, and platelet PL.

MATERIALS AND METHODS

Study subjects

Blood samples were collected with EDTA as anticoagulant in the fasting state (for a minimum of 12 h) from 163 CF patients randomly selected from a total of 297 children and adolescents attending the CF clinic.

Abbreviations: EFA, essential fatty acid; CF, cystic fibrosis; PL, phospholipid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PUFA, polyunsaturated fatty acid; RBC, red blood cells; TLC, thin-layer chromatography.

[1]To whom reprint requests should be addressed at: Pediatric Research Center, Hôpital Ste-Justine, 3175 Ste-Catherine road, Montréal, Québec, Canada, H3T 1C5.

TABLE 1. Clinical characteristics of cystic fibrosis patients

Groups	Age	Z Scores for Growth[a]		Clinical Score[b]	Fecal Fat (N: <5 g/24 h)	Vit E/TG (N:0.009–0.019)	Liver Function Tests		
		Weight	Height				Bilirubin (N: <0.8 mg/dL)	ALT (N: <35 U/L)	GGT (N: <45 U/L)
	yr								
EFA-sufficient[c] (n = 10)	11.6 ± 0.6	−0.61 ± 0.32	−0.40 ± 0.38	80 ± 3.4	10.5 ± 2.5	0.011 ± 0.001	0.2 ± 0.05	15.4 ± 2.4	10.5 ± 1.5
EFA-deficient (n = 7)	13.9 ± 1.7	−1.22 ± 0.30	−1.66 ± 0.32	71.9 ± 4.6	21.1 ± 5.5	0.005 ± 0.001	0.4 ± 0.06	37.4 ± 17.9	43.7 ± 10.6
Significance (*P* value)	NS	NS	0.02	NS	0.05[d]	0.02	0.02	0.05	0.01

[a]Standard deviation scores for French Canadian children (15).
[b]Modified Shawchman scores (16).
[c]Plasma 20:3(n-9)/20:4(n-6) ratios = 0.02 identify the EFA-sufficient group, and values 0.10, the EFA-deficient.
[d]One-tail *t* test.

These patients with an age range of 1 to 23 yr and a mean (± SD) of 9.6 ± 4.8 yr were encouraged to consume 35–40% of their calories as fat but no dietary records were kept. The control group consisted of 44 unaffected siblings aged 8–21 yr (13.1 ± 3.1 yr). Using the ratio of 20:3(n-9)/20:4(n-6) in plasma as a measure of essential fatty acid status, two subgroups consisting of EFA-deficient (n = 7) and EFA-sufficient (n = 10) subjects were chosen from the each tail of the distribution curve. The EFA-sufficient group had a 20:3(n-9)/20:4(n-6) ratio that did not differ from that of 44 controls whereas the ratio for the EFA-deficient children was >0.1. The two groups of CF children were asked to stop all medications for 10 days except pancreatic enzymes and vitamins. The clinical characteristics of the CF children (**Table 1**) show that the EFA-deficient group differed from the EFA-sufficient patients in terms of Z score for height, steatorrhea, vitamin E status, and liver function tests. Furthermore, three of the seven EFA-deficient patients had clinical, biochemical, and ultrasound evidence of chronic liver disease. The study protocol was approved by the Ethics Committee of l'Hôpital Ste-Justine, November 6, 1986.

Methods

For the screening study, 4 ml of blood was collected into ice-cooled EDTA (1.5 g/l) tubes. Plasma was kept at −50°C and total fatty acid analysis on 100 μl was performed within 1 month of collection as described previously (14) and summarized below.

In patients and controls selected for more complete evaluation of their EFA status, 500 μl of plasma was also processed for fatty acid analysis of the various species of PL. Total lipids were extracted by the method of Folch, Lees, and Sloane Stanley (17). The PL were separated by TLC (18) and visualized with iodine vapor. Each one of the five spots corresponding to sphingomyelin, phosphatidylcholine (PC), phosphatidylserine, phosphatidylinositol, and phosphatidylethanolamine (PE) was scraped off and directly transesterified with 200 μl of acetyl chloride, after addition of 250 nmol of 13:0 as internal standard, in 2 ml of methanol–benzene 4:1 (v/v) (14). Red blood cells (RBC) were washed three times with isotonic saline before being frozen at −50°C until determination of the fatty acid pattern of the five species of PL using the procedure described above for plasma.

Platelet PL required the collection of 15 ml of whole blood flowing freely without the help of a tourniquet into tubes containing sodium citrate (4 g/l) and citric acid (0.5 g/l). In order to obtain washed platelets, platelet-rich plasma was centrifuged at 1350 *g* for 15 min and platelets were resuspended in a 2% EDTA–saline solution. Three further washings were carried out as published elsewhere (19) before freezing the pellet at −50°C. In view of the

small size of the pellets, the assay was limited to the determination of total PL. Platelet PL were separated from other classes of lipids by TLC using the solvent system: hexane–diethyl ether–glacial acetic acid 80:20:3 (v/v/v).

Fatty acid methyl esters, products of a direct transesterification procedure with acetyl chloride (14), were injected into a 60-m fused silica capillary column coated with SP-2331. Analysis was performed on a Hewlett-Packard 5880 gas chromatograph as described earlier (20).

Statistical analysis

The data were evaluated by ANOVA and the difference between the means was analyzed by the Scheffe procedure for groups of unequal size. The level of significance was set at 0.05. The stepwise discriminant analysis technique (21) was used to identify which plasma FA % or FA ratio contributed to the identification of the EFA deficiency.

RESULTS

The plasma fatty acid pattern of the 163 CF children revealed that 77 (47%) had a 20:3(n-9)/20:4(n-6) ratio that was more than 2 SD above the values (mean ± SD) of 0.021 ± 0.007 obtained in the 44 controls. The corresponding ratios for the EFA-sufficient (n = 10) and EFA-deficient (n = 7) groups studied in greater detail with an appropriate number of controls (n = 13 to 44) were 0.029 ± 0.003 and 0.216 ± 0.103, respectively, at a P value <0.05 (one-tail t-test). Total plasma FA concentration in the EFA-sufficient group (9253 ± 510 μmol/l) did not differ from those of the EFA-deficient (8575 ± 676) and from controls (8957 ± 220).

Table 2 shows the plasma fatty acid pattern of the 44 controls and of the two CF groups. The EFA-sufficient patients did not differ from controls with the exception of a marginally lower level of linoleic acid 18:2(n-6) and changes in the n-3 family characterized by higher concentrations of both 18:3(n-3) as well as of 20:5(n-3). The changes observed in the EFA-deficient group were extensive and can be summarized as follows. *1*) As a group, the saturates had a tendency to be higher except for 18:0, 22:0, and 24:0. *2*) The n-7 family was strikingly increased. In comparison with both controls and EFA-sufficient, 6:1(n-7) and 18:1(n-7) were elevated by 180% and 82%, respectively. *3*) Concentrations of FA belonging to the n-9 family were all increased. Of note is the fact that in both controls and EFA-sufficient children, 20:3(n-9) accounted for a significant percentage of total FA (0.17 ± 0.01 and 0.21 ± 0.02, respectively). However, in the EFA-deficient group, there was more than a fourfold increase of 20:3(n-9), originating from oleic acid (18:1(n-9). *4*) Of the two families of essential fatty acids, n-3 and n-6, differences between the EFA-deficient group and controls were limited to the n-6 family. They were all decreased, but linoleic acid was more significantly affected.

TABLE 2. Plasma total fatty acids[a]

Fatty Acid	Controls (44)	Cystic Fibrosis	
		EFA-Sufficient (10)	EFA-Deficient (7)
	mol %	*mol%*	
12:0	0.17 ± 0.02	0.32 ± 0.13	0.44 ± 0.13[b]
14:0	1.15 ± 0.05	1.54 ± 0.09[c]	2.02 ± 0.12[c]
16:0	22.11 ± 0.22	23.23 ± 0.40	27.41 ± 1.04[c]
18:0	7.57 ± 0.11	7.46 ± 0.16	6.60 ± 0.32[b]
20:0	0.39 ± 0.02	0.49 ± 0.03	0.54 ± 0.08
22:0	0.72 ± 0.02	0.58 ± 0.02[c]	0.41 ± 0.03[c]
24:0	0.28 ± 0.02	0.24 ± 0.04	0.25 ± 0.04
16:1(n-7)	1.84 ± 0.08	2.40 ± 0.17	5.16 ± 0.82[c]
18:1(n-7)	1.54 ± 0.04	1.61 ± 0.07	2.81 ± 0.27[c]
18:1(n-9)	19.58 ± 0.36	21.16 ± 0.84	24.53 ± 1.54[c]
20:3(n-9)	0.17 ± 0.01	0.21 ± 0.02	0.89 ± 0.22[c]
24:1(n-9)	0.42 ± 0.03	0.58 ± 0.06	0.79 ± 0.09[b]
18:2(n-6)	28.75 ± 0.48	25.08 ± 1.44[c]	15.96 ± 2.33[c]
20:2(n-6)	0.24 ± 0.01	0.27 ± 0.10	0.20 ± 0.01[c]
20:3(n-6)	1.73 ± 0.04	1.90 ± 0.11	1.48 ± 0.07[c]
20:4(n-6)	8.03 ± 0.17	7.09 ± 0.33	5.99 ± 0.83[b]
22:4(n-6)	0.38 ± 0.02	0.30 ± 0.02	0.32 ± 0.02
18:3(n-3)	0.55 ± 0.02	0.78 ± 0.13[c]	0.46 ± 0.10
20:5(n-3)	0.70 ± 0.07	1.11 ± 0.15[c]	0.64 ± 0.06
22:5(n-3)	0.79 ± 0.06	0.69 ± 0.04	0.44 ± 0.04
22:6(n-3)	1.69 ± 0.07	1.62 ± 0.13	1.23 ± 0.18

[a]Results are expressed as mol% of total fatty acids present. All data represent mean values ± SEM.
[b]Different from controls.
[c]Different from the other two groups.

Totals for the various species and families of fatty acids as well as ratios of relevant FA for assessment of EFA-deficiency and for calculation of desaturase index activities are shown in **Table 3**. As expected from the data in Table 2, the percentage of saturated FA and of polyunsaturated fatty acids (PUFA) and the ratios between EFA/non-EFA as well as PUFA/saturated were altered in EFA-deficient when compared with EFA-sufficient subjects and controls. Of interest are the observations that the EFA-sufficient also had lower EFA/non-EFA and PUFA/saturated ratios than controls. Tabulation of the percentage of total FA accounted for by individual families did not offer any difference except for the fact that the n-3 family was higher in the EFA-sufficient children than in the other two groups. The most striking finding in the EFA-deficient was a twofold increase of the n-7 family. The commonly used index for essential FA deficiency, the ratio of 20:3(n-9)/20:4(n-6), was 10 times higher in EFA-deficient than in EFA-sufficient and controls while that of 16:1(n-7)/18:2(n-6) also proved to be a good discriminant. The relationship between these two ratios in the three populations is shown in **Fig. 1** and **Fig. 2**. There was a close inverse correlation for both but less scatter was seen

TABLE 3. Plasma total fatty acids and desaturase index activity

Fatty Acid	Controls (44)	Cystic Fibrosis	
		EFA-Sufficient (10)	EFA-Deficient (7)
Plasma total fatty acids			
Saturated (%)	32.78 ± 0.30	34.31 ± 0.62	38.32 ± 1.05[a]
PUFA (%)	43.00 ± 0.48	39.03 ± 1.60	26.71 ± 3.27[a]
PUFA/saturated[b]	1.320 ± 0.026	1.148 ± 0.064[a]	0.710 ± 0.100[a]
Total n-3 (%)	3.36 ± 0.12	4.38 ± 0.27[a]	2.78 ± 0.26
Total n-6 (%)	39.64 ± 0.55	34.65 ± 1.51[a]	23.94 ± 3.11[a]
Total n-7 (%)	3.39 ± 0.09	4.01 ± 0.21	7.97 ± 1.06[a]
Total n-9 (%)	20.19 ± 0.36	21.98 ± 0.87	26.26 ± 1.64[a]
16:1(n-7)/18:2(n-6)	0.067 ± 0.004	0.102 ± 0.013	0.426 ± 0.121[a]
20:3(n-9)/20:4(n-6)	0.021 ± 0.001	0.029 ± 0.003	0.216 ± 0.103[a]
EFA/non-EFA[c]	0.777 ± 0.018	0.657 ± 0.043	0.385 ± 0.062[a]
Desaturase index activity			
Δ^6 (20:3(n-6)/18:2(n-6))	0.061 ± 0.002	0.077 ± 0.005[a]	0.104 ± 0.013[a]
Δ^9 (18:1(n-9)/18:0)	2.625 ± 0.076	2.840 ± 0.100	3.800 ± 0.370[a]
Δ^9 (16:1(n-7)/16:0)	0.083 ± 0.003	0.103 ± 0.006	0.185 ± 0.026[a]

[a]Different from the other two groups.
[b]Double bond index.
[c](n-6) + (n-3)/(n-7) + (n-9) + saturated.

around the regression line for n-7 family/18:2(n-6) (Fig. 2) than for 20:3(n-9)/20:4(n-6) (Fig. 1). From the 21 plasma FA (Table 2) and the total of 13 variables obtained through the analysis of plasma FA (Table 3), a stepwise discriminant analysis (21) was performed to identify the best discriminants for EFA deficiency. The percentage of total n-7 (16:1(n-7) + 18:1(n-7)) proved to be the best discriminant. Derivative/precursor ratios taken as indices of FA desaturase activities illustrate that Δ^6 desaturase was higher in EFA-deficient as well as in EFA-sufficient groups than in controls. However, the two indices for Δ^9 desaturase show a higher activity only in the EFA-deficient group.

The FA pattern of plasma phosphatidylcholine which represents 78% of total PL is shown in **Table 4** and mirrors the one tabulated for total plasma FA (Table 2). However, it does seem to be a more sensitive index of EFA deficiency in that a somewhat larger percentage of 16:1(n-7) and 20:3(n-9) are found in the plasma PC FA pattern than in the total FA pattern. Furthermore, the precursor

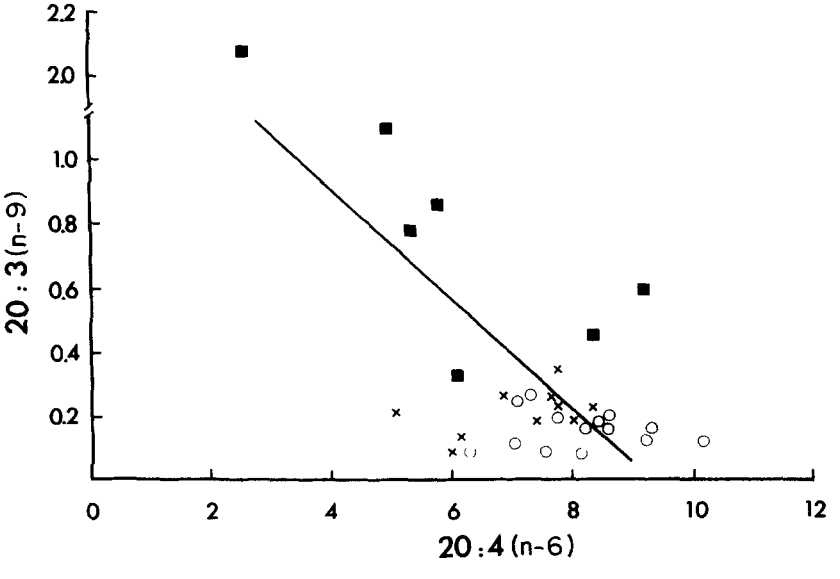

Fig. 1. Linear plot of the relationship between plasma values (percentage of total) of 20:4(n-6) and 20:3(n-9) in controls (○), CF with essential fatty acid sufficiency (×), and CF with essential fatty acid deficiency (■). The equation is $y = -0.17x + 1.58$ ($P < 0.001$), $r = -0.667$.

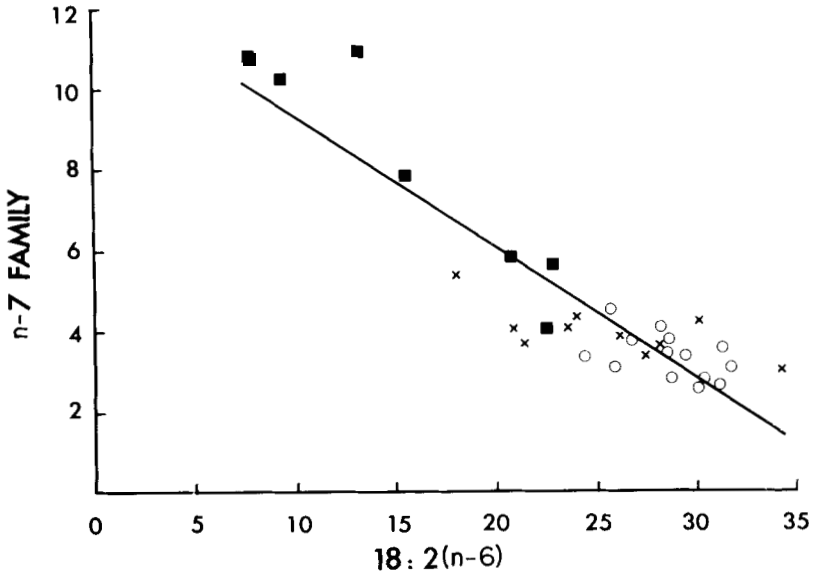

Fig. 2. Linear plot of the relationship between plasma values (percentage of total) of linoleic acid and the total n-7 family (16:1(n-7) + 18:1(n-7)) in controls (○), CF with essential fatty acid sufficiency (X), and CF with essential fatty acid deficiency (■). The equation is $y = -0.33x + 12.64$ ($P < 0.0001$), $r = -0.91$.

of arachidonic acid, 20:3(n-6), which was only slightly increased in total plasma, showed a significant augmentation in the EFA-sufficient children.

The values for total RBC PL (mmol/l) of 1.59 ± 0.08 in controls, 1.70 ± 0.10 in EFA-sufficient subjects, and 1.75 ± 0.11 in EFA-deficient subjects did not differ, nor did the percentage of individual species of PL. Of the fatty acid patterns obtained from each PL fraction, those obtained for PC and PE, which account for close to two thirds of total PL, are shown in **Table 5** and **Table 6**. The fatty acid pattern of RBC PL showed that 16:1(n-7), 18:1(n-7), and 20:3(n-9) were decreased to the same extent in the PC as in the PE of EFA-deficient children. However, for both 18:2(n-6) and 20:4(n-6), trends to lower values were observed but significance was only documented for PC 18:(n-6) (Tables 5 and 6). There was no difference between the platelet PL (nmol/mg protein) values of 94.1 ± 7.3 in controls, 82.5 ± 5.0 in EFA-sufficiency, and 112.4 ± 21.1 in EFA-deficiency. However, the EFA-deficient group showed only a marginally lower percentage of 18:2(n-6) and of 20:4(n-6), whereas 16:1(n-7), 18:1(n-7), and 20:3(n-9) were increased (**Table 7**).

DISCUSSION

This study has shown that there was a high percentage (47%) of CF children with biochemical evidence of EFA deficiency despite the fact that they regularly attended a CF clinic staffed by health professionals who advocate the consumption of a high energy diet with 35–40% fat. A further conclusion is that with the added precision of direct transesterification and the better separation of fatty acids with a 60-m capillary column, total FA analysis on 100 μl of plasma provides as good an assessment of EFA

TABLE 4. Fatty acids in plasma phosphatidylcholine[a]

Fatty Acid	Controls (18)	Cystic Fibrosis	
		EFA-Sufficient (10)	EFA-Deficient (7)
	mol %	*mol %*	
12:0	0.04 ± 0.01	0.06 ± 0.01	0.06 ± 0.01
14:0	0.43 ± 0.03	0.63 ± 0.04[b]	0.70 ± 0.05[b]
16:0	34.84 ± 0.65	34.84 ± 0.48	40.97 ± 0.94[c]
18:0	15.65 ± 0.50	16.38 ± 0.54	13.41 ± 1.17[c]
20:0	0.08 ± 0.01	0.08 ± 0.01	0.08 ± 0.01
22:0	0.06 ± 0.01	0.07 ± 0.01	0.07 ± 0.01
24:0	0.05 ± 0.01	0.06 ± 0.01	0.04 ± 0.01
16:1(n-7)	0.48 ± 0.04	0.67 ± 0.07	1.86 ± 0.36[c]
18:1(n-7)	1.81 ± 0.08	1.62 ± 0.07	2.52 ± 0.22[c]
18:1(n-9)	12.41 ± 0.33	12.14 ± 0.58	16.71 ± 1.64[c]
20:3(n-9)	0.16 ± 0.01	0.23 ± 0.02	1.07 ± 0.33[c]
24:1(n-9)	0.10 ± 0.01	0.10 ± 0.01	0.12 ± 0.01
18:2(n-6)	19.01 ± 0.53	17.60 ± 0.79	12.16 ± 1.52[c]
20:2(n-6)	0.28 ± 0.01	0.34 ± 0.02[c]	0.26 ± 0.02
20:3(n-6)	2.50 ± 0.09	3.10 ± 0.22[c]	2.11 ± 0.11
20:4(n-6)	7.73 ± 0.25	7.11 ± 0.33	5.37 ± 0.96[b]
22:4(n-6)	0.34 ± 0.02	0.39 ± 0.04	0.31 ± 0.02
18:3(n-3)	0.17 ± 0.01	0.24 ± 0.03[c]	0.15 ± 0.02
20:5(n-3)	0.50 ± 0.03	0.83 ± 0.10[c]	0.48 ± 0.06
22:5(n-3)	0.67 ± 0.05	0.84 ± 0.06	0.41 ± 0.09
22:6(n-3)	1.54 ± 0.09	1.58 ± 0.21	0.84 ± 0.19[c]

[a]Results are expressed as mol% of total fatty acids present. All data represent mean values ± SEM.
[b]Different from controls.
[c]Different from the other two groups.

TABLE 5. Fatty acids in red cell phosphatidylcholine[a]

Fatty Acid	Controls (13)	Cystic Fibrosis EFA-Sufficient (9)	Cystic Fibrosis EFA-Deficient (7)
	mol %	*mol%*	
12:0	0.07 ± 0.01	0.09 ± 0.01	0.10 ± 0.02
14:0	0.60 ± 0.05	0.91 ± 0.06[b]	0.96 ± 0.07[b]
16:0	40.17 ± 1.00	40.05 ± 0.51	43.13 ± 1.26
18:0	12.98 ± 0.53	13.19 ± 0.31	9.16 ± 0.73[c]
22:0	0.12 ± 0.01	0.13 ± 0.02	0.31 ± 0.22
24:0	0.12 ± 0.01	0.16 ± 0.02	0.24 ± 0.10
16:1(n-7)	0.38 ± 0.03	0.54 ± 0.05	1.61 ± 0.30[c]
18:1(n-7)	1.92 ± 0.07	1.79 ± 0.06	2.73 ± 0.26[c]
18:1(n-9)	16.90 ± 0.33	16.75 ± 0.42	21.08 ± 1.67[c]
20:3(n-9)	0.10 ± 0.01	0.11 ± 0.01	0.62 ± 0.19[c]
24:1(n-9)	0.29 ± 0.01	0.37 ± 0.04	0.32 ± 0.02
18:2(n-6)	16.27 ± 0.86	16.04 ± 0.60	11.23 ± 1.15[c]
20:2(n-6)	0.29 ± 0.02	0.31 ± 0.02	0.25 ± 0.01
20:3(n-6)	1.44 ± 0.11	1.77 ± 0.11	1.49 ± 0.06
20:4(n-6)	4.70 ± 0.27	4.32 ± 0.27	3.78 ± 0.42
22:4(n-6)	0.24 ± 0.01	0.23 ± 0.02	0.22 ± 0.02
18:3(n-3)	0.14 ± 0.01	0.21 ± 0.02	0.29 ± 0.06[b]
20:5(n-3)	0.27 ± 0.05	0.42 ± 0.04	0.35 ± 0.03
22:5(n-3)	0.28 ± 0.03	0.32 ± 0.03	0.23 ± 0.03
22:6(n-3)	0.88 ± 0.09	0.86 ± 0.05	0.57 ± 0.09

[a]Results are expressed as mol% of total fatty acids present. All data represent mean values ± SEM.
[b]Different from controls.
[c]Different from the other two groups.

TABLE 6. Fatty acids in red cell phosphatidylethanolamine[a]

Fatty Acid	Controls (13)	Cystic Fibrosis EFA-Sufficient (9)	Cystic Fibrosis EFA-Deficient (7)
	mol %	*mol%*	
12:0	0.14 ± 0.01	0.20 ± 0.04	0.17 ± 0.02
14:0	0.41 ± 0.05	0.73 ± 0.20	0.51 ± 0.09
16:0	23.73 ± 0.80	24.57 ± 0.57	27.47 ± 0.75[b]
18:0	10.11 ± 0.28	9.78 ± 0.35	9.81 ± 0.53
20:0	0.41 ± 0.03	0.45 ± 0.06	0.52 ± 0.04
22:0	0.28 ± 0.03	0.35 ± 0.03	0.27 ± 0.04
24:0	0.47 ± 0.04	0.58 ± 0.08	0.52 ± 0.08
16:1(n-7)	0.26 ± 0.02	0.32 ± 0.04	0.93 ± 0.15[c]
18:1(n-7)	1.65 ± 0.05	1.52 ± 0.06	2.37 ± 0.27[c]
18:1(n-9)	23.65 ± 0.55	23.65 ± 0.87	22.73 ± 0.61
20:3(n-9)	0.12 ± 0.01	0.14 ± 0.01	0.54 ± 0.17[c]
24:1(n-9)	0.39 ± 0.04	0.43 ± 0.06	0.41 ± 0.04
18:2(n-6)	6.25 ± 0.31	6.36 ± 0.31	5.27 ± 0.58
20:2(n-6)	0.34 ± 0.01	0.35 ± 0.03	0.25 ± 0.02[c]
20:3(n-6)	1.02 ± 0.08	1.26 ± 0.11	1.22 ± 0.19
20:4(n-6)	17.18 ± 0.64	16.22 ± 0.91	15.15 ± 0.78
22:4(n-6)	4.78 ± 0.27	4.34 ± 0.29	3.71 ± 0.36
18:3(n-3)	0.16 ± 0.01	0.22 ± 0.02	0.27 ± 0.06[b]
20:5(n-3)	0.75 ± 0.06	1.00 ± 0.08	0.94 ± 0.09
22:5(n-3)	2.03 ± 0.15	2.18 ± 0.16	1.91 ± 0.15
22:6(n-3)	2.77 ± 0.21	2.54 ± 0.30	2.18 ± 0.22

[a]Results are expressed as mol% of total fatty acids present. All data represent mean values ± SEM.
[b]Different from controls.
[c]Different from the other two groups.

TABLE 7. Fatty acids in total platelet phospholipids[a]

Fatty Acid	Controls (15)	Cystic Fibrosis EFA-Sufficient (7)	Cystic Fibrosis EFA-Deficient (5)
	nmol/mg protein	*nmol/mg protein*	
12:0	0.13 ± 0.02	0.09 ± 0.02	0.18 ± 0.10
14:0	0.46 ± 0.04	0.52 ± 0.03	0.77 ± 0.23
16:0	21.46 ± 0.67	22.32 ± 1.05	28.10 ± 3.72[b]
18:0	23.08 ± 0.66	21.60 ± 0.70	22.10 ± 1.70
20:0	2.10 ± 0.08	1.97 ± 0.11	1.84 ± 0.10
22:0	4.09 ± 0.13	3.70 ± 0.16	3.87 ± 0.46
24:0	1.46 ± 0.08	1.47 ± 0.10	1.50 ± 0.24
16:1(n-7)	0.20 ± 0.01	0.28 ± 0.03	0.61 ± 0.13[c]
18:1(n-7)	1.15 ± 0.04	1.16 ± 0.02	1.48 ± 0.14[c]
18:1(n-9)	14.10 ± 0.28	14.60 ± 0.50	12.70 ± 1.5
20:3(n-9)	0.19 ± 0.01	0.21 ± 0.02	0.50 ± 0.13[c]
24:1(n-9)	1.59 ± 0.08	1.84 ± 0.07	1.62 ± 0.09
18:2(n-6)	4.06 ± 0.15	4.28 ± 0.43	3.05 ± 0.58
20:2(n-6)	0.46 ± 0.05	0.38 ± 0.05	1.24 ± 0.64
20:3(n-6)	1.03 ± 0.08	1.28 ± 0.08	1.08 ± 0.03
20:4(n-6)	18.57 ± 1.10	18.90 ± 1.30	16.10 ± 3.40
22:4(n-6)	1.70 ± 0.16	1.52 ± 0.15	1.66 ± 0.20
18:3(n-3)	tr	tr	tr
20:5(n-3)	0.25 ± 0.02	0.38 ± 0.04[b]	0.37 ± 0.06[b]
22:5(n-3)	1.03 ± 0.11	1.01 ± 0.07	1.38 ± 0.06
22:6(n-3)	1.14 ± 0.11	0.91 ± 0.11	1.32 ± 0.12

[a]Results are expressed as a % (nmol/mg protein) of total fatty acids present. All data represent mean values ± SEM.
[b]Different from controls.
[c]Different from the other two groups.

status as the FA profile of plasma, red cell, and platelet PL.

The ratio of triene/tetraene first proposed by Holman (22) and redefined since the advent of gas–liquid chromatography as the ratio of 20:3(n-9)/20:4(n-6) has stood the test of time (23, 24). The refinements of technology used in the present study permitted the identification of the so-called abnormal metabolite of oleic acid, 20:3(n-9), even in our control population made up of healthy siblings of CF patients. As a result, the 20:3(n-9)/20:4(n-6) ratio (mean ± SD) obtained in controls (0.021 ± 0.007) was lower than the value (0.10 ± 0.07) published 10 years ago by Holman, Smyth, and Johnson (25). More recently, Siguel et al. (24), using a 50-meter capillary column, described ratios of 0.013 ± 0.006 in their control population. This small difference between their results and ours appears to be accounted for by a somewhat lower level of 20:3(n-9) and a higher percentage of 20:4(n-6) in the 56 healthy adults he studied.

Almost 30 years ago, Holman (22) suggested that the EFA status of animals could be assessed equally as well from plasma as from the heart or from red cells. There is a common belief that FA patterns are closely related to the diet. Although this may be true of lipid components such as nonesterified FA, triglycerides, and cholesteryl esters, it does not appear to be true of PL (26). In fact, FA patterns

of plasma and red cell PL in humans and animals with EFA deficiency have been found to be similar to those of tissue lipids (23). However, critiques have been leveled at measurements of total PL because some individual PL fractions are reportedly more sensitive than others to EFA deficiency. Our observations confirm the fact that the fatty acid composition of PC in plasma and of both PC and PE in red cells, which represent 79% and 64% of total PL in plasma and red cells, respectively, are much more sensitive to EFA deficiency than the other PL species. However, they represent no advantage over total plasma FA.

Platelets, like erythrocytes, occur as free-floating cells in blood but they are an important source of eicosanoids. It is, therefore, not surprising that the major eicosanoid precursor, arachidonic acid, is more readily incorporated into phospholipids than nonprecursor FA such as palmitic, stearic, oleic, and linoleic acids (27). Results of platelet phospholipids are consonant with those obtained in red cells and plasma since the criteria for EFA deficiency—20:3(n-9), 20:3(n-9)/20:4(n-6) and the n-7 family (16:1(n-7) + 18:1(n-7))—are all significantly increased in the EFA-deficient subjects. Had FA patterns of PC, PE, and PI been done individually, perhaps platelets would have proven to be more sensitive. However, only total PL could be done because of the limited amount of blood that could be drawn from these children.

The use of the fatty acid composition of plasma and tissue lipids as predictors of EFA intake and metabolism is based on the fact that there is homeostatic control exerted over the fatty acid pattern of plasma and structural lipids (23). Studies of the parameters controlling the kinetics of EFA metabolism have underlined the fact that there is a close interdependency between saturated, monounsaturated, and PUFA through competition for the same enzymatic machinery. Each step of the biosynthesis of the n-3, n-6, n-9, and n-7 series of PUFA is catalyzed by microsomal chain elongation or desaturase systems with affinities n-3 > n-6 > n-9 > n-7 (28), consistent with in vitro studies of enzyme substrate preferences (29). The ratio of 20:3(n-9)/20:4(n-6) for EFA deficiency has stood the test of time but other indices have been suggested. We have found in the EFA-deficient group a high ratio of 16:1(n-7)/18:2(n-6) in total plasma FA as well as in phospholipids of plasma, red cells, and platelets thereby confirming a recent report (24). Upon correction of EFA deficiency, it was noted that the increase in linoleic acid was accompanied by a fall in the previously increased palmitoleic acid (16:1(n-7)) to normal values (30). In our hands, the n-7 family (16:1(n-7) + 18:1(n-7)) proved to be the most sensitive discriminant for EFA deficiency.

Polyunsaturated fatty acids are synthesized by a sequence of desaturation of elongation reactions in microsomes and are affected by different dietary conditions. Δ^6 Desaturation is the determining factor in arachidonic acid synthesis and its activity is increased in EFA deficiency along with that of Δ^9 desaturase (29, 31). Our results are in accord with these observations. It is likely that the increase in Δ^6 desaturase activity in EFA deficiency is a physiological response to maintain the unsaturated/saturated FA ratio and fluidity as well as function of membranes (29). Similarly, increased Δ^9 desaturase in membranes of EFA-deficient animals has been shown to be correlated with decreased fluidity assessed by fluorescent probes (32).

The high incidence of EFA deficiency in CF is consistent with other studies (33) and is worrisome especially in the context of our current understanding of the role of EFA in human biology and of the likely impact of subclinical EFA deficiency in CF. Easier control of chest infections, marginally better respiratory function, and perhaps extended survival following supplementary nutrition programs may in part be due to correction of EFA deficiency but this has yet to be documented. Although an early study showed that selective administration of EFA appeared to improve the clinical and/or biochemical status of patients (34), this has not been confirmed by others (6, 12, 30, 35). The only programs that were successful in improving the EFA status of their patients were those providing a high level of energy (12). In view of these reports and of our findings, we recommend that the nutritional intake of calories be maximized in order to correct EFA deficiency (36, 37) especially since arachidonic acid and eicosanoids are important regulators of chloride transport (38), currently thought to be the basic defect associated with CF (39).

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REFERENCES

1. Kraemer, R., A. Rudeborg, B. Hadorn, and E. Rossi. 1978. Relative underweight in cystic fibrosis and its prognostic value. *Acta. Paediatr. Scand.* **67:** 33–37.
2. Parsons, H. G., P. Beaudry, A. Dumas, and P. B. Pencharz. 1983. Energy needs and growth in children with cystic fibrosis. *J. Pediatr. Gastroenterol. Nutr.* **2:** 44–49.
3. Bell, L., P. Durie, and G. G. Forstner. 1984. What do children with cystic fibrosis eat? *J. Pediatr. Gastroenterol. Nutr.* **3** (Suppl 1): S137–S146.
4. Soutter, V. L., P. Kristidis, M. A. Gruca, and K. J. Gaskin. 1986. Chronic undernutrition/growth retardation on cystic fibrosis. *Clin. Gastroenterol.* **15:** 137–155.
5. Shepherd, R. W., T. L. Holt, L. Vasques-Velasquez, W. A. Coward, A. Prentice, and A. Lucas. 1988. Increased energy

expenditure in young children with cystic fibrosis. *Lancet.* **1:** 1300–1303.
6. Lloyd-Still, J. D., S. B. Johnson, and R. T. Holman. 1981. Essential fatty acid status in cystic fibrosis and the effects of safflower oil supplementation. *Am. J. Clin. Nutr.* **34:** 1–7.
7. Farrell, P. M., E. H. Mischler, M. J. Engle, D. J. Brown, and S-M. Lau. 1985. Fatty acid abnormalities in cystic fibrosis. *Pediatr. Res.* **19:** 104–109.
8. Biggeman, B., M. D. Laryea, A. Schuster, M. Griese, D. Reinhardt, and H. J. Bremer. 1987. Status of plasma and erythrocyte fatty acids and vitamins A and E in young children with cystic fibrosis. *Scand. J. Gastroenterol.* **23:** (Suppl 143): 135–141.
9. Dimand, R. J., C. T. W. Moonen, S. C. Chu, E. M. Bradbury, G. Kurland, and K. L. Cox. 1988. Adipose tissue abnormalities in cystic fibrosis: noninvasive determination of mono- and polyunsaturated fatty acids by carbon-13 topical magnetic resonance spectroscopy. *Pediatr. Res.* **24:** 243–246.
10. Spector, A. A., and M. A. York. 1985. Membrane lipid composition and cellular function. *J. Lipid Res.* **26:** 1015–1035.
11. Marcus, A. J. 1984. The eicosanoids in biology and medicine. *J. Lipid Res.* **25:** 1511–1516.
12. Mischler, E. H., S. W. Parrell, P. M. Farrell, W. J. Raynor, and R. J. Lemen. 1986. Correction of linoleic acid deficiency in cystic fibrosis. *Pediatr. Res.* **20:** 36–41.
13. Gibson, R. A., J. K. Teubner, K. Haines, D. M. Cooper, and G. P. Davidson. 1986. Relationships between pulmonary function and plasma fatty acid levels in cystic fibrosis patients. *J. Pediatr. Gastroenterol. Nutr.* **5:** 408–415.
14. Lepage, G., and C. C. Roy. 1986. Direct transesterification of all classes of lipids in a one-step reaction. *J. Lipid Res.* **27:** 114–120.
15. Demirjian, A., M. Jenicek, and M. B. Dubuc. 1972. Les normes staturo-pondérales de l'enfant urbain canadien français d'âge scolaire. *Can. J. Public Health.* **63:** 14–30.
16. Shwachman, H., and L. L. Kulczycki. 1958. Long-term study of 105 patients with cystic fibrosis. *Am. J. Dis. Child.* **96:** 6–15.
17. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissue. *J. Biol. Chem.* **226:** 497–509.
18. Mahadevappa, V. G., and B. J. Holub. 1982. The molecular species composition of individual diacyl phospholipids in human platelets. *Biochim. Biophys. Acta.* **713:** 73–79.
19. Mustard, J. F., D. W. Perry, N. G. Ardlie, and M. A. Packham. 1972. Preparation of suspensions of washed platelets from humans. *Br. J. Haematol.* **22:** 193–204.
20. Lepage, G., and C. C. Roy. 1988. Specific methylation of plasma nonesterified fatty acids in a one-step reaction. *J. Lipid Res.* **29:** 227–235.
21. Afifi, A. A., and V. Clark. 1984. Computer-aided multivariate analysis. Lifetime Learning Publications, Belmont, CA. 246–285.
22. Holman, R. T. 1960. The ratio of trienoic:tetraenoic acids in tissue lipids as a measure of essential fatty acid requirement. *J. Nutr.* **70:** 405–410.
23. Holman, R. T. 1986. Control of polyunsaturated acids in tissue lipids. *J. Am. Coll. Nutr.* **5:** 183–211.
24. Siguel, E. N., K. M. Chee, J. Gong, and E. J. Schaefer. 1987. Criteria for essential fatty acid deficiency in plasma as assessed by capillary column gas–liquid chromatography. *Clin. Chem.* **33:** 1869–1873.
25. Holman, R. T., L. Smythe, and S. Johnson. 1979. Effect of sex and age on fatty acid composition of human serum lipids. *Am. J. Clin. Nutr.* **32:** 2390–2399.
26. Horrobin, D. F., Y. S. Huang, S. C. Cunnane, and M. S. Manku. 1984. Essential fatty acids in plasma, red blood cells and liver phospholipids in common laboratory animals as compared to humans. *Lipids.* **19:** 806–811.
27. Neufeld, E. J., D. B. Wilson, H. Sprecker, and P. W. Majerus. 1983. High affinity esterification of eicosanoid precursor fatty acids by platelets. *J. Clin. Invest.* **72:** 214–220.
28. Siguel, E. N., and M. Maclure. 1987. Relative activity of unsaturated fatty acid metabolic pathways in humans. *Metabolism.* **36:** 664–669.
29. Brenner, R. R. 1977. Regulatory function of Δ^6 desaturase — key enzyme of polyunsaturated fatty acid synthesis. *Adv. Exp. Med. Biol.* **83:** 85–101.
30. Christophe, A., G. Verdonk, E. Robberecht, and R. Mahathanakhun. 1985. Effect of supplementing medium chain triglycerides with linoleic acid-rich monoglycerides on severely disturbed serum lipid fatty acid patterns in patients with cystic fibrosis. *Ann. Nutr. Metab.* **29:** 239–245.
31. De Alaniz, M. J. T., I. N. T. De Gomez Dumm, and R. R. Brenner. 1986. Effect of different acids with delta 9,12-dienoic structures on delta 9 desaturation activity in rat liver microsomes. *Lipids.* **21:** 425–429.
32. Pugh, E. L., M. Kates, and A. G. Szabo. 1982. Studies on fluorescence polarization of 1-acyl-2-*cis*- or *trans*-parinaroyl *sn*-3-glycerophosphorylcholines in model systems and microsomal membranes. *Chem. Phys. Lipids.* **30:** 55–69.
33. Hubbard, V. S. 1983. What is the association of essential fatty acid status with cystic fibrosis? *Eur. J. Pediatr.* **141:** 68–70.
34. Elliott, R. B. 1976. A therapeutic trial of fatty acid supplementation in cystic fibrosis. *Pediatrics.* **57:** 474–479.
35. Davidson, G. P., P. D. Phelan, and R. R. Trownley. 1978. A controlled trial using intravenous infusion of soya oil emulsion in the treatment of children with cystic fibrosis. *Aust. Paediatr. J.* **14:** 80–82.
36. Landon, C., J. A. Kerner, R. Castillo, L. Adams, R. Whalen, and N. J. Lewiston. 1981. Oral correction of essential fatty acid deficiency in cystic fibrosis. *J. Parenter. Enteral Nutr.* **5:** 501–504.
37. Lester, L. A., R. M. Rothberg, G. Dawson, A. L. Lopez and Z. Corpuz. 1986. Supplemental parenteral nutrition in cystic fibrosis. *J. Parenter. Enteral. Nutr.* **10:** 289–295.
38. Eling, T. E., R. M. Danilowicz, D. C. Henke, K. Sivarajah, J. R. Yankaskas, and R. C. Boucher. 1986. Arachidonic acid metabolism by canine tracheal epithelial cells. Product formation and relationship to chloride secretion. *J. Biol. Chem.* **261:** 12841–12849.
39. Welsh, M. J., and R. B. Frick. 1987. Cystic fibrosis. *J. Clin. Invest.* **80:** 1523–1526.