Unsaturated fatty acid bioavailability in growing rats fed low or adequate protein diets with sunflower or soybean oils

Mahmoud Bouziane, Josiane Prost, and Jacques Belleville

Research Department Cellular and Metabolic Nutrition, Sciences Faculty, Mirande University of Burgundy

The relationship of serum very low density lipoproteins (VLDL) to hepatic lipid composition was studied after 28 days of protein depletion to determine the interactions between dietary protein levels and the essential fatty acid (EFA) availability. This was examined in rats using a dietary combination of 20% or 2% casein with 5% vegetable oils, variable in their n-6:n-3 fatty acid ratios. Rats were divided into four groups, SFC (20% casein + 5% sunflower oil); SFd (2% casein + 5% sunflower oil); SC (20% casein + 5% soybean oil); Sd (2% casein + 5% soybean oil). Dietary protein depletion decreased phospholipid and protein concentrations in liver and VLDL, whereas triacylglycerol amounts were enhanced in liver, but lowered in VLDL. Dietary protein depletion strongly depressed VLDL apolipoproteins. Protein-deficient groups (SFd and Sd) exhibited, in both liver and VLDL, decreased linoleic acid in triacylglycerol fractions and depressed both arachidonic and linoleic acids in phospholipid fractions. In spite of short periods of dietary treatment, protein depletion involved an impairment in EFA availability. Total n-6 polyunsaturated fatty acids contents were diminished in liver and VLDL lipids, while total n-3 polyunsaturated fatty acids contents were diminished in only VLDL triacylglycerol and phospholipid. Furthermore, sunflower oil amplified this impairment, and the lack of α-linolenic acid involved a greater diminution in n-3 polyunsaturated fatty acids and enhanced 20:3 n-9 and 22:5 n-6, especially in phospholipid fractions. In this experiment, in spite of a short period of dietary treatment, protein depletion strongly impairs EFA metabolism and accentuates the α-linolenic acid deficiency.

Keywords: protein deficiency; α-linolenic deficiency; liver lipids; VLDL; rats

Introduction

Linoleic acid [18:2 n-6] and α-linolenic acid [18:3 n-3] are considered essential fatty acids (EFA) for mammals. These fatty acids are transformed by desaturation and elongation into more polyunsaturated fatty acids (PUFA). Some PUFA [i.e., 20:4 n-6 and 20:5 n-3] are precursors of prostaglandins and leukotrienes. α-Linolenic, linoleic, and oleic acids compete with each other, especially for the microsomal Δ6 desaturase activity.[1]

EFA deficiency involves impairments in liver lipids (i.e., fatty liver).[2] Liver steatosis is the main biochemical sign that occurs in children suffering from kwashiorkor. Impaired transport of triacylglycerol by very low density lipoprotein (VLDL) from the liver has been incriminated in the hepatic steatosis observed in rats fed a low protein diet.[3] We showed in a previous study that the low level of VLDL may be attributable to a reduction in the synthesis of VLDL apolipoproteins.[4] Protein deprivation affects metabolism of PUFA.[5] Hill and Holman[6] reported the effects of various dietary protein levels (5–40%) in rats using hydrogenated coconut oil on EFA availability, and indicated that signs observed in EFA deficiency are amplified with low protein intake. But in their study, EFA deficiency concerned both linoleic and α-linolenic acids. Koletzko et al.[7] showed that analysis of the different plasma lipid classes in malnourished Nigerian children revealed the presence of EFA deficiency.

This work was supported by research grants 86 MES 25 from Ministère de l'Industrie et de la Recherche (Health and Development program) and by the Ministère de l'Education Nationale (Biologie 86 program).
Address reprint requests to Professor Jacques Belleville at Unité de Recherches de Nutrition Cellulaire et Métabolique, Université de Bourgogne, Faculté des Sciences Mirande, BP 138, 21004 Dijon Cedex, France.
Received May 14, 1992; accepted December 30, 1992.

Thus, data obtained in those studies indicated a relationship between protein level and the development of EFA deficiency. However there is scarce data on the metabolism of EFA in protein malnutrition. Indeed, in children it is difficult to study these interferences with accuracy because numerous dietary deficits are usually associated with protein deficiency. On the other hand, EFA deficiency generally includes both linoleic and α-linolenic acids. Similarly, in studies of protein deprivation in animals, little attention has been accorded to PUFA in protein-depleted diets. Feeding these diets may increase EFA requirements and precipitate marginal EFA deficiency.

In this study, EFA and protein intakes were determined exactly, and diets were provided for a short period. The aim was to assess the qualitative differences in liver and VLDL fatty acid compositions and the EFA bioavailability in growing rats fed dietary combinations of adequate and low levels of casein with sunflower oil (deficiency in α-linolenic acid) or soybean oil (adequate in both α-linolenic and linoleic acids), and to determine if short periods of protein deficiency would also involve diminished n-3 and n-6 PUFA bioavailabilities.

Materials and methods

Animals and diets

Forty male Wistar rats (Iffa Credo, l'Arbresle, Lyon, France) weighing 80 ± 6 g at the beginning of experiment were allowed free access to an adequate diet (20% casein and 5% olive oil) for 10 days. After this adaptation period (when their weight was 115 ± 7 g) they were randomized into four equal groups. For 28 days, two groups (controls) were fed diets containing 20% casein and either 5% sunflower oil (0.1% α-linolenic acid and 65.5% linoleic acid) (composition as percentage of total fatty acids: 16:0, 6.5; 16:1 n-7, 0.1; 18:0, 4.7; 18:1 n-9, 21.3; 18:2 n-6, 65.5; 18:3 n-3, 0.1; 20:0, 0.4; 20:1 n-9, 0.1; 22:0, 0.8; 24:0, 0.2) (SFC), or 5% soybean oil (7.5% α-linolenic acid and 54.8% linoleic acid (composition as percentage of total fatty acids: 16:0, 10.3; 16:1 n-7, 0.1; 18:0, 3.9; 18:1 n-9, 22.1; 18:2 n-6, 54.8; 18:3 n-3, 7.5; 20:0, 0.4; 20:1 n-9, 0.2; 22:0, 0.4) (SC). Two other groups were fed a low-protein diet (2% casein) and either 5% sunflower oil [SFd], or 5% soybean oil (Sd). Compositions of the diets are shown on *Table 1*.

Diets were isoenergetic (16280 KJ/kg) and contained the same amounts of lipid, vitamin, mineral, and fiber. Animals were kept in wire-bottom cages at constant temperature (24° C) and humidity (60%), with a light cycle (07.00-19.00). They ate and drank ad libitum. We followed the general guidelines for the care and use of laboratory animals recommended by the European Community.[8]

Blood samples

On day 28 of the experiment, after 6 hours of fasting, rats were bled from the abdominal aorta under anesthesia (pentobarbital-Na, 60 mg/kg body weight). Plasma was obtained by low speed centrifugation and preserved with 0.26 mmol disodium EDTA and 3 mmol sodium azide.

Isolation of VLDL fraction from total lipoproteins

VLDL fraction was isolated by a single spin discontinuous gradient according to the method of Redgrave et al.[9] modified by Meghelli-Bouchenak et al.[4] Purified VLDL fraction ($d < 1.006$) was dialyzed against 0.15mol/L NaCl, 1mmol/L disodium EDTA, at pH 7.4, at 4° C for 24 hours, in spectra/Por 2 dialysis tubing (Spectrum Medical Industries, Inc; Los Angeles, CA USA).

Apolipoprotein (Apo) electrophoresis

After partial delipidation, VLDL-apolipoproteins (VLDL-Apo) were estimated using sodium dodecyl sulfate gradient gel electrophoresis (SDS PAGE, 2.5--->20%) using the method of Meghelli-Bouchenak et al.[4] Electrophoresis was performed in an LKB 2001-001 ventrical electrophoresis unit (LKB Produkte, Broma, Sweden) at 4° C for 18 hr with 20 mA / gel slab. After staining with Coomassie brilliant blue G 250, gels were scanned at 600 nm with a densitometer (Model Profil 26 Sebia, Lille, France). Apolipoprotein was quantified semiquantitatively with the densitometer tracings. The percentage of the area relative to each apolipoprotein was multiplied by the total apolipoprotein concentration of each serum sample. Data were expressed as arbitrary units (AU).

Livers

Livers were removed, washed with cold saline, quickly excised, blotted, and weighed. About 1 g of the greatest lobe was homogenized in an ultraturax for lipid extraction. About 100 mg of the same lobe was homogenized at 4° C in a Potter Elvejhem homogenizer and used for protein determination.

Chemical analysis

Total lipids of plasma VLDL and liver were extracted according to Folch et al.[10] Phospholipids (PL) and triacylglycerols (TG) fractions were isolated by thin layer chromatography according to Stahl et al.[11] PL and TG fractions were methylated, then fatty acid analysis of PL and TG fractions were performed by gas liquid chromatography,[12] [Becker Gas Chromatograph Packard 417 (Becker Instruments, Downer Groves, IL, USA), equipped with a glass column: length 39 m; internal diameter: 0.3 mm; stationary phase: carbowax 20 M; flow rate: H_2, 6 mL/min; inlet heater: 202° C; detector temperature: 240° C] using heptadecanoic acid as internal standard. Identification of fatty acids was performed with commercial standards by means of relative retention times. Areas were calculated with an ENICA 21 integrator (Delsi Instrument, Suresnes, France). PL were quantified by the measure of phosphorous, according to Bartlett's method.[13] Protein contents were evaluated by the technique of Lowry et al.[14] using bovine serum albumin as standard.

Statistical analysis

Statistical evaluation of the data was carried out by analysis of variance (ANOVA) and by classification of the means using Duncan's new multiple range test.[15] Differences were considered significant if $P < 0.05$.

Results

Body weights and food intake (Table 2)

In spite of similar food and energy daily intakes per kg body weight, SFd and Sd groups weighed only 50% of their respective control groups.

Table 1 Diet compositions

	Sunflower oil diets		Soybean oil diets	
	SFC 20% casein	SFd 2% casein	SC 20% casein	Sd 2% casein
	g/kg diet			
Milk Casein[a]	200	20	200	20
Dl-Methionine[b]	3	3	3	3
Corn starch[c]	587	767	587	767
Sucrose	50	50	50	50
Fiber (agar-agar)[a]	50	50	50	50
Salt mix[d]	40	40	40	40
Vitamin mix[e]	20	20	20	20
Sunflower oil[f]	50	50		
Soybean oil[f]			50	50

The diets were isoenergetic (16280KJ/kg) and given as powdered form.
[a]Prolabo, Paris, France.
[b]Merck, Darmstadt, Germany.
[c]Ets Louis François, Saint Maur les Fossés, France.
[d]UAR B205 (Villemoisson 91360 Epinay-S/Orge, France). Minerals provided the following amounts (g/kg diet): calcium, 4; potassium, 2.4; sodium, 1.6; magnesium, 0.4; Iron, 0.12; trace elements: manganese, 0.032; copper, 0.005; zinc, 0.018; cobalt, 0.0004; iodine, 0.0002.
[e]UAR 200 (Villemoisson 91360 Epinay-S/Orge, France). Vitamin mixture provided the following amounts (mg/kg diet): thiamin, 40. riboflavin, 30; nicotinic acid, 140; pyridoxine, 20; pyridoxal, 300; cyanocobalamin, 0.1; ascorbic acid, 1600; dl α-tocopherol, 340; menadione, 80; calcium panthotenate, 200; choline, 2720; folic acid, 10; paraaminobenzoic acid, 100; biotine, 0.6; retinol, 12; cholecalciferol, 0.125.
[f]Lesieur, Boulogne Billancourt, France.

Table 2 Food intake, body weight, liver, and VLDL lipid compositions from rats fed adequate or deficient protein diets with sunflower or soybean oils

	Sunflower oil diets		Soybean oil diets	
	SFC (20% casein)	SFd (2% casein)	SC (20% casein)	Sd (2% casein)
Food intake (g/24h.kg body weight)	68 ± 6	71 ± 13	76 ± 11	69 ± 8
Energetic intake (KJ/24h.kg body weight)	1108 ± 97	1157 ± 211	1238 ± 179	1124 ± 130
Body weight (g)	292 ± 17[a]	149 ± 27[b]	291 ± 46[a]	144 ± 24[b]
Liver (g)	8.76 ± 0.23[a]	5.89 ± 0.20[b]	8.85 ± 0.42[a]	5.52 ± 0.13[b]
Liver				
Relative liver weight (g/100g body weight)	3.01 ± 0.09[b]	3.93 ± 0.25[a]	3.04 ± 0.19[b]	3.82 ± 0.18[a]
Protein (mg/g liver)	177.0 ± 11.6[a]	168.0 ± 7.4[ab]	181.0 ± 12.0[a]	158.0 ± 7.8[b]
Total lipid (mg/g liver)	51.2 ± 10.4[b]	141.0 ± 8.0[a]	46.0 ± 3.1[b]	112.0 ± 17.5[a]
Lipid/protein (mg/mg)	0.28 ± 0.05[b]	0.83 ± 0.07[a]	0.25 ± 0.03[b]	0.72 ± 0.08[a]
Phospholipid (mg/g liver)	43.9 ± 2.4[a]	34.1 ± 3.6[bc]	37.0 ± 3.1[b]	32.0 ± 1.1[c]
Triacylglycerol (mg/g liver)	5.4 ± 0.4[c]	62.1 ± 3.2[a]	4.6 ± 0.3[c]	42.2 ± 2.7[b]
VLDL				
Proteins (g/L serum)	0.030 ± 0.004[a]	0.012 ± 0.004[b]	0.030 ± 0.008[a]	0.010 ± 0.003[b]
Phospholipid (mmol/L serum)	9.10 ± 1.16[a]	3.74 ± 0.74[b]	8.29 ± 1.22[a]	3.39 ± 0.51[b]
Triacylglycerol (mmol/L serum)	0.86 ± 0.01[b]	0.33 ± 0.05[d]	1.09 ± 0.10[a]	0.45 ± 0.03[c]

SFC, 20% casein + 5% sunflower oil; SFd, 2% casein + 5% sunflower oil; SC, 20% casein + 5% soybean oil; Sd, 2% casein + 5% soybean oil. Values are given as means ± SEM for six rats per group. After ANOVA, classification of the means was performed with Duncan's new multiple range test. Means with different superscript letters in the row are significantly different ($P < 0.05$).

Absolute and relative liver weights (Table 2)

In SFd and Sd groups, livers weighed 62 and 72% of their respective control groups. The relative liver weights were enhanced by dietary protein depletion.

Protein, triacylglycerol and phospholipid liver contents (Table 2)

In control groups sunflower enhanced liver PL contents compared with soybean oil. Protein deficiency involved a slight decrease in protein (for Sd) and PL (SFd and Sd) contents, but a high gain in total lipids, which was essentially due to TG contents. In SFd group, total lipid and TG contents were major in comparison with Sd values.

VLDL (Table 2)

Protein and PL and TG contents of VLDL were lower in both protein-deficient groups. Compared with sunflower oil, soybean oil increased VLDL-TG contents. Moreover, VLDL-TG contents were diminished by sun-

flower oil in control group (SFC) compared with SC group.

Fatty acid composition of liver-TG

Overall fatty acid contents (mg/g liver) were elevated with dietary protein depletion [*Table 3a*]. These increases were more pronounced in the SFd group than in the Sd group, except for 20:5(n-3) and 22:6(n-3). Dietary protein depletion enhanced 20:4(n-6) to 18:2(n-6) and 20:3(n-9) to 20:4(n-6) ratios.

Relative fatty acid composition [*Table 3b*] showed total (n-6) fatty acid (particularly linoleic acid) decrease and 22:5(n-3) increase in both protein deficient groups.

Compared with sunflower oil, soybean oil enhanced 22:5(n-3) and lowered 22:5(n-6). Moreover, 20:3(n-9) to 20:4(n-6) and 20:4(n-6) to 18:2(n-6) ratios were depressed with soybean oil diets.

Fatty acid composition of liver-PL (Tables 4a & 4b)

Total (n-6) fatty acid contents (arachidonic and linoleic acids) and unsaturated to saturated fatty acid ratio were diminished by dietary protein depletion. 20:3(n-9) and 22:5(n-6) contents, 20:3(n-9) to 20:4(n-6) and 20:4(n-6) to 18:2(n-6) ratios were enhanced in Sd and SFd groups (with a greater increase in SFd group). 16:1(n-7) was diminished in both protein-deficient groups, whereas 18:1(n-9) was raised in Sd group, but decreased in SFd group. Furthermore, in this latter group, total (n-3) fatty acids were more strongly depressed.

Compared with sunflower oil, soybean oil lowered total (n-6) fatty acids and the unsaturated to saturated fatty acid ratio, but raised total (n-3) fatty acids. Total saturated fatty acids in SFC group and total monounsaturated fatty acids in SC group were depressed.

Fatty acid composition of VLDL-TG

Except for 18:0, 22:0, and 24:1(n-9), overall fatty acid contents (mg/100 mL serum) were diminished with dietary protein depletion (*Table 5a*). Total (n-6) and (n-3) fatty acid contents were depressed in both protein deficient groups. 22:5(n-6) and 20:3(n-9) contents were particularly enhanced in SFd group. Relative fatty acid composition (*Table 5b*) indicated a decrease in linoleic acid and total (n-6) fatty acids in both protein-deficient groups and an enhancement in 20:4(n-6) to 18:2(n-6) ratio. Moreover, with dietary protein depletion the unsaturated to saturated fatty acid ratio was depressed.

Compared with sunflower oil, soybean oil enhanced total (n-6) and (n-3) fatty acid contents, and in proportion only total (n-3) fatty acids.

Fatty acid composition of VLDL-PL

Except for 16:1(n-7) and 24:0 in Sd group and 22:5(n-3) in SFd group, overall fatty acid contents were diminished with dietary protein depletion (*Table 6a*). Total (n-6) and (n-3) fatty acids were depressed in both protein-deficient groups. The percentages of fatty acid compositions (*Table 6b*) showed that dietary protein depletion diminished total (n-6) fatty acids (arachidonic and linoleic acids). Moreover, SFd group involved the highest 22:5(n-6) level and the lowest 22:6(n-3) level. In Sd group the unsaturated to saturated fatty acid ratio was the most reduced.

Compared with sunflower oil, soybean oil depressed

Table 3a Fatty acid contents of hepatic triacylglycerols from rats fed adequate or deficient protein diets with sunflower or soybean oils (mg/g liver)

	Sunflower oil diets		Soybean oil diets	
	SFC 20% casein	SFd 2% casein	SC 20% casein	Sd 2% casein
Fatty acids		(mg/g liver)		
14:0	0.12 ± 0.02^{c}	1.17 ± 0.02^{a}	0.09 ± 0.00^{c}	0.88 ± 0.08^{b}
16:0	2.00 ± 0.14^{b}	20.08 ± 2.10^{a}	1.89 ± 0.14^{b}	16.17 ± 0.79^{a}
16:1 (n-7)	0.20 ± 0.06^{b}	1.11 ± 0.43^{a}	0.19 ± 0.05^{b}	1.47 ± 0.29^{a}
18:0	0.28 ± 0.06^{c}	6.69 ± 1.05^{a}	0.34 ± 0.05^{c}	3.94 ± 0.29^{b}
18:1 (n-9)	1.30 ± 0.09^{c}	21.08 ± 1.30^{a}	1.08 ± 0.08^{c}	12.48 ± 0.46^{b}
18:2 (n-6)	0.49 ± 0.06^{c}	4.03 ± 0.24^{a}	0.50 ± 0.04^{c}	2.60 ± 0.71^{b}
20:0	0.05 ± 0.01^{c}	0.37 ± 0.12^{a}	0.09 ± 0.02^{c}	0.12 ± 0.04^{b}
20:1 (n-9)	0.03 ± 0.01^{c}	0.68 ± 0.24^{a}	0.02 ± 0.00^{c}	0.16 ± 0.04^{b}
20:3 (n-9)	0.05 ± 0.01^{c}	1.98 ± 0.43^{a}	0.01 ± 0.00^{d}	0.37 ± 0.08^{b}
20:4 (n-6)	0.16 ± 0.02^{c}	1.48 ± 0.06^{a}	0.11 ± 0.01^{c}	0.71 ± 0.17^{b}
20:5 (n-3)	ND	ND	0.06 ± 0.01^{b}	0.12 ± 0.04^{a}
22:5 (n-3)	0.02 ± 0.00^{d}	0.43 ± 0.06^{b}	0.05 ± 0.01^{c}	0.13 ± 0.25^{a}
22:5 (n-6)	0.13 ± 0.02^{c}	2.10 ± 0.18^{a}	0.03 ± 0.01^{d}	0.46 ± 0.13^{b}
22:6 (n-3)	0.09 ± 0.02^{bc}	0.67 ± 0.24^{a}	0.06 ± 0.01^{c}	0.12 ± 0.04^{b}
24:0	0.03 ± 0.00^{c}	0.18 ± 0.06^{a}	0.01 ± 0.01^{b}	0.50 ± 0.17^{ab}
Total (n-6)	0.78 ± 0.1^{c}	7.61 ± 0.48^{a}	0.64 ± 0.06^{c}	3.77 ± 1.01^{b}
Total (n-3)	0.11 ± 0.02^{c}	1.10 ± 0.30^{a}	0.21 ± 0.03^{b}	1.37 ± 0.33^{a}

Values are means ± SEM for six rats. SFC, 20% casein + 5% sunflower oil; SFd, 2% casein + 5% sunflower oil; SC, 20% casein + 5% soybean oil; Sd, 2% casein + 5% soybean oil. After ANOVA, classification of the means was performed with Duncan's new multiple range test. Means with different superscript letters in a row for the same fatty acid are significantly different ($P < 0.05$). ND = not detected.

Table 3b Fatty acid composition of hepatic triacylglycerols from rats fed adequate or deficient protein diets with sunflower or soybean oils (mg/100mg fatty acids)

	Sunflower oil diets		Soybean oil diets	
	SFC 20% casein	SFd 2% casein	SC 20% casein	Sd 2% casein
Fatty acids	wt % (mg/100mg fatty acids)			
Total saturated	49.4 ± 5.5	45.4 ± 6.0	52.0 ± 3.5	51.5 ± 3.3
20:3 (n-9)	1.0 ± 0.2^{b}	3.2 ± 0.7^{a}	0.3 ± 0.1^{c}	0.9 ± 0.2^{b}
Total monounsaturated	17.3 ± 1.5	14.7 ± 1.3	14.5 ± 2.0	18.8 ± 2.2
18:2 (n-6)	9.8 ± 1.2^{a}	6.5 ± 0.4^{b}	10.8 ± 1.7^{a}	6.2 ± 1.1^{b}
20:4 (n-6)	3.1 ± 0.5^{a}	2.4 ± 0.1^{b}	2.4 ± 0.4^{b}	1.7 ± 0.4^{b}
22:5 (n-6)	2.6 ± 0.5^{a}	3.4 ± 0.3^{a}	0.7 ± 0.2^{b}	1.1 ± 0.3^{b}
Total (n-6)	15.5 ± 2.2^{a}	12.3 ± 0.8^{b}	13.9 ± 2.3^{ab}	9.0 ± 1.8^{c}
20:5 (n-3)	ND	ND	1.3 + 0.1^{a}	0.3 ± 0.1^{b}
22:5 (n-3)	0.4 ± 0.1^{d}	0.7 ± 0.1^{c}	1.1 ± 0.2^{b}	2.7 ± 0.6^{a}
22:6 (n-3)	1.8 ± 0.4^{a}	1.1 ± 0.4^{a}	1.3 ± 0.1^{a}	0.3 ± 0.1^{b}
Total (n-3)	2.2 ± 0.5^{b}	1.8 ± 0.8^{b}	3.7 ± 0.4^{a}	3.3 ± 0.8^{ab}
Unsaturated/saturated	1.02	1.20	0.88	0.88
20:4 (n-6)/18:2 (n-6)	0.31	0.37	0.22	0.27
20:3 (n-9)/20:4 (n-6)	0.32	1.33	0.12	0.53

Values are means ± SEM for six rats. SFC, 20% casein + 5% sunflower oil; SFd, 2% casein + 5% sunflower oil; SC, 20% casein + 5% soybean oil; Sd, 2% casein + 5% soybean oil. After ANOVA, classification of the means was performed with Duncan's new multiple range test. Means with different superscript letters in a row for the same fatty acid are significantly different ($P < 0.05$). ND = not detected.

Table 4a Fatty acid contents of hepatic phospholipids from rats fed adequate or deficient protein diets with sunflower or soybean oils (mg/g liver)

	Sunflower oil diets		Soybean oil diets	
	SFC 20% casein	SFd 2% casein	SC 20% casein	Sd 2% casein
Fatty acids	(mg/g liver)			
14:0	1.4 ± 0.3	1.3 ± 0.1	1.0 ± 0.2	1.1 ± 0.1
16:0	10.0 ± 0.9	9.2 ± 0.4	9.9 ± 0.6	9.3 ± 0.6
16:1 (n-7)	1.1 ± 0.2^{a}	0.6 ± 0.1^{b}	1.1 ± 0.3^{a}	0.6 ± 0.1^{b}
18:0	5.8 ± 0.5^{b}	5.4 ± 0.5^{b}	7.4 ± 0.7^{a}	5.2 ± 0.7^{b}
18:1 (n-9)	6.5 ± 0.6^{a}	4.2 ± 0.4^{c}	4.2 ± 0.4^{c}	5.4 ± 0.4^{b}
18:2 (n-6)	5.2 ± 0.6^{a}	2.2 ± 0.2^{c}	3.4 ± 0.7^{b}	1.6 ± 0.6^{c}
18:3 (n-6)	0.3 ± 0.1	0.2 ± 0.0	ND	ND
20:1 (n-9)	0.4 ± 0.1^{a}	0.3 ± 0.0^{a}	0.2 ± 0.0^{b}	0.1 ± 0.0^{b}
20:3 (n-9)	ND	0.7 ± 0.1^{a}	ND	0.4 ± 0.1^{b}
20:3 (n-6)	0.6 ± 0.0^{a}	0.5 ± 0.2^{ab}	0.4 ± 0.1^{b}	traces
20:4 (n-6)	9.9 ± 0.1^{a}	4.9 ± 0.4^{c}	6.9 ± 0.5^{b}	4.4 ± 0.3^{c}
20:5 (n-3)	0.4 ± 0.1	traces	traces	traces
22:0	0.3 ± 0.1^{a}	0.3 ± 0.0^{a}	traces	0.1 ± 0.0^{b}
22:5 (n-3)	0.3 ± 0.0^{b}	traces	1.2 ± 0.2^{a}	0.9 ± 0.3^{a}
22:5 (n-6)	0.8 ± 0.4^{bc}	1.6 ± 0.2^{a}	0.3 ± 0.1^{c}	0.6 ± 0.1^{b}
22:6 (n-3)	0.4 ± 0.1^{bc}	0.3 ± 0.1^{c}	0.9 ± 0.1^{a}	0.6 ± 0.1^{b}
24:0	0.3 ± 0.1	0.3 ± 0.1	traces	0.2 ± 0.1
Total (n-6)	16.8 ± 1.2^{a}	9.4 ± 1.0^{b}	11.0 ± 1.4^{b}	6.6 ± 1.0^{c}
Total (n-3)	1.1 ± 0.2^{b}	0.3 ± 0.1^{c}	2.1 ± 0.3^{a}	1.5 ± 0.4^{ab}

Values are means ± SEM for six rats. SFC, 20% casein + 5% sunflower oil; SFd, 2% casein + 5% sunflower oil; SC, 20% casein + 5% soybean oil; Sd, 2% casein + 5% soybean oil. After ANOVA, classification of the means was performed with Duncan's new multiple range test. Means with different superscript letters in a row for the same fatty acid are significantly different ($P < 0.05$). ND = not detected.

22:5(n-6) and enhanced 22:5(n-3) contents. Soybean oil enhanced the relative proportion of total (n-3) fatty acids.

VLDL apolipoproteins (Figure 1)

Overall VLDL apolipoproteins were strongly diminished by dietary protein depletion. Soybean oil diets involved lower B_{48} and higher A_{IV} values compared with values obtained with sunflower oil diets.

Discussion

The relationships between serum VLDL and liver lipids in kwashiorkor have been studied,[4,16] but never with consideration for qualitative changes in EFA.

Table 4b Fatty acid composition of hepatic phospholipids from rats fed adequate or deficient protein diets with sunflower or soybean oils (mg/100mg fatty acids)

	Sunflower oil diets		Soybean oil diets	
	SFC 20% casein	SFd 2% casein	SC 20% casein	Sd 2% casein
Fatty acids		wt % (mg/100mg fatty acids)		
Total saturated	40.7 ± 3.7^{b}	51.5 ± 3.5^{a}	49.9 ± 4.4^{a}	52.2 ± 3.8^{a}
20:3 (n-9)	ND	2.2 ± 0.4^{a}	ND	1.3 ± 0.5^{b}
Total monounsaturated	18.3 ± 1.7^{a}	15.7 ± 1.6^{ab}	14.8 ± 2.1^{b}	18.4 ± 1.3^{a}
18:2 (n-6)	11.9 ± 1.2^{a}	6.8 ± 0.7^{b}	9.1 ± 1.9^{a}	5.3 ± 2.1^{b}
20:4 (n-6)	22.5 ± 1.9^{a}	15.3 ± 1.3^{c}	18.6 ± 1.4^{b}	14.2 ± 0.6^{c}
22:5 (n-6)	1.8 ± 0.6^{b}	5.0 ± 1.0^{a}	0.9 ± 0.2^{c}	1.7 ± 0.4^{b}
Total (n-6)	37.9 ± 4.3^{a}	29.0 ± 3.6^{b}	29.6 ± 3.6^{b}	21.3 ± 3.1^{c}
20:5 (n-3)	1.0 ± 0.3^{a}	0.1 ± 0.0^{b}	0.2 ± 0.0^{b}	0.1 ± 0.0^{b}
22:5 (n-3)	0.6 ± 0.1^{b}	0.2 ± 0.0^{c}	3.2 ± 0.7^{a}	3.0 ± 1.0^{a}
22:6 (n-3)	1.0 ± 0.2^{b}	0.8 ± 0.2^{b}	2.3 ± 0.4^{a}	1.9 ± 0.3^{a}
Total (n-3)	2.6 ± 0.6^{b}	1.1 ± 0.2^{c}	5.7 ± 1.1^{a}	5.0 ± 1.3^{a}
Unsaturated/Saturated	1.44	0.93	1.00	0.88
20:4 (n-6)/18:2 (n-6)	1.9	2.3	2.0	2.7
20:3 (n-9)/20:4 (n-6)		0.14		0.09

Values are means ± SEM for six rats. SFC, 20% casein + 5% sunflower oil; SFd, 2% casein + 5% sunflower oil; SC, 20% casein + 5% soybean oil; Sd, 2% casein + 5% soybean oil. After ANOVA, classification of the means was performed with Duncan's new multiple range test. Means with different superscript letters in a row for the same fatty acid are significantly different ($P < 0.05$). ND = not detected.

Table 5a Fatty acid contents of VLDL triacylglycerols from rats fed adequate or deficient protein diets with sunflower or soybean oils (mg/100mL serum)

	Sunflower oil diets		Soybean oil diets	
	SFC 20% casein	SFd 2% casein	SC 20% casein	Sd 2% casein
Fatty acids		(mg/100mL serum)		
14:0	3.1 ± 0.8^{a}	0.7 ± 0.1^{b}	2.6 ± 0.6^{a}	1.0 ± 0.4^{b}
16:0	19.0 ± 1.2^{a}	8.9 ± 1.0^{b}	22.8 ± 1.7^{a}	12.0 ± 1.4^{b}
16:1 (n-7)	4.6 ± 0.6^{a}	2.8 ± 0.3^{b}	4.0 ± 0.6^{a}	2.9 ± 0.6^{b}
18:0	1.9 ± 0.5	2.5 ± 0.3	3.0 ± 0.9	2.5 ± 0.8
18:1 (n-9)	27.2 ± 5.0^{a}	8.8 ± 0.5^{b}	33.8 ± 2.9^{a}	12.6 ± 2.3^{b}
18:2 (n-6)	9.3 ± 2.1^{a}	0.9 ± 0.3^{b}	13.3 ± 2.4^{a}	1.9 ± 0.5^{b}
18:3 (n-3)	0.6 ± 0.1^{b}	0.1 ± 0.0^{c}	1.0 ± 0.2^{a}	0.5 ± 0.0^{b}
18:3 (n-6)	ND	ND	0.6 ± 0.1^{a}	0.2 ± 0.1^{b}
20:0	0.8 ± 0.1^{a}	0.2 ± 0.0^{b}	0.5 ± 0.1^{b}	0.4 ± 0.1^{b}
20:1 (n-9)	0.9 ± 0.3^{ab}	0.3 ± 0.1^{c}	1.3 ± 0.2^{a}	0.7 ± 0.2^{b}
20:3 (n-9)	ND	0.1 ± 0.0	ND	ND
20:3 (n-6)	0.4 ± 0.1^{ab}	0.5 ± 0.1^{a}	0.2 ± 0.0^{b}	traces
20:4 (n-6)	1.8 ± 0.4^{a}	0.4 ± 0.2^{b}	2.4 ± 0.3^{a}	0.7 ± 0.0^{b}
20:5 (n-3)	0.4 ± 0.1^{b}	0.2 ± 0.0^{c}	0.9 ± 0.4^{a}	0.3 ± 0.0^{b}
22:0	1.0 ± 0.5	0.5 ± 0.1	1.0 ± 0.4	0.8 ± 0.4
22:5 (n-3)	traces	traces	0.5 ± 0.1	traces
22:5 (n-6)	0.4 ± 0.1^{c}	0.7 ± 0.0^{b}	2.0 ± 0.2^{a}	0.8 ± 0.2^{b}
22:6 (n-3)	0.6 ± 0.1^{b}	0.1 ± 0.0^{c}	2.0 ± 0.5^{a}	0.5 ± 0.1^{b}
24:0	0.8 ± 0.1^{a}	0.3 ± 0.0^{b}	0.7 ± 0.1^{a}	0.5 ± 0.3^{ab}
24:1 (n-9)	1.9 ± 0.7^{ab}	0.9 ± 0.3^{b}	1.1 ± 0.5^{ab}	2.1 ± 0.5^{a}
Total (n-6)	11.9 ± 2.8^{b}	2.5 ± 0.6^{d}	18.5 ± 3.0^{a}	3.6 ± 0.8^{c}
Total (n-3)	1.6 ± 0.3^{b}	0.4 ± 0.1^{c}	4.4 ± 1.2^{a}	1.3 ± 0.2^{b}

Values are means ± SEM for six rats. SFC, 20% casein + 5% sunflower oil; SFd, 2% casein + 5% sunflower oil; SC, 20% casein + 5% soybean oil; Sd, 2% casein + 5% soybean oil. After ANOVA, classification of the means was performed with Duncan's new multiple range test. Means with different superscript letters in a row for the same fatty acid are significantly different ($P < 0.05$). ND = not detected.

Table 5b Fatty acid composition of VLDL triacylglycerols from rats fed adequate or deficient protein diets with sunflower or soybean oils (mg/100mg fatty acids)

	Sunflower oil diets		Soybean oil diets	
	SFC 20% casein	SFd 2% casein	SC 20% casein	Sd 2% casein
Fatty acids		wt % (mg/100mg fatty acids)		
Total saturated	35.1 ± 4.5[b]	44.0 ± 5.4[a]	32.2 ± 4.4[b]	43.1 ± 5.2[a]
20:3 (n-9)	ND	0.5 ± 0.1	ND	ND
Total monounsaturated	45.7 ± 8.8	43.8 ± 3.8	43.4 ± 4.5	43.3 ± 8.8
18:2 (n-6)	12.3 ± 2.9[a]	3.3 ± 0.5[b]	14.0 ± 2.7[a]	4.9 ± 1.3[b]
20:4 (n-6)	2.4 ± 0.5	1.3 ± 0.7	2.5 ± 0.3	1.8 ± 0.2
22:5 (n-6)	0.6 ± 0.3[b]	2.6 ± 0.1[a]	2.1 ± 0.6[a]	2.1 ± 0.6[a]
Total (n-6)	15.9 ± 3.9[a]	9.1 ± 1.7[b]	19.5 ± 3.7[a]	9.4 ± 2.5[b]
20:5 (n-3)	0.6 ± 0.1	0.8 ± 0.2	1.0 ± 0.4	0.8 ± 0.0
22:5 (n-3)	traces	traces	0.5 ± 0.1	traces
22:6 (n-3)	0.8 ± 0.2[b]	0.4 ± 0.1[c]	2.1 ± 0.6[a]	1.2 ± 0.2[b]
Total (n-3)	2.2 ± 0.4[b]	1.6 ± 0.4[b]	4.7 ± 1.4[a]	3.3 ± 0.3[a]
Unsaturated/saturated	1.81	1.22	2.10	1.29
20:4 (n-6)/18:2 (n-6)	0.2	0.4	0.2	0.4
20:3 (n-9)/20:4 (n-6)		0.38		

Values are means ± SEM for six rats. SFC, 20% casein + 5% sunflower oil; SFd, 2% casein + 5% sunflower oil; SC, 20% casein + 5% soybean oil; Sd, 2% casein + 5% soybean oil. After ANOVA, classification of the means was performed with Duncan's new multiple range test. Means with different superscript letters in a row for the same fatty acid are significantly different ($P < 0.05$). ND = not detected.

Table 6a Fatty acid contents of VLDL phospholipids from rats fed adequate or deficient protein diets with sunflower or soybean oils (mg/100mL serum)

	Sunflower oil diets		Soybean oil diets	
	SFC 20% casein	SFd 2% casein	SC 20% casein	Sd 2% casein
Fatty acids		(mg/100mL serum)		
14:0	0.9 ± 0.1[a]	0.3 ± 0.0[b]	0.9 ± 0.2[a]	0.3 ± 0.0[b]
16:0	4.5 ± 0.3[a]	1.8 ± 0.2[b]	5.7 ± 0.8[a]	2.5 ± 0.2[b]
16:1 (n-7)	1.7 ± 0.2[a]	0.6 ± 0.1[b]	0.8 ± 0.2[b]	0.3 ± 0.0[b]
18:0	5.3 ± 1.0[a]	2.6 ± 0.3[b]	3.7 ± 1.0[ab]	1.8 ± 0.3[c]
18:1 (n-9)	3.5 ± 0.5[a]	2.3 ± 0.2[b]	2.9 ± 0.4[ab]	1.6 ± 0.2[c]
18:2 (n-6)	4.0 ± 0.3[a]	1.3 ± 0.1[b]	4.2 ± 0.5[a]	1.2 ± 0.1[b]
20:0	0.3 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
20:1 (n-9)	0.3 ± 0.1[a]	0.1 ± 0.0[b]	0.5 ± 0.1[a]	0.1 ± 0.0[b]
20:4 (n-6)	3.8 ± 0.5[a]	1.1 ± 0.2[b]	3.9 ± 0.5[a]	1.0 ± 0.2[b]
22:0	0.4 ± 0.4[a]	0.1 ± 0.0[b]	0.4 ± 0.1[a]	0.1 ± 0.0[b]
22:5 (n-3)	0.3 ± 0.1[b]	0.2 ± 0.0[bc]	0.7 ± 0.1[a]	0.1 ± 0.0[c]
22:5 (n-6)	0.9 ± 0.1[a]	0.5 ± 0.1[b]	0.5 ± 0.0[b]	0.2 ± 0.0[c]
22:6 (n-3)	0.6 ± 0.1[a]	0.1 ± 0.0[b]	0.7 ± 0.1[a]	0.2 ± 0.1[b]
24:0	0.6 ± 0.2[a]	0.3 ± 0.0[b]	0.2 ± 0.0[b]	0.1 ± 0.0[b]
24:1 (n-9)	0.7 ± 0.3[a]	0.3 ± 0.0[b]	0.5 ± 0.2[a]	0.2 ± 0.0[b]
Total (n-6)	8.7 ± 0.9[a]	2.9 ± 0.4[b]	8.4 ± 1.1[a]	2.4 ± 0.3[b]
Total (n-3)	0.9 ± 0.3[a]	0.3 ± 0.0[b]	1.4 ± 0.2[a]	0.3 ± 0.1[b]

Values are means ± SEM for six rats. SFC, 20% casein + 5% sunflower oil; SFd, 2% casein + 5% sunflower oil; SC, 20% casein + 5% soybean oil; Sd, 2% casein + 5% soybean oil. After ANOVA, classification of the means was performed with Duncan's new multiple range test. Means with different superscript letters in a row for the same fatty acid are significantly different ($P < 0.05$).

The aim of this experiment was to evaluate the bioavailability of EFA and their derivatives by the study of detailed quantitative and qualitative changes in liver and VLDL fatty acid compositions in rats subjected to low protein diets in combination with two oils differing in their α-linolenic acid contents.

In both protein-deficient groups, the increment in total hepatic lipids was extensive, as observed in a previous experiment.[4] Data showed that the TG accumulation in liver observed with dietary protein depletion was in relation with the decreased synthesis of VLDL-apo and perhaps with a greater peripheral uptake of VLDL by other tissues than liver.[17]

Generally, α-linolenic acid deficiency is obtained when rats are fed a free α-linolenic acid diet over very long periods or for two or three generations.[18] Bourre et al.[19] estimated α-linolenic and linoleic acids requirements for developing rats to be about 0.4% and 2.4% of total calories intake or 2 g/kg and 12 g/kg of food intake, respectively. In this study, sunflower and soy-

Table 6b Fatty acid composition of VLDL phospholipids from rats fed adequate or deficient protein diets with sunflower or soybean oils (mg/100mg fatty acids)

	Sunflower oil diets		Soybean oil diets	
	SFC 20% casein	SFd 2% casein	SC 20% casein	Sd 2% casein
Fatty acids		wt % (mg/100mg fatty acids)		
Total saturated	43.5 ± 6.5	43.9 ± 6.3	42.5 ± 8.3	47.3 ± 6.3
20:3 (n-9)	ND	ND	ND	ND
Total monounsaturated	22.2 ± 3.7^{ab}	28.1 ± 3.6^{a}	18.4 ± 3.5^{b}	22.5 ± 4.0^{ab}
18:2 (n-6)	14.4 ± 0.9^{a}	10.9 ± 1.1^{b}	16.1 ± 1.9^{a}	11.7 ± 1.2^{b}
20:4 (n-6)	13.7 ± 1.7^{a}	9.2 ± 1.3^{b}	15.1 ± 2.2^{a}	10.0 ± 2.2^{b}
22:5 (n-6)	2.2 ± 0.4^{b}	4.2 ± 0.8^{a}	2.1 ± 0.4^{b}	2.2 ± 0.4^{b}
Total (n-6)	30.3 ± 3.0^{a}	24.3 ± 3.2^{b}	33.3 ± 4.5^{a}	23.9 ± 3.8^{b}
20:5 (n-3)	ND	ND	ND	ND
22:5 (n-3)	1.2 ±0.4^{b}	1.7 ± 0.4^{ab}	2.6 ± 0.7^{a}	1.4 ± 0.7^{ab}
22:6 (n-3)	2.0 ± 0.3^{a}	1.2 ± 0.4^{b}	2.8 ± 1.1^{a}	2.2 ± 0.5^{ab}
Total (n-3)	3.2 ± 1.1^{b}	2.9 ± 0.8^{b}	5.4 ± 1.8^{a}	3.6 ± 1.2^{ab}
Unsaturated/saturated	1.28	1.26	1.34	1.0
20:4 (n-6)/18:2 (n-6)	0.95	0.84	0.94	0.85

Values are means ± SEM for six rats. SFC, 20% casein + 5% sunflower oil; SFd, 2% casein + 5% sunflower oil; SC, 20% casein + 5% soybean oil; Sd, 2% casein + 5% soybean oil. After ANOVA, classification of the means was performed with Duncan's new multiple range test. Means with different superscript letters in a row for the same fatty acid are significantly different ($P < 0.05$). ND = not detected.

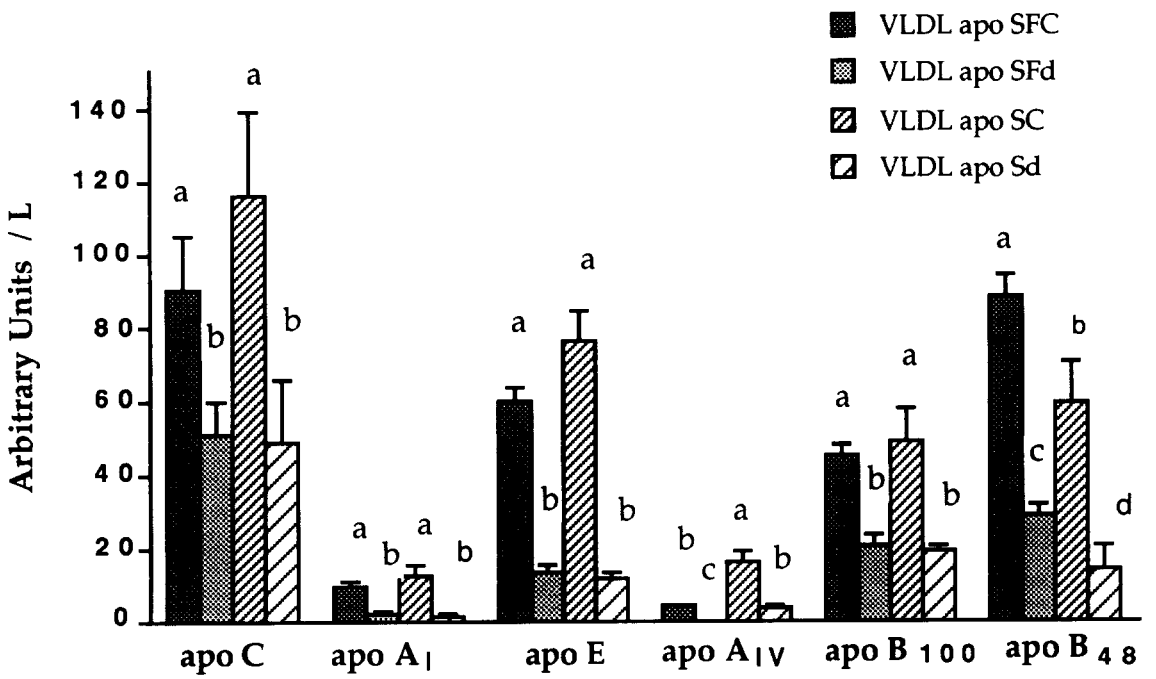

Figure 1 VLDL-apolipoprotein (VLDL-apo) distribution from young rats fed adequate or deficient protein diets with sunflower or soybean oils for 28 days. Values are expressed as arbitrary units (AU)/L serum. Each column represents means ± SEM for six rats per group. SFC, 20% casein + 5% sunflower oil; SFd, 2% casein + 5% sunflower oil; SC, 20% casein + 5% soybean oil; Sd, 2% casein + 5% soybean oil. Data were compared for each apo. Statistical evaluation of the data was conducted by ANOVA and classification of the means with Duncan's new multiple range test. Means in a panel with different superscript letters were significantly different ($P < 0.05$).

bean oil diets contained 0.05 g and 2.5 g of α-linolenic acid and 29 g and 28 g of linoleic acid, per kg diet respectively. Therefore, overall diets brought an adequate linoleic acid content, but both sunflower oil diets were strongly depleted in α-linolenic acid.

After 28 days of experiment, in both protein-deficient groups, in spite of adequate level of 18:2(n-6) in diets, the relative fatty acid composition showed decreased total (n-6) fatty acids, mainly linoleic acid, in overall fractions studied. The fatty acid contents decreased similarly, except in liver TG, in which they were enhanced by dietary protein depletion. Absolute and relative arachidonic acid values were only depressed in PL fractions (liver and VLDL). In liver PL the decrease in both

arachidonic and linoleic acids, essentially originating from membranes, indicated that the bioavailability of these PUFA was decreased in liver by protein-deficient diets. In this experiment, the 20:4(n-6) to 18:2(n-6) ratio was enhanced by protein-deficient diets in liver, VLDL TG, and liver PL, whereas DeTomas et al.[20] showed that partial protein deprivation is associated with a decreased 20:4(n-6) to 18:2(n-6) ratio in PL from rat liver. These conflicting data may be explained by differences in the age of rats or in the duration, degree, and type of protein malnutrition.

Increased 20:3(n-9) content and 20:3(n-9) to 20:4(n-6) ratio are generally accepted as indicators of EFA deficiency. In our study, in liver TG and PL this ratio value exceeded the normal range (<0.2) defined by Holman.[6] Furthermore, 20:3(n-9) in VLDL-TG was detected only in the SFd group. EFA deficiency obtained with hydrogenated coconut oil (deficient in both α-linolenic and linoleic acids) and associated with a protein-deficient diet involves a higher increase in 20:3(n-9) and a lower decrease in 20:4(n-6) concentrations in fatty acid composition of heart and hepatic PL than those obtained with EFA deficiency alone.[21] 20:3(n-9) to 20:4(n-6) ratio and 20:4(n-6) to 18:2(n-6) ratio were always greater in SFd group than in Sd group. Deficiency in α-linolenic acid (sunflower oil diets) seemed to involve a decreased competition in microsomal Δ6 desaturase activities, which appeared unaltered by dietary protein depletion. This consequently increased (n-6) fatty acid desaturation. Total (n-3) fatty acid contents were depressed in VLDL (TG and PL) in both protein-deficient groups. The relative fatty acid composition showed decreased (n-3) PUFA in liver PL for the SFd group only. Dietary protein depletion enhanced 22:5(n-6) in overall lipid fractions studied, and to a larger extent in the SFd group, suggesting that the elongase and desaturase activity of (n-6) fatty acids were still operating, but also perhaps that the utilization of 22:5(n-6) might be decreased. On the other hand, compared with sunflower oil, soybean oil enhanced total (n-3) fatty acid contents and especially 22:5(n-3), and diminished total (n-6) fatty acid contents, especially 22:5(n-6). But in VLDL-TG, total (n-6) and (n-3) fatty acid contents were more elevated with soybean oil, because VLDL-TG concentrations were majored with this oil compared with sunflower oil values. However, the relative fatty acid composition in VLDL-TG only showed increased (n-3) fatty acids. Linolenate deficiency involves a reduction in the proportions of series (n-3) fatty acids in liver of rats[22] and leads to an accumulation of 22:5(n-6), which replaces the deficient 22:6(n-3).[19] Dietary α-linolenic acid deficiency studied over three generations shows a great decrease of series (n-3) PUFA in liver PL, particularly in 22:6 (n-3), which is compensated for by a very high increase in 22:5 (n-6).[18] As in our experiment, α-linolenic acid-deficient rats (in both sunflower oil diets) show the greatest 22:5 (n-6) and the lowest 22:6(n-3) values in liver PL, particularly in the SFd group. Therefore dietary protein depletion seems to accentuate α-linolenic acid impairment. Increment in 22:5 (n-6) could be attributable to higher Δ4 desaturase activity or to lower utilization [Δ4 desaturase activity involved the desaturation of 22:5 (n-3) to 22:6 (n-3) or 22:4 (n-6) to 22:5 (n-6)]. Rats fed sunflower oil diets were deficient in 18:3 (n-3) then in 22:5 (n-3) and it could decrease the competition between 22:5 (n-3) and 22:4 (n-6) for Δ4 desaturase activity. This activity increases with linolenate deficiency in rat liver.[23]

In our study, absolute values of overall apos were decreased by dietary protein depletion. The reduction in synthesis of total VLDL-apos has been observed in perfused livers by Yagasaki and Kametaka[24] and would originate from the decrease in rough endoplasmic reticulum of hepatic cells of rats fed on a low-protein diet. Data obtained in this study and previous reports[4,25] proved that the reduced synthesis of liver VLDL-apo is a cause of impaired export of hepatic TG that involves fatty liver. Apo B and apo C, the major VLDL-apos of rat, mainly originating from liver, which are impaired by dietary protein depletion, are particularly low in protein deficient rats.

In conclusion, protein depletion involves an impairment in EFA availability. Total (n-6) PUFA contents are diminished in liver and VLDL, while total (n-3) PUFA contents are lowered in VLDL TG and PL only. Furthermore sunflower oil amplifies this impairment and the lack of α-linolenic acid involves a greater diminution in (n-3) PUFA and enhances 20:3(n-9) and 22:5(n-6), especially in PL fractions. In this experiment, in spite of a short period of dietary treatment, protein deficiency strongly impairs EFA metabolism and accentuates the α-linolenic acid deficiency.

Acknowledgments

The authors thank Anne Magnet, a linguist at the University of Burgundy, for editing the manuscript in English.

References

1 Mohrhauer, H. and Holman, R.T. (1963). Effect of linolenic acid upon the metabolism of linoleic acid. *J Nutr.* **81,** 67–74
2 Sinclair, A.J. and Collins, F.D. (1968). Fatty livers in rats deficient in essential fatty acids. *Biochem. Biophys. Acta* **152,** 498–510
3 Flores, H., Pak, N., Maccioni, A., and Monckeberg, F. (1970). Lipid transport in kwashiorkor. *Brit. J. Nutr.* **24,** 1005–1011
4 Meghelli-Bouchenak, M., Boquillon, M., and Belleville, J. (1989). Serum lipoprotein composition and amounts during the consumption of two different low protein diets followed by a balanced diet. *Nutr. Rep. Int.* **39,** 323–343
5 Holman, R.T. (1986). Nutritional and functional requirements for essential fatty acids. *Progr. Clin. Biol. Res.* **222,** 211–228
6 Holman, R.T. (1960). The ratio of trienoic:tetraenoic acids in tissue lipids as a measure of essential fatty acid requirement. *J. Nutr.* **70,** 405–411
7 Koletzko, B., Abiodun, P.O., Laryea, M.D., and Bremer, H.J. (1986). Fatty acid composition of plasma lipids in Nigerian children with protein-energy malnutrition. *Eur. J. Pediatr.* **145,** 109–115
8 Conseil des Communautés Europeénnes (1986). Directive du conseil, concernant la protection des animaux utilisés à des fins expérimentales ou à d'autres fins scientifiques. 86/609/CEE. *Journal officiel des Communautés européennes* **L 358,** 1–28
9 Redgrave, T.G., Roberts, D.C.K., and West, C.E. (1975). Sep-

aration of plasma lipoprotein by density gradient ultracentrifugation. *Anal. Biochem.* **65,** 42–49
10 Folch, J., Lees, M., and Sloane-Stanley, G.H. (1957). A simple method for isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226,** 497–509
11 Stahl, E., Schröter, G., Kraft, G., and Renz, E. (1956). Thin layer chromatography (the method affecting factors, and a few examples of application). *Pharmazie.* **11,** 633–637
12 Slover, H.T. and Lanza, E. (1979). Quantitative analysis of food fatty acids by capillary gas chromatography. *J Am. Oil. Chem. Soc.* **56,** 933–943
13 Bartlett, G.R. (1958). Phosphorus assay in column chromatography. *J. Biol. Chem.* **234,** 466–468
14 Lowry, O.H., Rosenbrough, N.J., Farr, A.L., and Randall, R.I. (1951). Protein measurement with folin phenol reagent. *J. Biol. Chem.* **193,** 265–275
15 Duncan, D.B. (1955). Multiple range and multiple F tests. *Biometrics* **11,** 1–42
16 Truswell, A.S., Hansen, J.D.L., Watson, C.E., and Wannenburg, P. (1969). Relation of serum lipids and lipoproteins to fatty liver in kwashiorkor. *Am. J. Clin. Nutr.* **22,** 568–576
17 Meghelli-Bouchenak, M., Boquillon, M., Belleville, J., and Prost, J. (1991). Metabolism of serum VLDL-, LDL-, and HDL-apoproteins in rats on two different low protein diets. *Nutr. Res.* **11,** 575–586
18 Guesnet, P., Pascal, G., and Durand G. (1988). Effects of dietary α-linolenic acid deficiency during pregnancy and lactation on lipid fatty acid composition of liver and serum in the rat. *Reprod. Nutr. Develop.* **28,** 275–292
19 Bourre, J.M., François, M., Youyou, A., Dumont, O., Piciotti, M., Pascal, G., and Durand, G. (1989). The effects of dietary α-linolenic acid on the composition of nerve membranes, enzymatic activity, amplitude of electrophysiological parameters, resistance to poisons and performance of learning tasks in rats. *J. Nutr.* **119,** 1880–1892
20 De Tomas, M.E., Mercuri, O., and Rodrigo, A. (1980). Effects of dietary protein and EFA deficiency on liver Δ5, Δ6 and Δ9 desaturase activities in the early developing rat. *J. Nutr.* **110,** 595–599
21 Hill, E.G. and Holman, R.T. (1980). Effect of dietary protein level upon essential fatty acid (EFA) deficiency. *J. Nutr.* **110,** 1057–1060
22 Tinoco, J., Babcock, R., Hincenbergs, B., and Medwadowski, B. (1979). Linolenic acid deficiency. *Lipids* **14,** 166–173
23 Hagve, T.A. and Chrisophersen, B.O. (1984). Effect of dietary fats on arachidonic acid and eicosapentanoic acid biosynthesis and conversion to C22 fatty acids in isolated rat liver cells. *Biochem. Biophys. Acta* **796,** 205–217
24 Yagasaki, K. and Kametaka, M. (1984). Apolipoprotein synthesis by the fatty liver of rats fed a low whole egg protein diet. *Nutr. Rep. Int.* **29,** 533–540
25 Meghelli-Bouchenak, M., Boquillon, M., and Belleville, J. (1987). Time course of changes in rat serum apolipoproteins during consumption of different low protein diets followed by a balanced diet. *J. Nutr.* **117,** 641–649

Abbreviations

Apo	apolipoprotein
EFA	essential fatty acids
PL	phospholipid
PUFA	polyunsaturated fatty acids
SC	20% casein + 5% soybean oil
Sd	2% casein + 5% soybean oil
SFC	20% casein + 5% sunflower oil
SFd	2% casein + 5% sunflower oil
TG	triacylglycerol