Combined (n-3 and n-6) essential fatty deficiency is a potent modulator of plasma lipids, lipoprotein composition, and lipolytic enzymes

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Abstract Essential fatty acids (EFA) are important structural and functional components of cell membranes. Their deficiency has been associated with several clinical and biochemical abnormalities. In the present study, the lipid profile as well as the concentration, composition, and metabolism of lipoproteins were examined in rats rendered EFA-deficient over a period of 12 weeks. Changes in plasma fatty acids mainly induced an increase of palmitoleic (16:1 n-7) and eicosatrienoic (20:3 n-9) acids, while linoleic (18:2 n-6), arachidonic (20:4 n-6), linolenic (18:3 n-3), and docosahexaenoic (22:6 n-3) acids were decreased. The results show increased concentrations of free fatty acids (FFA) ($P<0.001$), triglycerides ($P<0.001$), total cholesterol ($P<0.02$), free cholesterol ($P<0.005$), and phospholipids ($P<0.05$) when compared to pair-fed controls. Similar levels of cholesteryl esters were found in the two groups, and lecithin: cholesterol acyltransferase activity (nmol/100 μl plasma per h) (8.98 ± 1.44 vs 8.72 ± 0.50) did not differ. On the other hand, postheparin extrahepatic lipoprotein lipase (LPL) activity was significantly ($P<0.002$) decreased (5.96 ± 0.29 vs 7.29 ± 0.68 μmol FFA/ml per h) and could account for the hypertriglyceridemia as well for the relative triglyceride enrichment of very low density lipoprotein, intermediate density lipoprotein, and low density lipoprotein particles. This enzymatic depletion of LPL was mainly due to the adipose tissue, since a higher level ($P<0.001$) of hepatic lipase (325.8 ± 16.0 vs 130.8 ± 9.5 nmol FFA/mg protein per h) was found in liver acetone powder extracts. As neither LCAT nor cholesterol transfer protein can explain these findings, we suggest that the lipoprotein abnormalities induced by EFA deficiency can be largely attributed to decreased lipoprotein lipase activity coupled with an increase of hepatic lipase. —**Levy, E., L. Thibault, C. Garofalo, M. Messier, G. Lepage, N. Ronco, and C. C. Roy.** Combined (n-3 and n-6) essential fatty acid deficiency is a potent modulator of plasma lipids, lipoprotein composition, and lipolytic enzymes. *J. Lipid Res.* 1990. **31:** 2009-2017.

Supplementary key words lipoprotein lipase • hepatic lipase • lecithin:cholesterol acyltransferase

Essential fatty acid (EFA) deficiency leads to a depletion of (n-6) and to a lesser extent of (n-3) followed by an accumulation of (n-9) fatty acids (1). As a result, various tissues display a high eicosatrienoic acid/arachidonic acid [20:3 (n-9)/20:4 (n-6)] ratio. Unusual findings characteristic of EFA deficiency include salt-dependent hypertension (2), impaired fertility (3), epidermal dysfunction (4), as well as alteration of eicosanoid metabolism (5), of inflammatory response (6), and of lung function (7).

Despite the wide and growing knowledge of the clinical repercussions of EFA deficiency, only limited information is available with regard to the biological effects of the depleted state. Evidence indicates that EFA-depletion is accompanied by marked changes in lipid composition as well as in membrane physiochemical properties, such as their fluidity, discoid shape, and Na^+K^+ATPase activity (8). As a consequence, many organs are affected including the liver (9) and the intestine (10) which are known to play a major role in lipoprotein metabolism.

We have recently developed an accurate and a sensitive procedure for the measurement of all fatty acid classes (11). It has allowed us to document a high incidence of EFA deficiency based on a ratio 20:3 (n-9)/20:4 (n-6) greater than 0.1, in patients with cystic fibrosis (12). When these subjects were studied, the lipid profile as well as lipoprotein concentration, composition, size, and metabolism were profoundly affected (13). Although there appeared to be a good relationship between the lipid and lipoprotein abnormalities and EFA status, a definitive interpretation of the role of EFA deficiency could only be surmised, in view of the many other variables associated with the spectrum of manifestations and complications of

Abbreviations: EFAD, essential fatty acid deficiency; VLDL, very low density lipoproteins; IDL, intermediate density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; TC, total cholesterol; TG, triglycerides; FC, free cholesterol; CE, cholesteryl esters; FFA, free fatty acids; PR, protein; PL, phospholipid; HTGL, hepatic triglyceride lipase; LPL, lipoprotein lipase; LCAT, lecithin:cholesterol acyltransferase.

the disease. In this context we elected to examine the effects of combined EFA deficiency (n-3 and n-6) on the lipid profile and lipoprotein composition, and on the activity of specific enzymes such as lipoprotein lipase, hepatic lipase, and lecithin:cholesterol acyltransferase in rats with dietary-induced EFAD.

MATERIALS AND METHODS

Animals and diets

Male Sprague-Dawley rats were purchased from Charles River Breeding Laboratories (Montreal, Quebec, Canada). The rats weighing about 80 g were allowed free access to water and food. After 1 week of acclimatization, the rats were randomly divided into two groups: one was assigned to an essential fatty acid-deficient diet (ICN Biochemicals) and the other was pair-fed with standard chow diet. The composition of the diets is detailed in **Table 1** and **Table 2**. The rats were maintained at 22°C with constant lighting (7 AM to 7 PM). Body weights and food intakes were recorded three times weekly to monitor growth. After 12 weeks on these diets, rats were fasted overnight before receiving 50 mg sodium pentobarbital/kg body weight intraperitoneally (Nembutal, Abbott Laboratories, Montreal, Quebec, Canada). Blood was collected from the jugular vein for analyses, and the heart, the liver, and adipose tissue from the rats were promptly removed and prepared for lipoprotein lipase assay.

Isolation of lipoproteins

Blood samples were collected in tubes containing 1 mg EDTA/ml and were separated immediately by low speed centrifugation (2,500 rpm, 20 min) at 4°C. Lipoproteins were isolated from fresh plasma by conventional discontinuous density gradient ultracentrifugation as previously described (13, 14). Briefly, after preliminary centrifugation to remove chylomicrons (25,000 rpm for 30 min) in a Beckman L5-65 preparative ultracentrifuge using a Ti-50 rotor, 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, at 40,000 rpm for 18 h at 5°C. The separation of high density lipoprotein (HDL) subpopulations was performed at 40,000 rpm 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 by their equilibrium density and dialyzed intensively against 0.15 M NaCl, 0.001 M EDTA, pH 7.0.

TABLE 1. Composition of diets

	g/100 g
Sucrose	50.0
Corn starch	15.0
Casein, vitamin free	20.0
Fat[a]	5.0
Fiber (non nutritive)	5.0
Mineral mixture[b]	3.5
Vitamin mixture[c]	1.0
DL-Methionine	0.3
Choline bitartrate	0.2

[a] See Table 2.

[b] Providing (mg/kg mix): $CaHPO_4$, 500: NaCl, 74; K_2SO_4, 52; MgO, 24; KIO_4, 0.01; $Crk(SO_4)\cdot 12H_2O$, 0.55; $MnCO_3$, 3.5; $ZnCO_3$, 1.6; potassium citrate, 220; and ferric citrate, 6.0.

[c] Providing (mg/g mix): thiamine hydrochloride, 600; riboflavin, 600; pyridoxine hydrochloride, 700; nicotinic acid, 3.0; D-calcium pantothenate, 1.6; folic acid, 200; D-biotin, 20; vitamin B-12, 10; retinyl palmitate, 1.6; DL-alpha-tocopherol acetate, 20; cholecalciferol, 250; and menaquinone, 5.0.

TABLE 2. Fatty acid composition of the diets

	Diet	
Fatty Acid	Control	EFAD
12:0	ND	0.12
14:0	1.06	3.25
15:0	0.13	0.77
16:0	17.19	47.12
17:0	0.15	1.84
18:0	3.49	44.56
22:0	0.20	ND
24:0	0.17	0.03
18:3 n-3	6.09	ND
18:4 n-3	0.24	ND
20:5 n-3	1.20	ND
22:5 n-3	0.17	ND
22:6 n-3	2.13	ND
18:2 n-6	47.60	0.78
18:3 n-6	0.28	0.48
20:2 n-6	0.02	ND
20:4 n-6	0.13	ND
22:4 n-6	0.10	ND
16:1 n-7	1.07	0.51
18:1 n-7	1.45	ND
20:1 n-7	0.09	ND
18:1 n-9	16.97	0.45
22:1 n-9	0.06	0.02
Saturates	22.39	97.68
PUFA	57.97	1.27
Total n-3	9.83	ND
Total n-6	48.14	1.27
Total n-7	2.61	0.51
Total n-9	17.03	0.48

ND, nondetectable.

Lipid and lipoprotein analysis

Plasma concentrations of total cholesterol (TC), free cholesterol (FC), and triglycerides were measured enzymatically by a commercial kit (Boehringer Mannheim, Montreal) as reported previously (14). 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. (15) with bovine

serum albumin as a standard. Phospholipids (PL) were determined by the Bartlett method (16). HDL-cholesterol (HDL-C) was measured after precipitation of VLDL and LDL with phosphotungstic acid (17). Apolipoprotein content of plasma lipoproteins was qualitatively assayed using both SDS (18) and urea (19) polyacrylamide gel 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 by standards of different molecular weights. The densitometric distribution of apolipoproteins was assayed as described previously (14).

Plasma lipolytic activity measurement

Postheparin plasma was taken from jugular puncture 10 min after an intravenous injection of heparin (100 units/kg body weight). Lipolytic activity was measured with an emulsion of tri[1-^{14}C]oleoylglycerol as substrate (20). Hepatic triglyceride lipase (HL) activity was assayed in the presence of protamine sulfate which was verified to inhibit completely peripheral lipoprotein lipase (LPL). Extraction of FFA was performed by the procedure of Belfrage and Vaughan (21) as previously described (13, 14).

Preparation of tissue for assay of lipoprotein lipase

Heart, adipose tissue, and liver were weighed and homogenized in ice-cold acetone using the Polytron homogenizer. The homogenates were centrifuged at 4,000 rpm for 20 min at 4°C, and the supernatants were discarded. The residues were reextracted three times with 50 ml of ice-cold acetone and twice with diethyl ether. The defatted preparations were dried at 0°C under nitrogen, and designated "acetone powder." The defatted preparations were suspended in 0.025 M NH_3-HCl buffer, pH 8.1, containing 2 IU of heparin/ml. Clear supernatants were obtained after centrifugation (4,000 rpm for 15 min at 4°C) and used for LPL studies. LPL activity was assayed as described previously (20), and was calculated as the difference between the total activity and the lipolytic activity remaining after the addition of 1 M NaCl in the assay mixture.

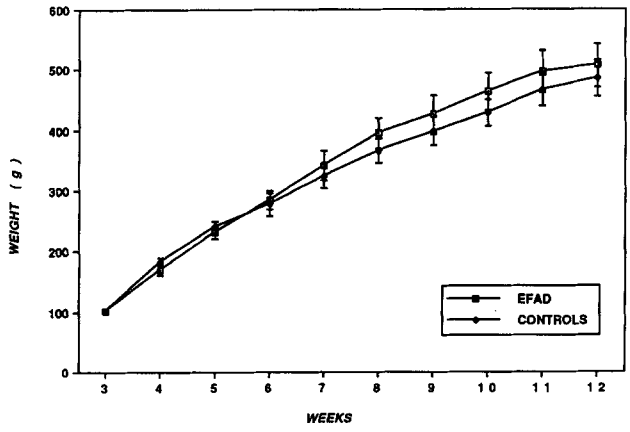

Fig. 1. Weekly weight gain of EFAD and control rats.

Lecithin:cholesterol acyltransferase (LCAT)

The plasma LCAT activity was measured according to the original procedure of Stokke and Norum (22).

Statistical analysis

All values were expressed as the mean ± standard error (SEM). Statistical differences were assessed by the Student's two-tail *t*-test.

RESULTS

Food consumption and body weight

Since pair-feeding was used in this study, no changes were observed in food consumption. Daily food intake was 29.8 ± 0.6 g for the EFAD group and 29.8 ± 0.5 g for the control group. As expected, no significant differences in growth rates were found between the EFAD and control groups (**Fig. 1**).

Plasma lipids

The plasma FA pattern was profoundly affected (**Table 3**). Striking changes were observed in polyunsaturated fatty acids of the EFAD group. In general, the (n-3) and (n-6) families were decreased and both the (n-7) and (n-9) families were increased. It is worth noting that linoleic acid (18:2 n-6) as well as arachidonic acid (20:4 n-6) were decreased by more than 5- and 2-fold, respectively, compared to control values, whereas palmitoleic acid (16:1 n-7) and eicosatrienoic acid were increased by 3- and 167-fold, respectively. These extensive alterations led to an elevation of the commonly used index for essential FA deficiency (20:3 (n-9)/20:4 (n-6)). Similarly, the 16:1 (n-7)/18:2 (n-6) ratio which has been shown to be a good discriminant for EFAD (12), was 16 times higher in the EFAD group than in controls.

The patterns of plasma lipids and lipoproteins in the two groups of animals are shown in **Table 4**. Elevated values of FFA (32% above controls) were associated with hypertriglyceridemia, hypercholesterolemia, and hyperphospholipidemia. The increase in TG (49%) was almost twice that of TC (25%). As expected, because of the high total plasma cholesterol concentration, both cholesterol fractions, FC and EC, were markedly increased. However, the percentage esterification of cholesterol did not differ from controls. Although the HDL-C was reduced, the difference did not reach statistical significance.

TABLE 3. Plasma Total Fatty Acids

Fatty Acid	Control	EFA-Deficient
	mol %	
14:0	0.53 ± 0.05	0.79 ± 0.007
15:0	0.27 ± 0.002	0.19 ± 0.005
16:0	23.16 ± 0.87	24.46 ± 2.48
17:0	0.24 ± 0.14	0.28 ± 0.08
18:0	9.95 ± 0.35	12.05 ± 1.15
22:0	0.16 ± 0.01	0.09 ± 0.06
24:0	0.44 ± 0.02	0.35 ± 0.08
18:3 n-3	0.93 ± 0.09	0.05 ± 0.00*
20:5 n-3	1.27 ± 0.09	0.09 ± 0.01*
22:5 n-3	0.69 ± 0.05	0.08 ± 0.005*
22:6 n-3	7.62 ± 0.97	2.46 ± 0.30*
14:1 n-5	0.03 ± 0.005	0.08 ± 0.04
18:2 n-6	20.84 ± 0.97	3.70 ± 0.44**
18:3 n-6	0.22 ± 0.02	0.58 ± 0.007
20:2 n-6	1.12 ± 0.99	5.43 ± 0.74*
20:3 n-6	0.49 ± 0.07	0.33 ± 0.05
20:4 n-6	18.01 ± 0.78	7.23 ± 0.33**
22:4 n-6	0.10 ± 0.01	0.05 ± 0.005*
16:1 n-7	2.24 ± 0.16	6.59 ± 0.13**
18:1 n-7	1.63 ± 0.05	2.58 ± 0.28*
20:1 n-7	0.16 ± 0.03	0.43 ± 0.005*
18:1 n-9	9.14 ± 0.58	20.91 ± 1.24**
20:3 n-9	0.06 ± 0.006	10.06 ± 2.53***
22:1 n-9	0.04 ± 0.006	0.03 ± 0.005
24:1 n-9	0.38 ± 0.05	0.71 ± 0.24*
Saturated (%)	34.82 ± 1.11	38.32 ± 1.17
PUFA (%)	51.29 ± 1.81	30.05 ± 0.23**
PUFA/saturated	1.48 ± 0.10	0.52 ± 0.01***
Total n-3 (%)	10.51 ± 0.90	2.72 ± 0.36*
Total n-6 (%)	40.79 ± 1.07	17.33 ± 0.59**
Total n-7 (%)	4.03 ± 0.14	9.61 ± 0.14**
Total n-9 (%)	6.82 ± 1.80	31.73 ± 1.54***
16:1 (n-7)/18:2 (n-6)	0.108 ± 0.08	1.802 ± 0.180***
18:2 (n-6)/20:4 (n-6)	1.162 ± 0.073	0.515 ± 0.085
20:3 (n-9)/20:4 (n-6)	0.015 ± 0.012	1.410 ± 0.415***
EFA/non-EFA	1.06 ± 0.13	0.250 ± 0.005***

Data are mean ± SEM. Fatty acids contributing less than 0.1% of the total have been omitted from the Table. EFAD versus controls: $*P < 0.05$; $**P < 0.01$; $***P < 0.005$.

Lipid and apoprotein composition of lipoproteins

The VLDL faction (1.006 g/ml) was significantly enriched in triglyceride and cholesteryl ester (**Table 5**), and poorer in protein. These alterations led to greater ratios of TG/PR, TG/PL, CE/PR, and TG + CE/FC + PL + PR. It should be noted that the mass ratio of core constituents (TG + CE) to surface constituents (FC + PR + PL) can be used to make inferences on the size of spherical lipoproteins; lighter and larger particles are relatively enriched with core components when compared to the denser and smaller populations. The calculated values of these ratios indicated that VLDL particles were larger.

In both the IDL (1.019 g/ml) and LDL (1.063 g/ml) fractions, there was only a slight increase TG. This trend led to an increase, albeit not significant, of the ratios worked out for the VLDL population of particles. In contrast, HDL_2 (1.125 g/ml) and HDL_3 (1.21 g/ml) fractions presented a relative drop of TG, CE, and FC, accompanied by an elevation of PL and PR. As a result of these alterations, the TG/PR, TG/PL, CE/PR, and TG/(FC + PL + PR) ratios of HDL_2 and HDL_3 in the EFAD group showed a decrease, most of them statistically significant.

The VLDL preparations varied in the apolipoprotein pattern with respect to apoE and apoC (**Fig. 2**). The VLDL fraction derived from the EFAD group clearly showed a distinct relative enrichment in the lower molecular weight apoE isoforms, whereas a reduced proportion of the higher molecular weight apoC isoforms was observed. This was confirmed by densitometric distribution of VLDL apolipoproteins (results not shown). The apoC family was analyzed on tetramethylurea (TMU) gels (**Fig. 3**). There was no consistent difference between the apoC patterns of VLDL of EFAD and control rats. Pronounced apolipoprotein changes were also noted in the HDL_2 fraction of EFAD on 15% SDS-PAGE (Fig. 3). A depletion of apoE and an increase of apoA-I characterized the profile.

LCAT activity

Despite the fact that the percentage of esterified cholesterol in the plasma of the EFAD and control groups did not differ (Table 3), alterations of the proportion of cholesteryl esters were seen in the chemical composition of lipoproteins, particularly VLDL. However, LCAT activity showed no difference between the two groups (**Table 6**).

Plasma and tissue lipoprotein lipase activity

In order to determine whether the hypertriglyceridemia was associated with an abnormally low clearance of TG, plasma and tissue LPL activity was measured. While ex-

TABLE 4. Plasma lipids and lipoproteins

Group	FFA	TG	TC	FC	CE as % of TC	PL	HDL-C
		mg/dl			%	*mg/dl*	
EFAD (n = 17)	640 ± 13	112 ± 12	88 ± 6	18 ± 3	78 ± 2	374 ± 13	42 ± 3
Controls (n = 12)	343 ± 28	57 ± 6	66 ± 5	10 ± 6	79 ± 6	335 ± 11	48 ± 1
P	0.001	0.001	0.02	0.005	NS	0.05	NS

Abbreviations: EFAD, essential fatty acid deficient; FFA, free fatty acids; TG, triacylglycerols; TC, total cholesterol; FC, free cholesterol; CE, cholesteryl ester; PL, phospholipids; HDL-C, HDL-cholesterol; NS, not significant.

TABLE 5. Chemical composition of lipoproteins

Lipoprotein	TG	CE	FC	PL	PR	TG/PR	TG/PL	CE/PR	(TG + CE)/(FC + PL + PR)
					g/100 g				
VLDL (1.00 g/ml)									
EFAD	63.7 ± 2.4	6.0 ± 0.4	4.3 ± 0.3	17.1 ± 1.3	8.9 ± 0.7	7.31 ± 0.65	3.83 ± 0.34	0.68 ± 0.05	2.34 ± 0.19
Controls	51.2 ± 2.1	4.0 ± 0.1	4.1 ± 0.1	20.5 ± 1.9	20.1 ± 1.3	2.36 ± 0.30	2.57 ± 0.30	0.21 ± 0.02	1.19 ± 0.11
P	0.005	0.005	NS[a]	NS	0.001	0.001	0.025	0.001	0.001
IDL (1.019 g/ml)									
EFAD	26.4 ± 1.3	12.4 ± 2.7	5.2 ± 0.5	25.2 ± 1.1	30.8 ± 3.0	0.92 ± 0.32	1.06 ± 0.09	0.45 ± 0.14	0.64 ± 0.05
Controls	23.2 ± 4.0	12.0 ± 1.6	4.7 ± 0.3	27.8 ± 0.8	32.3 ± 3.3	0.89 ± 0.22	0.95 ± 0.20	0.30 ± 0.05	0.57 ± 0.10
P	NS	NS	NS	NS	NS	NS	NS	NS	NS
LDL (1.063 g/ml)									
EFAD	13.5 ± 2.4	24.1 ± 2.0	8.5 ± 0.6	29.2 ± 1.4	24.5 ± 1.1	0.61 ± 0.08	0.55 ± 0.07	0.92 ± 0.10	0.63 ± 0.03
Controls	10.7 ± 1.0	26.3 ± 1.3	8.4 ± 0.2	29.3 ± 0.3	25.3 ± 0.9	0.42 ± 0.03	0.36 ± 0.03	1.05 ± 0.10	0.59 ± 0.02
P	NS	NS	NS	NS	NS	NS	0.05	NS	NS
HDL_2 (1.125 g/ml)									
EFAD	0.20 ± 0.03	19.63 ± 0.70	3.55 ± 0.24	35.98 ± 0.80	40.33 ± 0.75	0.005 ± 0.001	0.005 ± 0.001	0.496 ± 0.019	0.252 ± 0.008
Controls	0.38 ± 0.03	22.80 ± 0.76	5.10 ± 0.24	33.40 ± 0.71	38.32 ± 0.58	0.011 ± 0.001	0.011 ± 0.001	0.596 ± 0.024	0.302 ± 0.013
P	0.005	0.02	0.005	0.05	NS[a]	NS	0.001	0.05	0.05
HDL_3 (1.21 g/ml)									
EFAD	0.35 ± 0.09	18.48 ± 0.52	2.27 ± 0.11	26.17 ± 0.96	52.87 ± 0.86	0.007 ± 0.002	0.013 ± 0.003	0.350 ± 0.005	0.232 ± 0.007
Controls	0.38 ± 0.04	20.62 ± 0.48	2.50 ± 0.05	21.02 ± 0.64	55.44 ± 0.56	0.007 ± 0.001	0.018 ± 0.002	0.372 ± 0.009	0.266 ± 0.007
P	NS	0.05	NS	0.005	0.05	NS	NS	NS	0.01

Data are means ± SEM; NS, not significant.

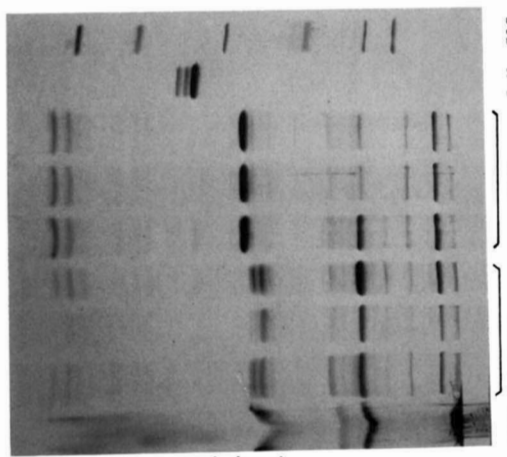

Fig. 2. SDS-PAGE (12.5% gels) of VLDL apolipoproteins. The location of apolipoprotein species from EFAD and control (CONT) rats was identified by comparison with human apolipoprotein VLDL, purified apoA-I, and molecular weight standards (STD): phosphorylase B (92,500), BSA (66,200), ovalbumin (45,000), carbonic anhydrase (31,000), soy bean trypsin inhibitor (21,500), and lysozyme (14,400).

trahepatic activity was significantly diminished, hepatic lipase activity was somewhat increased in animals with EFAD, resulting in an increase in the ratio of HL/LPL (**Table 7**). Lipoprotein lipase activities in adipose tissue, heart, and liver expressed as nmoles FFA/mg protein per h are shown in **Table 8**. The most striking finding was a 2.5-fold increase of activity in the liver of animals with EFAD, coupled with a modest decrease in the adipose tissue.

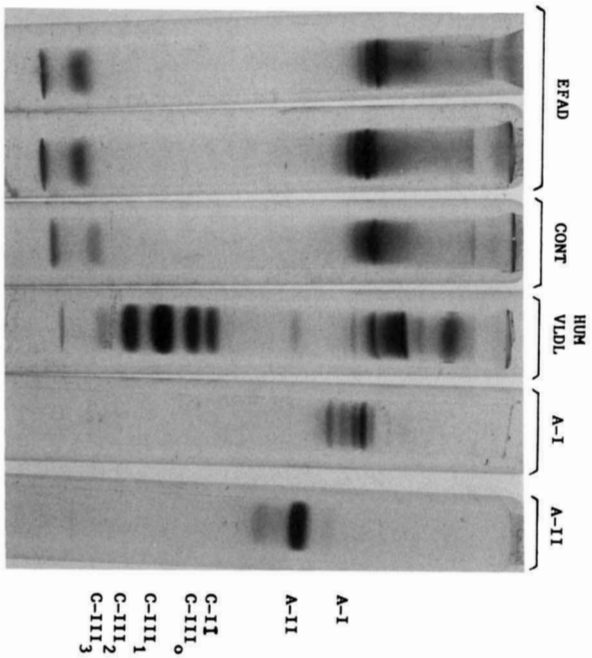

Fig. 3. Disc electrophoresis of VLDL in 10% polyacrylamide gels containing tetramethylurea. Human VLDL and purified A-I and A-II were run in parallel for comparison.

TABLE 6. Plasma LCAT activity

Group	CE/CT × 100/h	nmol/100 μl per h
EFAD	31.04 ± 0.93	8.98 ± 1.44
Controls	30.02 ± 1.34	8.72 ± 0.50
P	NS	NS

DISCUSSION

Several clinical and biochemical abnormalities have been described with EFAD (2-7). However, few studies have reported changes in lipoproteins and their metabolism (13, 23, 24). Since human EFAD is generally accompanied by other metabolic defects (13), there is no clear cut understanding of the role of EFA status on the composition and metabolism of lipoproteins. Thus, we have chosen to use an animal model of EFAD in order to improve our insight into the mechanisms involved.

Before attempting to study the role of EFAD on lipoprotein composition and metabolism, we validated the model of EFAD in the rat. The 12-week dietary regimen induced a severe degree of EFAD as defined by the triene/tetraene ratio (25). There was close to a 100-fold increase of the 20:3 (n-9)/20:4 (n-6) ratio, the widely accepted biochemical criterion of EFAD since the pioneer work of Holman (25). The EFA-deficient group also showed a high ratio of 16:1 (n-7)/18:2 (n-6) confirming our previous findings in cystic fibrosis patients with EFAD (12). Furthermore, there was a marked decrease of the n-3 family in contrast with the modest changes observed in our cystic fibrosis studies (13). Therefore, it is reasonable to assume that in the animal model all the lipid and lipoprotein abnormalities are mediated by the deficiency of both (n-3) and (n-6) families. Despite their limited ability to substitute for n-6 fatty acids to prevent or reverse some of the symptoms of the classic EFA deficiency syndrome, growing evidence points out that n-3 fatty acids are also essential, and that they have their own effects on the structure and function of many systems (26). It can therefore be anticipated that the repercussions of EFAD on lipoprotein composition and metabolism in this animal model could differ to some extent from those described in cystic fibrosis (13).

Striking changes in the lipid and lipoprotein profile were seen in the EFAD rats. They developed hypertriglyceridemia associated with abnormalities of the chemical composition of VLDL, IDL, and LDL. Increases of TG might be related to the reduced levels of extrahepatic lipoprotein lipase activity found in these animals. However, increased rates of triacylglycerol secretion into the plasma have been suggested to occur in EFAD rats (27). There is, nevertheless, no unanimous agreement on this point, given that other workers have reported increased concentrations of liver triacylglycerols in EFAD rats (28). This has been attributed to diminished VLDL secretion into the plasma because of the lack of EFA to form phospholipids required for lipoprotein formation and secretion (29, 30). Enhancement of lipogenic enzyme activities has also been shown and may result for the removal of the inhibitory effect of long chain polyunsaturated fatty acids. It could provide an alternative explanation for hepatic lipid accumulation in EFAD (31). Further work is necessary to investigate the ability of the liver of EFAD rats to synthesize, store, and secrete TG before concluding that decreased lipoprotein lipase activity accounts for the abnormalities of TG observed in clinical and experimental models of EFAD. Although the mechanism responsible for hyperlipidemia associated with raised FFA levels in experimental fatty acid deficiency (EFAD) remains in doubt, it is likely the result of increased lipolysis of stored triacylglycerols in adipose tissue. FFA are mainly taken up by the liver, reesterified to triacylglycerols, and released as VLDL, thereby contributing to the hyperlipidemia.

A possible explanation for the difference in plasma FFA between control and EFA-deficient animals might be an enhanced fat mobilization mediated by hormone-sensitive lipase. This enzyme is distinct from LPL and catalyzes intracellular triacylglycerol breakdown. Impaired uptake of FFA by the peripheral tissues is a second alternative explanation.

Both plasma free cholesterol and cholesteryl ester as well as phospholipid concentrations were found to be increased in the EFAD group. This rise might be attributed to the lower clearance of VLDL which could be attributable, in part, to a lower extrahepatic LPL activity. An abnormal clearance of VLDL is especially likely since

TABLE 7. Postheparin lipoprotein lipase

Group	Total PHLA[a]	LPL[b]	HTGL[c]	HTGL/LPL
EFAD	22.12 ± 0.32	5.96 ± 0.29	16.17 ± 0.22	2.79 ± 0.16
Controls	22.53 ± 0.68	7.29 ± 0.68	15.24 ± 0.22	2.18 ± 0.25
P	NS	0.02	0.05	0.05

[a] PHLA, postheparin lipolytic activity.
[b] LPL, extrahepatic lipoprotein lipase.
[c] HTGL, hepatic triglyceride lipase.

TABLE 8. Lipoprotein lipase activity in tissues

Group	Adipose Tissue	Heart	Liver
	nmol FFA/mg protein per h		
EFAD	292.7 ± 50.7	171.5 ± 27.7	325.8 ± 16.0
Controls	593.7 ± 93.8	119.0 ± 15.0	130.8 ± 9.5
P	0.05	NS	0.001

HDL, the cholesterol-carrying particle in rats, was decreased although not significantly. The profile of HDL, in fact, showed a decrease in the proportion of CE. Given that LCAT is preferentially active on the HDL fraction (32, 33), its level was determined to clarify its role. The possibility that LCAT could account for our findings is unlikely since we did not detect any decrease in its activity nor in its main activator, apoA-I, which is, in fact, increased (**Fig. 4**). These findings are at variance with previous observations of increased LCAT activity in EFA-deficient rats (34).

EFAD resulted in shifts of apoprotein content in VLDL and HDL. The pattern of apoE isoforms in VLDL proteins (Fig. 2) and depletion of apoE in the HDL fraction (Fig. 4) were the most marked anomalies of the lipoprotein-protein profile. These alterations of apoE presumably played a pivotal role, since apoE is primarily associated with HDL, the major lipoprotien fraction in the rat (35), and is involved in cholesterol transport (35). In their study, Ney, Ziboh, and Schneeman (23) also observed a trend toward lower apoE levels with induction of EFAD by continuous intragastric infusion of triolein-supplemented total parenteral solutions. Taken together, these findings suggest a decrease in cholesterol transport.

Alterations were also observed in the intensity of the protein band of VLDL, corresponding to albumin (Fig. 2). Less labeling was visualized in EFAD VLDL in accordance with their relative protein content (Table 5). Despite the fact that plasma of control and experimental animals was processed by the same procedure, and lipoprotein isolation was performed simultaneously, it is tempting to suggest that perhaps less albumin sticks to EFAD VLDL. Nevertheless the ratios of core lipids to protein or phospholipids show the same trend.

On the basis of the CE/PR and (TG + CE)/(FE + PL + PR) ratios it can be inferred that HDL_2 and HDL_3 are smaller in rats with EFAD. In a recent study of diet-induced EFA deficiency in rats, ultracentrifugal fractionation of the HDL into HDL_1 and HDL_2 also led to a drop in the proportion of the HDL_1 subpopulation and of the peak diameter of the HDL_2 (24). Therefore, we can conclude that the lack of EFA led to changes in particle size distribution of the subpopulation of HDL. LCAT catalyzes the removal and transfer of the *sn*-2 acyl chain from phosphatidylcholine to cholesterol (33). This reaction, activated by apoA-I, occurs preferentially on the surface of the smaller, spherical HDL subclasses and nascent discoidal HDL (32, 33). Therefore, the LCAT activity contributes to replenish the HDL core with CE and thereby increases the particle diameter. However, we could not demonstrate diminished activity of LCAT or of its activator, apoA-I, which could account for alterations in HDL size. On the other hand, our observations of increased hepatic triglyceride lipase (HTGL) activity in rats with

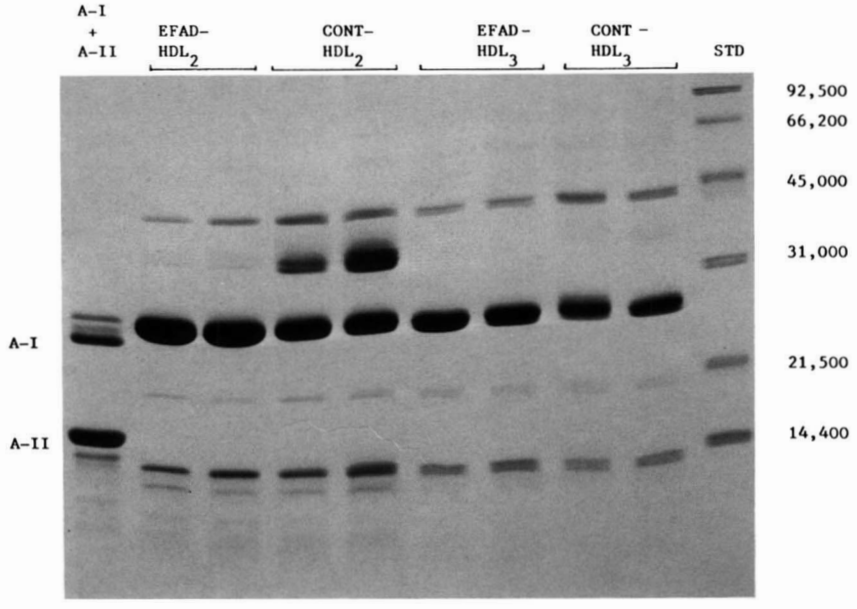

Fig. 4. SDS-polyacrylamide gel (15%) electrophoretograms of HDL_2 and HDL_3 from EFAD and control rats. The mobilities of purified apoA-I + apoA-II and molecular weight standard (STD) are indicated.

EFAD could provide an explanation for the alterations of HDL size. Although the physiological role of HTGL has not yet been established, several lines of evidence suggest that it could decrease HDL size by hydrolyzing phospholipids and triglycerides (36, 37). Additional effects of HTGL on high density lipoproteins include modulation of the rate of delivery of cholesterol from peripheral tissues to the liver which could again modify HDL size (38, 39). Therefore, we suggest that the increased activity levels of HTGL documented in this study may have contributed to the HDL changes.

Factors regulating HTGL activity are far from clear. Most of the studies have reported low hepatic lipase activity in liver disease (40), renal insufficiency (41), hyperlipoproteinemia (42, 43), was well as in a variety of other clinical states (44). A well-known consequence of EFAD is impairment of prostaglandin synthesis. In this context, it is interesting to note that hepatic lipase activity was also found to be increased after the administration of indomethacin which blocks the synthesis of prostaglandins (45).

Because lipoprotein lipase is the rate-limiting enzyme in the removal process of triglycerides, we propose that the reduction of its activity documented herein can lead to hypertriglyceridemia in EFAD. It is worth noting that in this work the postheparin plasma LPL correlated with adipose tissue LPL activity, but not with heart LPL activity. These interesting data are consistent with tissue-specific regulation of LPL in the sense that cardiac and adipose tissues responded differently to the same physiologic and hormonal signals (46, 47). Furthermore, the discrepancy between the lipolytic activity of the adipose tissue on the one hand and that of heart and liver on the other hand could be due to the decreased vulnerability of certain organs to EFAD. Lefkowith et al. (48) have demonstrated that, despite maximal restriction of dietary EFA, organs respond differently with regard to the content of arachidonate. For example, cardiac arachidonate increases with an EFA deficiency.

In conclusion, the present rat model provides clear evidence that EFA deficiency causes marked abnormalities in the concentration and composition of plasma lipids and lipoproteins. Further studies are, however, needed in order to explore the mechanism(s) by which EFA deficiency enhances hepatic lipase activity.

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