Relationship of decreased hepatic lipase activity and lipoprotein abnormalities to essential fatty acid deficiency in cystic fibrosis patients

E. Lévy, G. Lepage, M. Bendayan, N. Ronco, L. Thibault, N. Galéano, L. Smith, and C. C. Roy

Gastroenterology-Nutrition Research Unit, Centre de Recherche Pédiatrique, Hôpital Ste-Justine, and Departments of Pediatrics, Nutrition, and Anatomy, Université de Montréal, Montréal, Québec, Canada

Abstract Polyunsaturated fatty acids are known to affect plasma lipids and lipoproteins but there is no information on the effect of essential fatty acid (EFA) deficiency on lipoprotein composition. The purpose of this study was to characterize lipoproteins from 17 cystic fibrosis (CF) patients in relationship to their EFA status (eicosatrienoic/arachidonic acid ratio) and compare them with those of 10 healthy siblings (SIB) and of 10 unrelated controls. In 7 EFA-deficient (EFAD) and 10 EFA-sufficient (EFAS) patients, hypocholesterolemia was associated with a decrease of HDL-cholesterol and of LDL-cholesterol which was more marked in the EFAD group. Similarly, although triglyceride enrichment of VLDL, LDL, HDL_2, and HDL_3 with a concomitant reduction of cholesteryl esters from all particles except HDL_2 was observed in both CF groups, it was more sizable in the EFAD patients. These changes led to an increase in the particle size of VLDL, LDL, and HDL_2 whereas the distribution of HDL_3 was skewed to smaller particles. Alterations in the apoprotein composition of particles were greater in EFAD than in EFAS. A decrease of total postheparin lipolytic activity was observed in the two groups of CF patients as well as in siblings. It was entirely accounted for by hepatic lipase (μmol FFA/ml per h) which was more severely diminished in EFAD (2.8 ± 0.6) than in EFAS (4.4 ± 0.7) and SIB (5.1 ± 0.5). Although the two groups of CF children differed in terms of growth, severity of malabsorption, and vitamin E status, these data suggest that disturbance of lipoprotein concentration, composition, size, and metabolism (hepatic lipase) may be in part related to EFA deficiency. Further studies are necessary to explore the effect of EFA deficiency on hepatic lipase activity.—**Levy, E., G. Lepage, M. Bendayan, N. Ronco, L. Thibault, N. Galeano, L. Smith, and C. C. Roy.** Decreased hepatic lipase activity and lipoprotein abnormalities in cystic fibrosis patients: their relationship to essential fatty acid deficiency. *J. Lipid Res.* 1989. **30:** 1197–1209.

Supplementary key words lipoprotein lipase • vitamin E

Cystic fibrosis (CF) is the most common inherited disorder; it is transmitted in an autosomal recessive manner and affects 1 in 2000 live births in the United States. The disease is characterized by metabolic, gastrointestinal, pulmonary, and nutritional disturbances. Although the defective gene is associated with abnormal regulation of the activity of the Cl^- channel, the primary biochemical defect is not yet understood (1).

Among the nutritional alterations present in CF patients, those related to abnormalities of essential fatty acids (EFA) are receiving increased attention (2). Changes in plasma fatty acids include an increase of palmitic (16:0), palmitoleic [16:1 (n-9)], oleic acid (18:1), and eicosatrienoic [20:3 (n-9)], while linoleic [18:2 (n-6)] and arachidonic [20:4 (n-6)] acids are decreased in all lipid fractions from blood and tissues (3). Malabsorption of FA and reduced dietary intake of fats containing 18:2 (n-6), have been proposed to account for the abnormal fatty acid profiles (4,5). Other investigators have proposed that an enzymatic defect in fatty acid desaturation was directly related to the basic gene defect (6). More recently, it has been suggested that there is disordered regulation of the release of arachidonic acid from membranes (7). Thus, EFA deficiency may have far reaching consequences on membrane fluidity and function (8) as well as on the metabolism of eicosanoids (9), on inflammatory response (10), and lung function (11).

The only study of lipoproteins in CF (12) is limited in scope and does not provide characterization of their core and surface components, nor does it address their particle size and apoprotein patterns. Although there is evidence that EFA may affect lipoprotein concentration and com-

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CE, cholesteryl ester; CF, cystic fibrosis; EFA, essential fatty acid; EFAD, EFA-deficient; EFAS, EFA-sufficient; FC, free cholesterol; FFA, free fatty acid; GGT, γ-glutamyl transpeptidase; HDL, high density lipoprotein; HL, hepatic lipase; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; LPL, lipoprotein lipase; PHLA, post-heparin lipolytic activity; PL, phospholipid; PR, protein; SIB, sibling; TC, total cholesterol; TG, triglyceride; VLDL, very low density lipoprotein.

position (13-16), no study has assessed the relationship between EFA status and lipoproteins in CF.

The present study was, therefore, designed to examine lipid profiles and lipoproteins and to measure lipoprotein lipase activity in two groups of CF patients with and without biochemical evidence of EFA deficiency. The CF children were compared to non-CF siblings who, in turn, were contrasted with healthy unrelated controls.

METHODS

Subjects

Out of the total of 297 children and adolescents attending the CF clinic, 163 patients were examined for EFA deficiency. Seventy seven were found to be >2 SD above the mean ($\bar{x}$ ± SD) of 0.021 ± 0.001 for the plasma ratio of eicosatrienoic (Mead) acid/arachidonic acid [20:3 (n-9)/20:4 (n-6)] obtained in 44 healthy controls. Ten CF subjects who were EFA-sufficient (EFAS) and 7 EFA-deficient (EFAD) were recruited for the present study (**Table 1**). There was no difference between the two groups in terms of age and clinical score (17). Using growth charts for French Canadian children (18) they did not differ in their Z scores for weight, but the EFAD group had lower height scores. The EFAS group with a 20:3 (n-9)/20:4 (n-6) ratio <0.02 had a modest degree of steatorrhea persisting despite pancreatic enzymes, normal levels of vitamin E, and liver function tests that were within normal limits. In contrast, the EFAD patients with a 20:3 (n-9)/20:4 (n- 6) ratio >0.10 had a twofold higher fecal fat loss and vitamin E deficiency. Three of the 7 children in the EFAD group had biochemical (Table 1) and ultrasound evidence of chronic liver disease. This accounted for the higher levels of alanine aminotransferase (ALT) and of γ-glutamyl transpeptidase (GGT) (**Table 2**). However, since these 3 patients did not differ from the 4 others with EFAD in terms of plasma lipids, lipoprotein composition and lipoprotein lipase activity, they were considered as one group.

Ten age-matched non-CF siblings served as controls for the two CF groups. An equal number of healthy controls with no evidence of malnutrition, gastrointestinal or hepatobiliary disease were also studied and compared to siblings of the CF children. Informed consent was obtained from the parents and the project was approved by the Ethics Committee of Ste-Justine Hospital.

Isolation of lipoproteins

Blood samples were collected in 1 mg/ml EDTA after a 12-h overnight fast and plasma was separated immediately by low speed centrifugation (2,500 rpm, 20 min) at 4°C. The lipoprotein fractions were isolated by discontinuous density gradient ultracentrifugation in a Beckman L5-65 preparative ultracentrifuge using a Ti-50 rotor as

TABLE 1. Clinical characteristics of cystic fibrosis patients

Group	Age	Z Scores for Growth[a]		Clinical Score[b]	Fecal Fat[c]	Vit E/TG[d]	Liver Function Tests		
		Weight	Height				Bilirubin[e]	ALT[f]	GGT[g]
	yr				*g/24 h*		*mg/dl*	*U/l*	*U/l*
EFA-sufficient[h] (n = 10)	11.6 ± 0.6	−0.61 ± 0.32	−0.40 ± 0.38	80.0 ± 3.4	10.5 ± 2.5	0.111 ± 0.001	0.20 ± 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[i]	0.02	0.02	0.05	0.01

[a]Standard deviation scores for French Canadian children (18).
[b]Modified Shwachman scores (17).
[c]Normal = <5 g/24 h.
[d]Normal = 0.009-0.019.
[e]Normal = <0.8 mg/dl.
[f]Alanine aminotransferase, normal = <35 U/l.
[g]γ-Glutamyltranspeptidase, normal = <45 U/l.
[h]Plasma Mead acid [20:3(n-9)]/arachidonic acid [20:4(n-6)] ratios = 0.02 identify the EFA-sufficient group, and values >0.10 identify the EFA-deficient group.
[i]One-tail *t*-test.

TABLE 2. Characterization of liver function in cystic fibrosis patients

Patients	ALT	AST	γGT	Alkaline Phosphatase	Bilirubin Total/Direct	Albumin	Cholyl Glycine	Caffeine[a]	Prealbumin[b]	Liver Ultrasound	Hepatic Biopsy
		IU/l					*mg/dl*				
EFA-sufficient											
Case 7	13.0	37.0	11.0	373.0	0.1/0.1	4.21	10.0	0.00	22.0	N	
Case 8	22.0	34.0		445.0	0.5/0.1	3.63	32.0	0.30	20.0	Architecture heterogeneous, ? steatosis	
Case 10	24.0	34.0			0.1/0.0	2.78[c]	73.9[c]	0.30	10.0[c]	N	
Case 13	16.0	46.0	7.0		0.2/0.0	3.84	10.2	0.08	23.0	N	
Case 14	4.0	26.0	9.0		0.1/0.0	4.08	19.9	0.34	25.0	N	
Case 17	28.0	49.0	20.0	429.0	0.4/0.0	4.21	21.9	0.26	19.0	N	
Case 19	11.0	24.01	11.0	305.0	0.1/0.0	3.83	10.0	0.11		N	
Case 23	18.0	51.0	9.0		0.1/0.0	4.01	22.2	0.02	18.0	N	
Case 24	7.0	26.0	10.0		0.2/0.0	3.7	42.0	0.48	12.9[c]	N	
Case 29	11.0	29.0	2.0		0.3/0.0	4.53	10.0	0.30	24.0	N	
Mean ± SE	15.4 ± 2.4	36.5 ± 3.2	10.5 ± 1.5	388.0 ± 31.0	0.2 ± 0.05	3.88 ± 0.15	25.2 ± 0.3	0.22 ± 0.16	19.0 ± 2.0		
EFA-deficient											
Case 1	35.0	50.0	39.0	430.0	0.6/0.1	3.93	52.0	0.72	13.6[c]	Slight heterogeneity	
Case 2	29.0	13.0	11.0	390.0	0.3/0.0	4.18	11.0	0.36	25.0	N	
Case 3	15.0	32.0	18.0		0.3/0.0	4.15	66.8[c]	0.28	19.8	Architecture heterogeneous	Cirrhosis
Case 4	12.0	33.0	41.0	202.0	0.2/0.0	4.26	10.0	0.23	20.0	N	
Case 5	10.2[c]	98.0[c]	89.0[c]	455.0[c]	0.4/0.1	3.98	56.3	0.39	19.5	Vol.↓, heterogeneous, nodular, portal hypertension	Cirrhosis with portal hypertension
Case 6	28.0	45.0	35.0	102.0	0.5/0.1	3.37[c]	54.2	0.04	30.5	N	
Case 9	41.0[c]	54.0	73.0[c]	562.0[c]	0.6/0.1	3.5	111.0[c]	1.43[c]	18.2	Vol.↓, heterogeneous, nodular, portal hypertension, absent gallbladder	Cirrhosis with portal hypertension
Mean ± SE	37.4 ± 11.4	46.4 ± 10.0	43.7 ± 10.6	356.8 ± 64.9	0.41 ± 0.06	3.91 ± 0.13	194.3 ± 152.8	0.49 ± 0.17	19.51 ± 2.73		
Significance (*P*)	<0.05	NS	<0.01	NS	<0.02	NS	NS	NS	NS		
Normal range	<35	<55	<45	Variable dep. on age	<0.8	>3.5	<60	<0.9	>15		

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; γGT, γ-glutamyl transpeptidase.
[a]Loading test.
[b]More an index of malnutrition.
[c]Abnormal result.

previously reported (19). Briefly, after preliminary centrifugation to remove chylomicrons (25,000 rpm, 30 min), very low density (VLDL), intermediate density (IDL), and low density (LDL) lipoproteins were isolated at densities of 1.006 g/ml, 1.019 g/ml, and 1.063 g/ml, respectively, running at 100,000 *g* for 18 h at 5°C. The separation of high density lipoprotein (HDL) subpopulations was performed at 100,000 *g* for 48 h at the following densities: 1.125 g/ml for HDL_2 and 1.21 g/ml for HDL_3. The lipoprotein fractions were washed with salt solutions of their equilibrium density and dialyzed intensively against 0.15 M NaCl, 0.001 M EDTA, pH 7.0.

Lipid and lipoprotein analyses

The concentrations of total cholesterol (TC), free cholesterol (FC), and triglycerides (TG) were determined enzymatically by a commercial kit (Boehringer Mannheim, Montreal) as reported previously (19). Cholesteryl esters (CE) were calculated as the difference between total and unesterified cholesterol × 1.7. Lipoprotein-protein (PR) was quantified according to Lowry et al. (20) with bovine serum albumin as a standard. Phospholipids were measured by the Bartlett method (21). HDL-cholesterol (HDL-C) was determined after precipitation of VLDL and LDL with phosphotungstic acid (22) while LDL-cholesterol (LDL-C) was measured using polyvinylsulfate (Boehringer Mannheim) (23). VLDL-cholesterol (VLDL-C) was calculated from the difference between cholesterol in the polyvinylsulfate supernatant and HDL-C. Plasma concentration of apoB was determined by nephelometry using a commercial standard supplied by Hoerscht-Behring.

Apolipoprotein content of individual plasma lipoproteins was qualitatively assayed using both SDS (24) and urea (25) polyacrylamide electrophoresis. The gels were stained for 1 h with Coomassie blue and destained in 7% acetic acid. The bands for apolipoproteins were identified by comparison with the mobility of apolipoprotein standards and also by standards of molecular weight. The densitometric distribution of apolipoproteins was assayed as described previously (19). Electron microscopy of lipoprotein particles was performed on a Zeiss EM-10, using negative staining with 1% phosphotungstic acid (pH 7.2) as described previously (19). The diameter of 200–300 particles was then determined.

Lipolytic activity measurement

For assay of lipolytic activities, heparin was injected intravenously (10 units/kg body weight) and blood was taken 10 min later (26). Total lipolytic activity was measured in plasma with an emulsion of tri[1-^{14}C]oleoylglycerol as substrate (27). Hepatic lipase (HL) was quantified in the presence of protamine sulfate and lipoprotein lipase activity (LPL) was calculated as the difference between total lipase activity and HL activity. Extraction of FFA was performed by the procedure of Belfrage and Vaughan as previously described (28).

Statistical anaylsis

All values were expressed as the mean ± standard error (SEM). Statistical differences were assessed by analysis of variance for groups of unequal size and by Student's two-tail *t*-test unless indicated otherwise.

RESULTS

Plasma lipids

Although both groups with CF demonstrated abnormal values for plasma lipids, lipoproteins, and apolipoproteins, the group with EFA deficiency was more severely affected (**Table 3**). Triglyceride levels were higher in both groups of CF patients and there was a decrease in both total and free cholesterol, but it was more pronounced in EFAD than in EFAS when compared to siblings. In addition, only the EFAD group had a level of PL below that of siblings. The hypocholesterolemia was closely associated with a significant decrease of LDL-C, particularly marked in EFAD, and of HDL-C which affected both groups equally. As a result, the HDL/LDL ratio was significantly higher in EFAD children than in both EFAS and in siblings. On the other hand, VLDL-C levels were only marginally higher in both CF groups. As expected from the LDL-C and HDL-C findings, there was a more substantial decrease of apoB and apoA-I in the EFAD population. The only significant changes found between the groups of siblings and controls involved VLDL-C and HDL-C. The former had higher concentrations of VLDL-C and lower concentrations of HDL-C.

Lipid and apolipoprotein composition of lipoproteins

The composition of the lipoprotein classes obtained with sequential ultracentrifugation is reported in **Table 4**, and the results for EFAD patients with and without liver disease in **Table 5**. All lipoprotein fractions from CF patients were TG-enriched. There was no difference between the CF groups, except for the TG of the LDL fraction which was higher in the EFAD children. A reduction of CE was observed in the VLDL, LDL, and HDL_3 fractions of CF subjects but particularly in EFAD. With regard to FC, a lower proportion was detected in EFAD than in EFAS but it involved only the VLDL fraction. Table 4 shows that both PL and proteins were only marginally affected.

The major changes in the lipoprotein composition, i.e., triglyceride enrichment and cholesteryl ester depletion, led to abnormal TG/PL and CE/PL ratios. Furthermore, the ratio of core constituents (TG and CE) to surface components (FC, PL, PR) of all lipoprotein fractions except

TABLE 3. Plasma lipids, lipoproteins, and apolipoproteins

	Triglycerides	Phospholipids	Plasma Cholesterol TC	Plasma Cholesterol FC	Plasma Cholesterol EC as % of TC	Lipoprotein Cholesterol VLDL	Lipoprotein Cholesterol LDL	Lipoprotein Cholesterol HDL	HDL LDL	Apolipoproteins A-I	Apolipoproteins B
	mg/dl			*mg/dl*			*mg/dl*			*mg/dl*	
EFAS (n = 10)	101.5 ± 11.5	271.1 ± 12.8	124.3 ± 8.0[c]	71.0 ± 1.8[c]	71.0 ± 0.01	20.3 ± 2.3	68.2 ± 5.6[c]	35.7 ± 2.6	0.54 ± 0.04	124.4 ± 8.1	81.8 ± 5.9
EFAD (n = 7)	106.4 ± 11.8	233.4 ± 8.7[b]	102.3 ± 8.5[c]	33.6 ± 2.2[c]	67.0 ± 0.02	21.3 ± 2.4	48.6 ± 6.0[c,b]	32.2 ± 2.3[b]	0.69 ± 0.06[b]	108.1 ± 6.5[c]	66.6 ± 6.9[c]
SIB (n = 10)	87.8 ± 8.0	263.9 ± 8.0	158.3 ± 7.0	46.6 ± 1.4	70.2 ± 0.96	17.5 ± 1.8[c]	101.6 ± 8.5	41.2 ± 3.3[c]	0.44 ± 0.06	132.5 ± 7.6	86.8 ± 5.9
Controls (n = 10)	73.0 ± 3.0	ND	165.0 ± 1.5	ND	ND	11.4 ± 0.6	96.8 ± 0.9	54.5 ± 1.2	0.56 ± 0.01	142.3 ± 4.8	84.0 ± 1.6

Abbreviations: EFAS, essential fatty acid sufficient; EFAD, essential fatty acid deficient; SIB, siblings; ND, not determined.
[a]Different from siblings at $P < 0.05$.
[b]Different from EFAS at $P < 0.05$.
[c]Different from controls at $P < 0.05$ (analyzed only by two-tailed *t*-test).

for HDL_2 were altered. They showed an increment in VLDL and LDL with a significant decrease in HDL_3, more pronounced in EFAD than in EFAS. Since the TG + CE/FC + PL + PR ratio can be used to make inference of the size of spherical lipoprotein particles, the altered ratios in CF suggested that VLDL, LDL, and HDL_2 particles would be larger while HDL_3 particles would be smaller. Electron microscopy of CF plasma lipoproteins corroborated the biochemical estimation of particle size (**Fig. 1**). Thus, in CF, the diameter of VLDL, LDL, and HDL_2 was greater and that of HDL_3 smaller. The partitioning of particles according to particle size differed between the two groups of CF (Fig. 1a, 1b, 1c, 1d). For example, the distribution of VLDL was skewed to particles of increased size and this was more apparent in EFAD than in EFAS (Fig. 1a); an opposite trend was noticed with respect to HDL_3 (Fig. 1d). Even though regular and spherical shape generally characterized the CF lipoprotein particles, an abnormal form of high density lipoproteins migrating with the HDL fraction was found in one EFAD patient with liver disease. These discoid lipoproteins had the appearance of LPX.

Apolipoprotein distribution in isolated VLDL is illustrated in **Fig. 2**. The 10% SDS-PAGE analysis showed that the apoA-I and apoA-IV were increased in both groups of patients, but to a higher extent in EFAD for apoA-I. This was confirmed by densitometric distribution of VLDL apolipoproteins (**Table 6**). SDS-PAGE on 4% gels was performed to study the profile of apoB. There was no consistent difference between the apoB patterns of VLDL and LDL of patients, siblings, and controls (gel not shown). The apoC family was analyzed on tetramethyl urea (TMU) gels. Densitometric scanning of gels from samples of delipidated VLDL protein showed mean C-II/C-III ratios that did not differ between four CF children (two from each group) and three siblings. Values of 0.19 and 0.21 obtained in two controls were higher ($P = 0.028$, Mann Witney U test) than those measured in CF children (0.135 ± 0.005) and siblings (0.153 ± 0.021) (**Fig. 3**). ApoA-I and apoA-II determined by 15% SDS-PAGE in HDL fractions are shown in **Fig. 4**. ApoA-I/apoA-II ratios were higher in the HDL_2 (Fig. 4a) and lower in HDL_3 fractions (Fig. 4b) of both CF groups than in the siblings. Again, the changes in the EFAD group (11.1 and 5.51) were more pronounced than in the EFAS group (9.32 and 7.71) and in the siblings (5.23 and 8.56).

Plasma lipoprotein lipase activity

In order to determine whether impaired TG hydrolysis could account for the relative TG enrichment of CF lipoproteins, total heparin plasma lipoprotein lipase activity was measured (**Table 7**). Postheparin lipolytic activity (PHLA) was lower in EFAD than in siblings and there was a slight but significant difference between the two pa-

TABLE 4. Chemical composition of lipoproteins

Lipoprotein	Composition					Weight Ratios		
	TG	CE	FC	PL	PR	TG/PL	CE/PL	TG + CE / FC + PL + PR
VLDL (1.006 g/ml)								
EFAS	59.71 ± 0.94	7.96 ± 0.34	4.04 ± 0.09	16.80 ± 0.33	11.46 ± 0.90	3.57 ± 0.09	0.48 ± 0.03	2.11 ± 0.09
EFAD	58.29 ± 2.19	6.26 ± 0.28	3.76 ± 0.16	16.71 ± 1.08	11.72 ± 0.81	3.57 ± 0.25	0.38 ± 0.02	1.94 ± 0.16
SIB	52.95 ± 1.14	10.08 ± 0.45	4.79 ± 0.26	18.39 ± 0.62	13.90 ± 1.34	2.92 ± 0.14	0.56 ± 0.04	1.73 ± 0.10
EFAS vs SIB *P*	0.001	0.02	0.02	0.05	NS	0.005	NS	0.02
EFAD vs SIB *P*	0.05	0.001	0.005	NS	NS	0.05	0.005	NS
EFAS vs EFAD *P*	NS	0.005	NS	NS	NS	NS	0.02	NS
LDL (1.019<d<1.063 g/ml)								
EFAS	8.65 ± 0.68	37.23 ± 0.95	11.22 ± 0.43	18.17 ± 0.63	24.70 ± 0.75	0.48 ± 0.04	2.07 ± 0.10	0.85 ± 0.02
EFAD	14.68 ± 1.39	31.88 ± 2.02	11.32 ± 0.77	20.33 ± 0.69	21.70 ± 1.16	0.72 ± 0.08	1.58 ± 0.13	0.88 ± 0.04
SIB	5.94 ± 0.69	37.84 ± 1.01	11.20 ± 0.15	19.16 ± 0.68	25.81 ± 0.94	0.32 ± 0.04	2.00 ± 0.09	0.78 ± 0.02
EFAS vs SIB *P*	0.02	NS	NS	NS	NS	0.01	NS	0.02
EFAD vs SIB *P*	0.001	0.02	NS	NS	0.02	0.001	0.02	0.05
EFAS vs EFAD *P*	0.005	0.001	NS	0.05	0.05	0.02	0.01	NS
HDL_2 (1.063<d<1.125 g/ml)								
EFAS	6.22 ± 0.64	29.93 ± 1.79	7.07 ± 0.29	25.53 ± 0.83	32.86 ± 1.12	0.25 ± 0.03	1.20 ± 0.10	0.56 ± 0.04
EFAD	7.48 ± 0.59	25.10 ± 2.09	7.02 ± 0.18	27.02 ± 1.55	33.90 ± 0.99	0.26 ± 0.03	0.97 ± 0.14	0.48 ± 0.05
SIB	3.24 ± 0.42	26.76 ± 1.26	6.72 ± 0.31	26.74 ± 0.78	35.76 ± 1.30	0.13 ± 0.06	1.01 ± 0.07	0.44 ± 0.03
EFAS vs SIB *P*	0.005	NS	NS	NS	NS	0.005	NS	NS
EFAD vs SIB *P*	0.001	NS	NS	NS	NS	0.005	NS	NS
EFAS vs EFAD *P*	NS	NS	NS	NS	NS	NS	NS	NS
HDL_3 (1.125<d<1.21 g/ml)								
EFAS	3.26 ± 0.35	20.71 ± 0.74	2.92 ± 0.10	25.75 ± 0.46	47.28 ± 0.99	0.13 ± 0.01	0.81 ± 0.03	0.32 ± 0.01
EFAD	3.43 ± 0.40	17.64 ± 0.95	2.73 ± 0.12	25.44 ± 1.41	50.67 ± 2.35	0.13 ± 0.01	0.70 ± 0.03	0.27 ± 0.02
SIB	2.31 ± 0.25	22.41 ± 0.72	2.91 ± 0.09	25.31 ± 0.26	46.98 ± 0.69	0.09 ± 0.01	0.89 ± 0.03	0.33 ± 0.01
EFAS vs SIB *P*	0.05	NS	NS	NS	NS	0.05	NS	NS
EFAD vs SIB *P*	0.05	0.001	NS	NS	NS	0.025	0.001	0.005
EFAS vs EFAD *P*	NS	0.025	NS	NS	NS	NS	0.05	0.025

Data are means ± SEM; TG, triglyceride; CE, cholesteryl ester; FC, free cholesterol, PL, phospholipid; PR, protein; NS, nonsignificant.

tient groups. When hepatic lipase and extrahepatic lipoprotein lipase activities were differentiated by selective inhibition of the latter enzyme by preincubation with protamine sulfate, a decreased activity of PHLA was entirely accounted for by a decrease in hepatic lipase (HL) which was more pronounced in the group with EFA deficiency (Table 7). This was well reflected by the HL/LPL ratios. Of interest was the lower level of lipolytic activity in siblings by comparison with normal subjects. The chief contributing factor for this significant drop was again HL activity. Similarly, the results for EFAD patients with and without liver disease (**Table 8**) showed no significant differences, and the decreased hepatic lipase activity correlated best with indices of EFAD.

DISCUSSION

Essential fatty acid deficiency has been amply documented in CF (29–35). Although the classical definition of EFA deficiency is based on a ratio of 20:3 (n-9)/20:4 (n-6) larger than 0.2, this criterion is rarely met in CF patients (35). Recently, a more accurate and sensitive method was used for the measurement of fatty acids in plasma and phospholipids of red cells and platelets. It has allowed us to document a high incidence of EFA deficiency using a ratio of 20:3 (n-9)/20:4 (n-6) greater than 0.1 (36). Concentrations of lipoproteins in CF have been examined in a single study (12) but neither lipoprotein composition and size nor the major enzymatic activity responsible for their metabolism was determined. As PUFA are known to affect plasma lipids and lipoprotein composition (13–16) as well as lipoprotein lipase activity (37,38), which is also modulated by the nutritional status (39,40), the purpose of this study was to characterize lipoproteins from CF patients in relationship with their EFA status.

The data confirm previous observations documenting abnormally low plasma levels of LDL and HDL (12) and show that more profound alterations were seen in patients with EFA deficiency. Hypocholesterolemia has been reported previously in patients with malabsorption (41) and a number of investigators have stressed the role of the in-

TABLE 5. Relationship between liver disease in the EFAD group and their plasma lipids, lipoproteins, and apolipoproteins

Patients	TG	PL	Plasma Cholesterol			Lipoprotein Cholesterol			Apolipoproteins	
			TC	FC	EC as % of TC	VLDL	LDL	HDL	A-I	B
	mg/dl		*mg/dl*			*mg/dl*				
Abnormal[a]										
Case 3	114.0	243.9	105.5	35.1	66.7	22.8	45.4	37.3	113.0	6.7
Case 5	112.5	237.4	84.8	33.3	60.7	22.5	36.4	25.9	92.0	66.0
Case 9	50.9	200.8	77.6	26.3	66.1	10.2	35.9	31.6	93.0	37.0
Mean ± SE	92.5 ± 20.8	227.4 ± 13.4	87.3 ± 8.4	31.6 ± 2.6	64.5 ± 1.9	18.5 ± 4.1	39.2 ± 3.0	3.3 ± 3.3	99.3 ± 6.8	56.7 ± 9.8
No chronic liver disease										
Case 1	76.4	231.0	98.6	26.0	73.6	15.3	46.9	36.4	116.0	50.0
Case 2	126.9	267.6	145.5	41.5	71.4	25.4	83.1	37.0	136.0	90.0
Case 4	126.2	246.1	90.8	39.2	56.8	25.2	43.5	22.1	90.0	73.0
Case 6	137.9	207.2	113.0	33.6	70.2	27.6	50.1	35.3	117.0	83.0
Mean ± SE	116.8 ± 13.7	238.0 ± 12.7	112.0 ± 22.1	35.1 ± 3.4	68.0 ± 3.8	23.4 ± 2.7	55.9 ± 9.1	32.7 ± 3.5	114.7 ± 9.4	74.0 ± 8.7
Statistical difference	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Abbreviations: TG, triglyceride; PL, phospholipid; TC, total cholesterol; FC, free cholesterol; EC, esterified cholesterol.
[a]As judged by liver function tests and ultrasound of the liver and biliary tract.

testine in regulating plasma lipid and lipoprotein levels (42,43). Excessive losses of both neutral and acidic (bile acids) sterols occur in all patients with CF and vary with the severity of the malabsorption syndrome (44,45). It is therefore likely that both the low total cholesterol and low LDL-C result from interruption of the enterohepatic circulation (EHC) of bile acids and neutral sterols.

The contribution of lipoproteins to the hepatic pool of cholesterol used for bile acid synthesis and for cholesterol secretion in bile is considerable. There is evidence that HDL-C is preferentially utilized (46) and that cholesteryl esters constitute a more important substrate than free cholesterol (47). It is therefore surprising that HDL were more modestly decreased than LDL. This may have to do with the relationship between LDL receptors and the EHC of cholesterol and bile acids. The return of cholesterol to the liver causes feedback inhibition on cholesterol synthesis. Similarly, the return of bile acids is thought to inhibit the conversion of cholesterol into bile acids (48) but this has recently been disputed (49). It is now thought that the biosynthesis of bile acids is a function of the availability of hepatic cellular cholesterol (50). As CF patients lose considerable amounts of endogenous (mostly biliary) and dietary cholesterol as well as of bile acids, this leads to a reduction of the quantity of cholesterol in the liver cell and in turn to an increased number of LDL receptors which will then take up LDL-C. We would therefore propose that in CF there is a reduction in liver cell cholesterol through malabsorption of bile acids and cholesterol. This fall in hepatic cholesterol stimulates the synthesis of LDL receptors which then causes an increased uptake of LDL and a fall in plasma LDL levels.

The fact that all lipoprotein fractions in CF were TG-enriched is one of the most important findings of this study. Extrahepatic lipoprotein lipase (LPL) is mainly responsible for the catabolism of chylomicrons and VLDL (51,52). Hepatic lipase (HL), the only fraction of the post-heparin lipolytic activity (PHLA) that was decreased in CF, is also active against larger particles but its highest activities are against IDL, LDL, and HDL (53). Deficiency of hepatic lipase has previously been reported to be associated with elevations of both LDL and HDL triglycerides (54). Our findings confirm this observation. It is unlikely that the relative decrease in apoC-II and resultant increase in the apoC-II/apoC-III ratio in the CF children and their siblings (Table 6) is responsible for the low hepatic lipase activity. This is because apoC-II activates LPL and not HL which is presumably stimulated by apoA-II and inhibited by other apoproteins (55). Hepatic lipase is thought to have not only triglyceridase but also phospholipase activity. Therefore, its decreased activity could explain the decreased conversion of HDL_2 into HDL_3 particles observed in one study. In view of the decrease of the apoA-I/apoA-II ratio, the fall in HL of CF and siblings is possibly secondary to an increase in apoA-II.

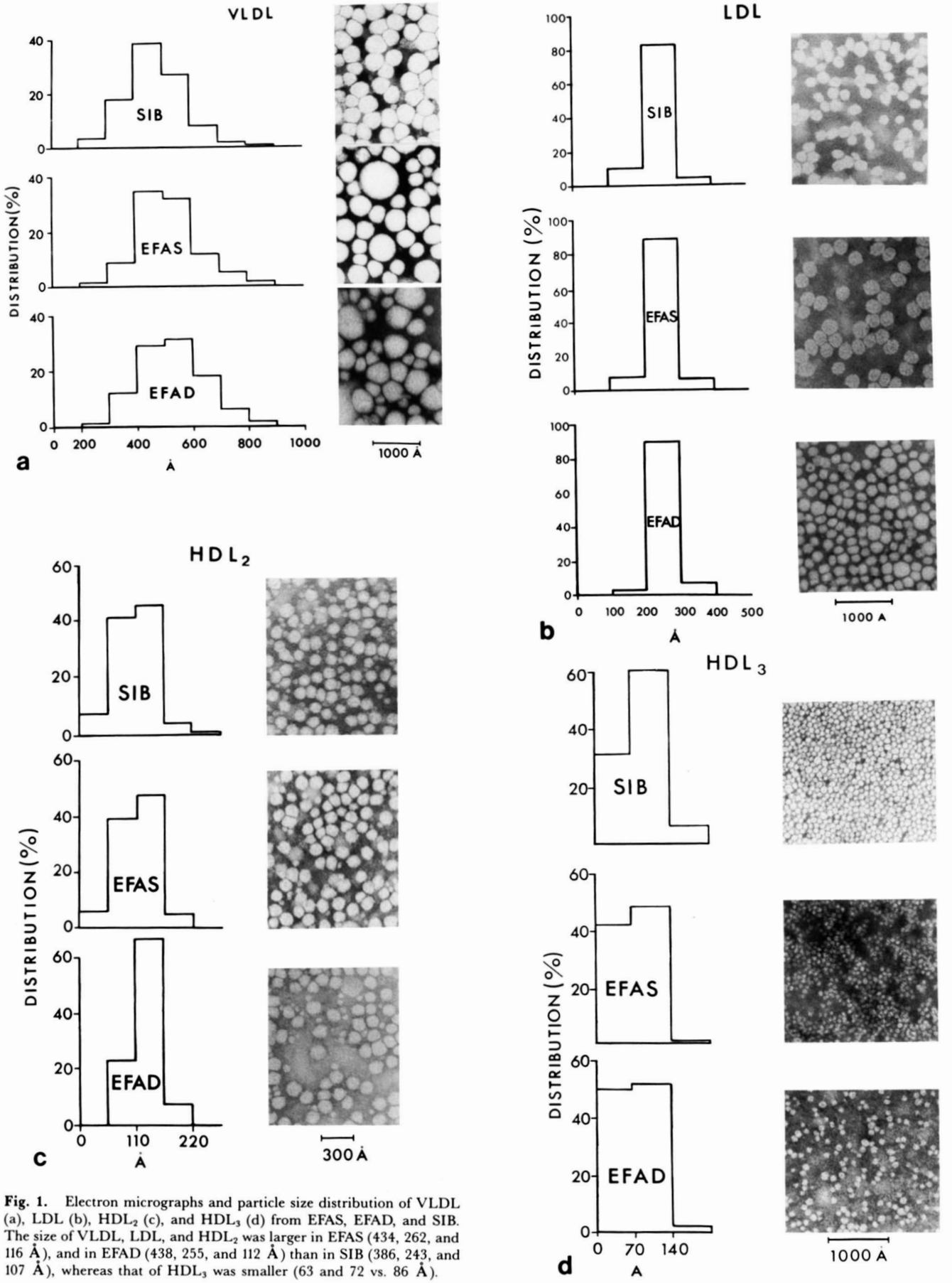

Fig. 1. Electron micrographs and particle size distribution of VLDL (a), LDL (b), HDL_2 (c), and HDL_3 (d) from EFAS, EFAD, and SIB. The size of VLDL, LDL, and HDL_2 was larger in EFAS (434, 262, and 116 Å), and in EFAD (438, 255, and 112 Å) than in SIB (386, 243, and 107 Å), whereas that of HDL_3 was smaller (63 and 72 vs. 86 Å).

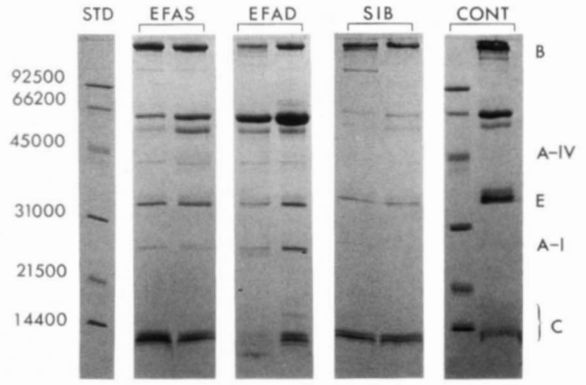

Fig. 2. SDS-PAGE (12.5% gels) of VLDL-apolipoproteins. The location of apolipoprotein species from SIB, EFAS, and EFAD was identified by comparison with a healthy subject (CONT) and with the following molecular weight standards (STD): phosphorylase B (92,500), BSA (66,200), ovalbumin (45,000), carbonic anhydrase (31,000), soybean trypsin inhibitor (21,500), and lysozyme (14,400).

TABLE 6. Distribution of VLDL apolipoproteins after the integration of SDS-PAGE densitometric tracings

	Apolipoproteins (%)				
Subjects	B	A-IV	E	A-I	C
EFAS	47.0	2.4	10.1	2.7	37.8
EFAD	40.0	1.6	12.2	16.5	29.6
SIB	47.6	ND	9.1	1.3	42.0
Controls	47.5	ND	21.4	ND	31.0

Values are means of measurements from two subjects in each group. The densitometric evaluation does not include the albumin fraction.

Besides triglyceride enrichment of lipoprotein fractions, the most consistent observation in the compositional analysis was a decrease in cholesteryl esters which particularly affected the EFAD group of CF. This indicates that there is a cholesteryl ester transfer defect from HDL to LDL and VLDL or that lecithin:cholesterol acyltransferase (LCAT) activity is decreased. The possibility that LCAT could account for our findings is unlikely since studies have shown that LCAT activity is increased in EFA-deficient rats (56). Therefore, the relative decrease of cholesteryl esters could perhaps be due to a reduced activity of cholesteryl ester transfer protein. Although liver disease may lead to impairment of cholesterol esterification, it is an unlikely explanation since both groups of CF were affected, albeit the EFAD group was more severely. Several studies have reported low hepatic lipase activity in liver disease (57) and the decrease in enzyme activity correlated with the severity of the disease (58). Other investigations have described an enzyme depletion in renal insufficiency (59), Type II hyperlipoproteinemia (60), hypothyroidism (61), as well as in a variety of other clinical situations such as alcoholism, viral hepatitis, pancreatitis, diabetes, and protein malnutrition (62). The findings in our study that the level of hepatic lipase activity was lower in EFAD than in EFAS cannot be explained by the fact that three of the former had liver disease since their HL values were identical to those of the EFAD without liver disease. On the other hand, malnutrition could have played a role (Table 2).

The profile and size of lipoproteins were generally consonant with the moderate degree of hypertriglyceridemia observed in CF. The data were consistent with the biochemical estimation of particle size obtained by calculated ratios. Although LPX particles were observed in one patient with chronic liver disease, it is clear that they can be formed in a number of in vivo situations where the formation of excess surface material may exceed the capacity of HDL with low cholesteryl esters to disrupt the vesicle and incorporate its constituents into HDL.

The results of this study showed that the siblings of CF patients had several biochemical alterations. At the beginning of this research, siblings were considered as suitable controls in order to cancel the environmental and nutritional factors. However, it rapidly became evident that they differed from healthy subjects in terms of higher VLDL-C and lower HDL-C as well as HL activity. They all had negative sweat tests and none had symptoms suggestive of CF. As no tests are currently available to detect heterozygosity, it is impossible to say whether the lipoprotein abnormalities noted are secondary to a basic defect present in heterozygotes. Indeed, the abnormalities described as being found in siblings as well as patients include alterations in autonomic and airway reactivity, the release of abnormal lysosomal enzymes, defective β-adrenergic responses of sweat glands in vivo and in vitro, ab-

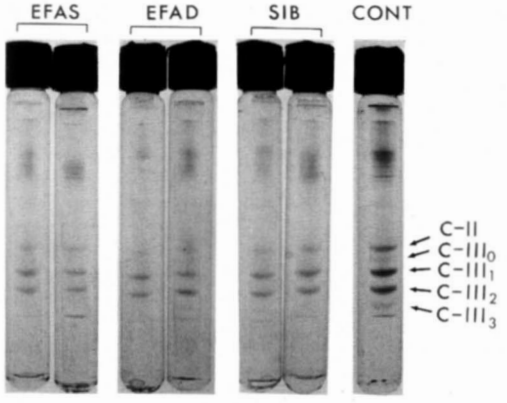

Fig. 3. Disc electrophoresis of VLDL in 10% polyacrylamide gels containing 8 M urea.

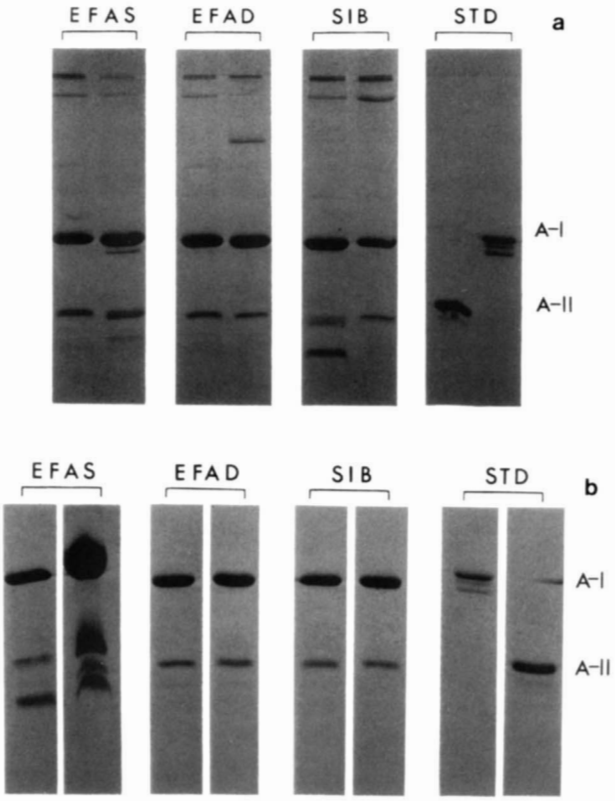

Fig. 4. SDS-polyacrylamide gel (15%) electrophoretograms of HDL_2 (a) and HDL_3 (b). EFAD gels were from one patient without (left) and one patient with (right) liver disease.

normalities of prostaglandin metabolism, disorders of platelet aggregation, and alterations in the composition of nonesterified fatty acids. In view of the confirmation of decreased secretion of chloride in intestinal cells, it has been suggested that the long-searched-for heterozygote advantage in CF may be an inherited resistance to the noxious effects of bacterial toxin causing secretory diarrhea (44, 63–71).

One of the aims of this study was to examine the relationship among essential fatty acid deficiency, plasma lipids, and lipoprotein composition. The hypothesis was that PUFA are essential as constituents of triglycerides, phospholipids, and cholesteryl esters for regulation of the metabolism of lipoproteins. The evidence indicating an inverse relationship between EFA intake and coronary heart disease was first documented 30 years ago and is being resurrected with studies showing that PUFA have a lipid-lowering effect (72). There is also work suggesting that LPL may have a more pronounced triglyceridase activity against unsaturated fatty acids than on saturates (38). In many respects, the lipoprotein compositional changes were observed in both CF groups buth they were more marked in the children with EFAD. Although speculative, it may be suggested that the exchange protein which plays a significant role for the transfer of TG, CE, and PL between lipoprotein fractions (73) could be affected by EFA deficiency. The fact that EFAD also had lower vitamin E levels brings in another variable. Because of the key role of tocopherols as scavengers of free radicals, there have been suggestions that vitamin E deficiency can lead to EFA deficiency (74). However, a recent study has not been able to confirm this (75). Although vitamin E supplementation has not been shown to alter lipoprotein patterns (76), there is no information on the possible effect of vitamin E deficiency.

In conclusion, this clinical study of CF children attracts attention to significant abnormalities in the concentration and composition of plasma lipids and lipoproteins as well as in hepatic lipase activity. Several changes were more prominent in EFAD children who, as a group, had more severe intestinal manifestations and in whom chronic liver disease was present in three of seven. The study of non-CF patients with EFAD but without interruption of their EHC for sterols and without liver disease would permit a more definitive interpretation of our findings. The observations that siblings shared several biochemical altera-

TABLE 7. Postheparin lipoprotein lipase

Subjects	Total PHLA[a]	LPL[b]	HL[c]	HL/LPL
EFAS (10)	8.98 ± 0.96	4.45 ± 0.43	4.41 ± 0.75	0.97 ± 0.15
EFAD (7)	6.08 ± 0.80[d,e]	3.69 ± 0.44	2.79 ± 0.56[d]	0.78 ± 0.14[d]
SIB (10)	8.93 ± 0.70[f]	4.11 ± 1.22	5.11 ± 0.54[f]	1.35 ± 0.16[f]
Controls (10)	13.79 ± 0.52	4.01 ± 0.65	9.84 ± 0.91	2.48 ± 0.71

[a]PHLA, postheparin lipolytic activity.
[b]LPL, extrahepatic lipoprotein lipase.
[c]HL, hepatic lipase.
[d]$P < 0.05$, EFAD versus SIB.
[e]$P < 0.05$, EFAD versus EFAS.
[f]$P < 0.05$, SIB versus Controls.

TABLE 8. Postheparin lipolytic activity in cystic fibrosis patients

Subjects	Total PHLA	LPL	HL	HL/LPL
		μmol FFA/ml per h		
EFAD, chronic liver disease				
Case 3	4.07	4.02	1.16	0.28
Case 5	7.17	3.19	4.15	1.30
Case 9	3.79	2.00	1.96	0.98
Mean ± SE	5.22 ± 1.01	3.13 ± 0.90	2.42 ± 0.89	0.85 ± 0.30
No liver disease				
Case 1	4.45	2.43	2.24	0.92
Case 2	4.96	4.35	1.29	0.30
Case 4	9.43	5.12	4.78	0.93
Case 6	8.04	4.53	3.96	0.87
Mean ± SE	6.72 ± 1.20	3.61 ± 1.07	3.06 ± 0.79	0.75 ± 0.87
Statistics (*P*)	NS	NS	NS	NS
Correlation with indices of EFAD (*r*)				
EFAD (n = 7)				
20:3(n-9)/20:4(n-6)	0.21	−0.15	0.36	0.53
16:1(n-7)/18:2(n-6)[a]	0.47	0.19	0.51	0.41
All CF (n = 17)				
20:3(n-9)/20:4(n-6)	−0.11	−0.22	0.05	0.17
16:1(n-7)/18:2(n-6)	−0.06	−0.09	−0.05	0.06

[a]Parameter of EFAD established in parallel study on tissue lipids in CF (ref. 36).

tions, namely a higher VLDL-C and a lower HDL-C along with decreased hepatic lipase activity, need to be explored further.

This study was supported by the Canadian Cystic Fibrosis Foundation (CCFF) and by the Medical Research Council of Canada (MT-4433). Emile Lévy has a "Chercheur-Boursier" (young investigator award) of "le Fonds de la Recherche en Santé du Québec" and Lesley Smith was the recipient of a CCFF and Wellcome Foundation travel grant. The authors are grateful to Line Chartier and Sylvie Tassé for typing the manuscript.

Manuscript received 22 September 1988 and in revised form 13 February 1989.

REFERENCES

1. Welsh, M. J., and R. B. Fick. 1987. Cystic fibrosis. *J. Clin. Invest.* **80:** 1523–1526.
2. Soutter, V. L., P. Kristidis, M. A. Gruca, and K. J. Gaskin. 1986. Chronic undernutrition/growth retardation in cystic fibrosis. *Clin. Gastroenterol.* **15:** 137–155.
3. 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.
4. Hubbard, V. S., G. Dewey-Dunn, and P. A. di Sant'Agnese. 1977. Abnormal fatty-acid composition of plasma-lipids in cystic fibrosis. A primary or a secondary defect? *Lancet.* **2:** 1302–1304.
5. Chase, P. H., M. A. Long, and M. H. Lavin. 1979. Medical progress. Cystic fibrosis and malnutrition. *J. Pediatr.* **95:** 337–347.
6. Elliot, R. B. 1976. A therapeutical trial of fatty acid supplementation in cystic fibrosis. *Pediatrics.* **57:** 474–479.
7. Carlstedt-Duke, J., M. Brönnegard, and B. Standvik. 1986. Pathological regulation of arachidonic acid release in cystic fibrosis: the putative basic defect. *Proc. Natl. Acad. Sci. USA.* **83:** 9202–9206.
8. Mead, J. F. 1984. The non-eicosanoid functions of the essential fatty acids. *J. Lipid Res.* **25:** 1517–1521.
9. Marcus, A. J. 1984. The eicosanoids in biology and medicine. *J. Lipid Res.* **25:** 1511–1516.
10. Kunkel, S. L. 1988. Editorial. The importance of arachidonate metabolism by immune and nonimmune cells. *Lab. Invest.* **58:** 119–121.
11. 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.
12. Vaughan, W. J., F. T. Lindgren, J. B. Whalen, and S. Abraham. 1978. Serum lipoprotein concentrations in cystic fibrosis. *Science.* **199:** 783–786.
13. Chait, A., A. Onitiri, A. Nicoll, R. Labaya, J. Davies, and B. Lewis. 1974. Reduction of serum triglyceride levels by polyunsaturated fat. Studies on the mode of action and on very low density lipoprotein composition. *Atherosclerosis.* **20:** 347–364.
14. Harris, W. S., W. E. Connor, and M. P. McMurry. 1983. The comparative reduction of the plasma lipids and lipoproteins by dietary polyunsaturated fats: salmon oil versus vegetable oils. *Metabolism.* **32:** 179–184.
15. Baudet, M. F., C. Dachet, M. Lasserre, O. Esteva, and B. Jacotot. 1984. Modification in the composition and metabolic properties of human low density and high density lipoproteins by different dietary fats. *J. Lipid Res.* **25:** 456–468.
16. Nestel, P. J., W. E. Connor, M. F. Reardon, S. Connor, S. Wong, and R. Boston. 1984. Suppression by diets rich in fish oil of very low density lipoprotein production in man. *J. Clin. Invest.* **74:** 82–89.
17. Shwachman, H., and L. L. Kulezycki. 1958. Long term study of one hundred and five patients with cystic fibrosis. *Am. J. Dis. Child.* **96:** 6–15.
18. Demirjian, A., M. Jenick, and M. B. Dubuc. 1972. Les normes staturopondérales de l'enfant urbain Canadien Fran-

çais d'age scolaire. *Can. J. Pub. Health.* **63:** 14–30.
19. Levy, E., L. A. Thibault, C. C. Roy, M. Bendayan, G. Lepage, and J. Letarte. 1988. Circulating lipids and lipoproteins in glycogen storage disease type I with nocturnal intragastric feeding. *J. Lipid Res.* **29:** 215–226.
20. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
21. Bartlett, G. R. 1959. Phosphorus assay in column chromatography. *J. Biol. Chem.* **234:** 466–468.
22. Lopez-Virella, M. F., P. Stone, S. Ellis, and J. A. Colwell. 1977. Cholesterol determination in high density lipoprotein separated by three different methods. *Clin. Chem.* **23:** 882–891.
23. Schriewer, B., W. Nolted, and G. Assmann. 1985. VLDL apolipoprotein B determination in blood serum following precipitation of LDL with polyvinylsulphate. *J. Clin. Chem. Clin. Biochem.* **23:** 349–353.
24. Weber, K., and M. Osborn. 1969. The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. *J. Biol. Chem.* **244:** 4406–4412.
25. Kane, J. 1973. A rapid electrophoretic technique for identification of subunit species of apoproteins in serum lipoproteins. *Anal. Biochem.* **53:** 350–364.
26. Deckelbaum, R. J., C. Dupont, J. Letarte, and P. Pencharz. 1983. Primary hypertriglyceridemia in childhood. *Am. J. Dis. Child.* **137:** 396–398.
27. Krauss, R. M., R. I. Levy, and D. S. Frederickson. 1974. Selective measurement of two lipase activities in post-heparin plasma from normal subjects and patients with hyperlipoproteinemia. *J. Clin. Invest.* **54:** 1107–1124.
28. Belfrage, P., and M. Vaughan. 1969. Simple liquid–liquid partition system for isolation of labeled oleic acid from mixtures with glycerides. *J. Lipid Res.* **10:** 341–344.
29. Chase, H. P., and J. Dupont. 1978. Abnormal levels of prostaglandins and fatty acids in blood of children with cystic fibrosis. *Lancet.* **2:** 236–238.
30. Hubbard, V. S., and G. D. Dunn. 1980. Fatty acid composition of erythrocyte phospholipids from patients with cystic fibrosis. *Clin. Chim. Acta.* **102:** 115–118.
31. Rogiers, V., I. Dab, R. Brokaert, and H. L. Vis. 1980. Long chain non-esterified fatty acid pattern in plasma of cystic fibrosis patients and their parents. *Pediatr. Res.* **14:** 1088–1091.
32. 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.
33. 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. *JPEN.* **5:** 501–504.
34. 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.
35. Farrel, P. M., E. H. Mischler, M. J. Engle, D. J. Brown, and S. Lau. 1985. Fatty acid abnormalities in cystic fibrosis. *Pediatr. Res.* **19:** 104–109.
36. Lepage, G., N. Ranco, L. Smith, N. Galeano, E. Levy and C. C. Roy. 1988. Are tissue lipids necessary to establish the diagnosis of EFA deficiency in CF? *FASEB J.* **2:** 1688 (Abstract).
37. Pawar, S., and H. Tidwell. 1968. Effect of ingestion of unsaturated fat on lipolytic activity of rat tissues. *J. Lipid Res.* **9:** 334–336.
38. Coiffier, E., R. Paris, and J. Lecerf. 1987. Effects of dietary saturated and polyunsaturated fat on lipoprotein lipase and hepatic triglyceride lipase activity. *Comp. Biochem. Physiol.* **88B:** 187–192.
39. Jansen, H., and W. C. Hülsmann. 1974. Liver and extrahepatic contributions to postheparin serum lipase activity of the rat. *Biochim. Biophys. Acta.* **369:** 387–396.
40. Spencer, I. M., A. Hutchinson, and D. S. Robinson. 1978. The effect of nutritional state on the lipoprotein lipase activity of isolated fat cells. *Biochim. Biophys. Acta.* **530:** 375–384.
41. Thompson, G. R., and J-P. Miller. 1973. Plasma lipid and lipoprotein abnormalities in patients with malabsorption. *Clin. Sci. Mol. Med.* **45:** 583–592.
42. Dietschy, J. M., and J. D. Wilson. 1970. Regulation of cholesterol metabolism. *N. Engl. J. Med.* **282:** 1128–1138, 1179–1183, 1241–1249.
43. Grundy, S. M. 1983. Absorption and metabolism of dietary cholesterol. *Annu. Rev. Nutr.* **3:** 71–96.
44. Weber, A. M., C. C. Roy, C. L. Morin, and R. Lasalle. 1973. Malabsorption of bile acids in children with cystic fibrosis. *N. Engl. J. Med.* **289:** 1001–1005.
45. Leroy, C., G. Lepage, C. L. Morin, J. M. Betrand, O. Dufour-Larue, and C. C. Roy. 1986. Effect of dietary fat and residues on fecal loss of sterols and on their microbial degradation in cystic fibrosis. *Dig. Dis. Sci.* **31:** 911–918.
46. Schwartz, C. C., L. G. Halloran, Z. R. Vlahcevic, D. H. Gregory, and L. Swell. 1978. Preferential utilization of free cholesterol from high-density lipoproteins for biliary cholesterol secretion in man. *Science.* **200:** 62–64.
47. Price, S. G. L., C. Cortese, and N. E. Miller. 1985. Are plasma lipoprotein cholesteryl esters utilized for biliary cholesterol and bile acid production in man? *Life Sci.* **36:** 2217–2222.
48. Shefer, S., S. Hauser, I. Bekersky, and E. H. Mosbach. 1969. Feedback regulation of bile acid biosynthesis in the rat. *J. Lipid Res.* **10:** 646–655.
49. Kubaska, W. M., E. C. Gurley, P.B. Hylemon, P.S. Guzelian, and Z. R. Vlahcevic. 1985. Absence of negative feedback control of bile acid biosynthesis in cultured rat hepatocytes. *J. Biol. Chem.* **260:** 13459–13463.
50. Borchardt, R. A., and R. A. David. 1987. Intrahepatic assembly of very low density lipoproteins. *J. Biol. Chem.* **262:** 16394–16402.
51. Quinn, D., K. Shirai, and R. L. Jackson. 1982. Lipoprotein lipase: mechanism of action and role in lipoprotein metabolism. *Prog. Lipid Res.* **22:** 35–78.
52. Vainio, P., J. A. Virtanen, P. K. Kinnunen, J. C. Voyta, L. C. Smith, A. M. Gotto, Jr., J. T. Sparrow, F. Pattus, and R. Verger. 1983. Action of lipoprotein lipase on phospholipid monolayers. Activation by apolipoprotein C-II. *Biochemistry.* **22:** 2270–2275.
53. Musliner, T. A., P. N. Herbert, and M. J. Kingston. 1979. Activation of lipoprotein lipase by native and acylated peptides of apolipoprotein C-II. *Biochim. Biophys. Acta.* **575:** 277–288.
54. Breckenridge, W. C., J. A. Little, P. Alaupovic, C. S. Wang, A. Kuksis, G. Kakis, F. Lindgren, and G. Gardiner. 1982. Lipoprotein abnormalities associated with a familial deficiency of hepatic lipase. *Atherosclerosis.* **45:** 161–179.
55. Shinomiya, M., N. Sasaki, R. L. Barnhart, K. Shirai, and R. L. Jackson. 1982. Effect of apolipoproteins on the hepatic lipase-catalyzed hydrolysis of human plasma high density lipoprotein$_2$-triacylglycerols. *Biochim. Biophys. Acta.* **713:** 292–299.
56. Sugano, M., and O. W. Portman. 1965. Essential fatty acid

deficiency and cholesterol esterification, activity of plasma and liver in vitro and in vivo. *Arch. Biochem. Biophys.* **109:** 302–315.
57. Freeman, M., L. Kuiken, J. B. Ragland, and S. M. Sabesin. 1977. Hepatic triglyceride lipase deficiency in liver disease. *Lipids.* **12:** 443–445.
58. Klose, G., J. Windelband, A. Weizel, and H. Greten. 1977. Secondary hypertriglyceridemia in patients with parenchymal liver disease. *Eur. J. Clin. Invest.* **7:** 557–562.
59. Mordasini, R., F. Frei, W. Flury, G. Klose, and H. Greten. 1977. Selective deficiency of hepatic triglyceride lipase in uremic patients. *N. Engl. J. Med.* **297:** 1362–1366.
60. Nikkila, E. A., J. K. Huttunen, and C. Ehnholm. 1976. Low postheparin plasma hepatic lipase activity in familial type IIa hyperlipoproteinemia. *Ann. Clin. Res.* **8:** 63–67.
61. Murase, T., and H. Uchimura. 1980. A selective decline of postheparin plasma hepatic triglyceride lipase in hypothyroid rats. *Metabolism.* **29:** 797–801.
62. Jackson, R. L. 1983. Lipoprotein lipase and hepatic lipase. *Enzyme.* **XVI.** 141–181.
63. Davis, P. B. 1984. Autonomic and airway reactivity in obligate heterozygotes for cystic fibrosis. *Am. Rev. Respir. Dis.* **129:** 911–914.
64. Hosrer, K., H. Kollberg, and E. Vogt. 1979. Operational definition of cystic fibrosis. *Monogr. Paediatr.* **10:** 90–95.
65. Sato, K., and F. Sato. 1984. Defective β-adrenergic response of CF sweat glands in vivo and in vitro. *J. Clin. Invest.* **73:** 1763–1771.
66. Betron, J. K., G. Hagiwara, N. J. Lewiston, P. M. Quinton, and J. J. Wine. 1987. Hyposecretion of β-adrenergically induced sweating in CF heterozygotes. *Pediatr. Res.* **22:** 271–276.
67. Burns, G. B., and J. A. Dodge. 1982. Elevated levels of 13,14-dihydro-15-keto prostaglandin metabolites and essential fatty acid deficiency in CF and heterozygote subjects. *In* Proceedings 11th Annual Meeting of the European Working Group for Cystic Fibrosis. D. Barman, editor. Acco Leuven. 76.
68. Hamdi, I., A. L. Bloom, M. C. Goodchild, and J. A. Dodge. 1979. Prostaglandins and cystic fibrosis. *Monogr. Paediatr.* **10:** 84–89.
69. Samuels, C.E., P. G. Robinson, and R. B. Elliott. 1975. Decreased inhibition of platelet aggregation by PGE, in children with cystic fibrosis and their parents. *Prostaglandins.* **10:** 617–621.
70. Rogiers, V., I. Dab, R. Crokaert, and H. L. Vis. 1980. Long chain nonesterified fatty acid pattern in plasma of cystic fibrosis patients and their parents. *Pediatr. Res.* **14:** 1088–1091.
71. Harrisson, G. C. 1988. Cystic fibrosis and chloride-secreting diarrhea. *Nature.* **333:** 711.
72. Boberg, M., B. Vessby, and I. Selinus. 1986. Effects of dietary supplementation with n-6 and n-3 long-chain polyunsaturated fatty acids on serum lipoproteins and platelet function in hypertriglyceridaemic patients. *Acta Med. Scand.* **220:** 153–160.
73. Deckelbaum, R. J., T. Olivecrona, and S. Eisenberg. 1984. Plasma lipoproteins in hyperlipidemias: roles of neutral lipid exchange and lipase. *In* Treatment of Hyperlipoproteinemia. L. A. Carlson and A. G. Olson, editors. Raven Press, New York. 85–93.
74. Witting, L. A. 1974. Vitamin E—polyunsaturated lipid relationship in diet and tissues. *Am. J. Clin. Nutr.* **27:** 952–956.
75. Subramanian, C. S., and J. F. Mead. 1986. A relationship between essential fatty acid and vitamin E deficiency. *Lipids.* **21:** 603–607.
76. Hatam, L. J., and H. J. Kayden. 1981. The failure of alpha-tocopherol supplementation to alter the distribution of lipoprotein cholesterol in normal and hyperlipoproteinemic persons. *Am. J. Clin. Pathol.* **76:** 122–126.