Changes in fatty acid compositions of total serum and lipoprotein particles, in growing rats given protein-deficient diets with either hydrogenated coconut or salmon oils as fat sources

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The present study examines the effects of dietary saturated (hydrogenated coconut oil) and polyunsaturated (salmon oil) fats on the composition and metabolism of lipoproteins in growing rats fed on protein-deficient diets. Four groups of rats were fed on the following diets for 28 d: 200 g casein + 50 g coconut oil (COC)/kg, 20 g casein + 50 g coconut oil (COd)/kg, 200 g casein + 50 g salmon oil (SAC)/kg, 20 g casein + 50 g salmon oil (SAd)/kg. Both protein-deficient groups exhibited low concentrations of protein and triacylglycerol (in serum, very-low-density lipoprotein (VLDL), low-density lipoprotein–high-density lipoprotein, (LDL–HDL_1) and HDL_{2-3}), of cholesterol (in LDL–HDL_1) and of phospholipids (in VLDL). Furthermore, serum and VLDL cholesterol concentrations were also reduced in the SAd group. Compared with rats given 200 g casein/kg diets, those fed on low-protein diets presented lower linoleic and arachidonic acid levels, in serum phospholipids and a dramatic decrease in the polyunsaturated:saturated fatty acid value. Relative amounts of linoleic and arachidonic acids in phospholipids of VLDL and HDL_{2-3} were also lowered in the COd group but not in the SAd group. However, proportions of 22:5*n*-6 and 22:6*n*-3 in VLDL and HDL_{2-3} phospholipid fractions were enhanced in the COd and SAd groups respectively. The most affected apolipoproteins (apo) were apo B_{100} and apo B_{48} in rats fed on protein-deficient diets, apo A_I and apo E in the COd group, and apo A_{IV} in the SAd group. Compared with rats fed hydrogenated coconut oil diets, those fed salmon oil diets had enhanced LDL–HDL_1 and HDL_{2-3} but lower VLDL total apolipoproteins (mainly due to a fall in apo B_{100} and apo B_{48}). Arachidonic and eicosapentaenoic acids, which are impaired by protein deficiency, are the precursors of prostaglandins, thromboxanes and leukotrienes which are implicated in a number of regulatory processes. Our results demonstrate that protein malnutrition is associated with impaired metabolism of arachidonic and eicosapentaenoic acids. Protein malnutrition and essential fatty acid (EFA) deficiency are characterized by many common clinical features and the link between the two may be an impaired production of eicosanoids, since arachidonic and eicosapentaenoic acids are the precursors of these important metabolic regulators. Because of the apparent involvement of EFA deficiency in the aetiology of protein malnutrition, it may be prudent to include adequate amounts of EFA in diets of infants suffering from kwashiorkor.

Protein deficiency: EFA deficiency: Blood lipids: Salmon oil: Coconut oil: Rat

High intakes of saturated fatty acids (hydrogenated coconut oil) promote, in serum phospholipids, a pattern of polyunsaturated fatty acids (PUFA) characteristic of essential fatty acid (EFA) deficiency (Holman, 1960). Coconut oil, which is rich in lauric acid, raises serum triacylglycerol (TG) concentrations (Hostmark *et al.* 1982). Hill & Holman (1980) have reported the effects of various dietary protein levels (50–400 g/kg) on EFA availability in rats fed on hydrogenated coconut oil, and have indicated that signs observed in EFA deficiency are amplified with low protein intake. It has been proposed that EFA deficiency

may contribute to a number of symptoms associated with kwashiorkor, e.g. fatty liver, scaly dermatitis with a tendency to superinfection, loss of hair, impaired wound healing and growth retardation, which are also observed in EFA deficiency alone (Koletzko *et al.* 1986).

On the other hand, PUFA affect lipid concentrations in plasma and lipoproteins. In fish oil the long-chain *n*-3 PUFA, namely eicosapentaenoic acid (20:5*n*-3; EPA) and docosahexaenoic acid (22:6*n*-3; DHA) are the active components in lowering plasma lipids. EPA and arachidonic acid serve as substrates for cyclooxygenases and lipoxygenases, the enzymes that initiate the synthesis of prostaglandins, thromboxanes, prostacyclins and leukotrienes. Fish oils (e.g. salmon oil) are known to affect platelet function by reducing platelet aggregation and adhesion (Hornstra, 1989), and plasma lipids by reducing plasma concentration of very-low-density lipoprotein (VLDL) and, thereby, plasma TG levels (Harris *et al.* 1983).

Protein deficiency, which affects the synthesis of enzymes involved in lipid metabolism (Narce *et al.* 1988), and apolipoprotein (apo) compositions (Meghelli-Bouchenak *et al.* 1989*a*), may be a component in the adverse effect on PUFA status in some human diseases (Holman, 1986). Thus, results obtained in those studies indicate relationships between protein intake and EFA utilization and metabolism. In addition to decreased intake and synthesis, the increased oxidative metabolism of PUFA may accelerate marginal EFA deficiency and decrease long-chain PUFA bioavailability, thus increasing EFA requirements. However, there is only scant information on the supply and metabolism of EFA in malnourished children. Food intakes of infants before their hospital admission are difficult to determine and the results of several studies were inconsistent (see Dhansay *et al.* 1991). Similarly, little attention has been accorded to PUFA supplies in protein-deficiency studies in animals. Variations may also be explained by the different degree and duration of protein malnutrition.

In the present study in the growing rat, EFA, other long-chain PUFA, and protein intakes were determined exactly. Diets were provided for a short period (28 d). The combined influence of dietary protein levels (200 or 20 g casein/kg) associated with saturated (coconut oil) or polyunsaturated (salmon oil) fatty acids was determined in serum and lipoprotein fractions to measure their capacity to carry PUFA.

MATERIALS AND METHODS

Animals and diets

Forty male Wistar rats (Iffa Credo, l'Arbresle, Lyon, France) weighing 80 (SD 6) g at the beginning of the experiment were allowed free access to an adequate diet (containing 200 g casein and 50 g olive oil/kg) for 10 d. After this adaptation period, when their body weight was 112 (SD 8) g, they were randomly divided into four groups. For 28 d, two groups (controls) were fed on adequate diets containing 200 g casein/kg and either 50 g hydrogenated coconut oil/kg (rich in saturated fatty acids; COC), or 50 g salmon oil (rich in EPA and DHA; SAC). Two other groups were given low-protein diets (20 g casein/kg) and either 50 g coconut oil/kg (COd), or 50 g salmon oil/kg (SAd).

The coconut oil composition (g/100 g total fatty acids) was as follows: 8:0 4·0, 10:0 6·0, 12:0 39·0, 14:0 19·0, 16:0 16·0, 18:0 4·6, 18:1 (*n*-9 + *n*-7) 8·9, 18:2*n*-6 1·6. The salmon oil composition was as follows: 14:0 2·0, 16:0 16·0, 16:1*n*-7 6·0, 18:0 4·0, 18:1(*n*-9 + *n*-7) 18·0, 18:2*n*-6 4·0, 20:1(*n*-9 + *n*-11) 10·0, 20:4*n*-6 1·1, 20:5*n*-3 9·9, 22:1(*n*-9 + *n*-11) 8·0, 22:5*n*-3 3·9, 22:6*n*-3 11·1, 24:1*n*-9 1·2. The composition of the diets is shown in Table 1.

Diets were isoenergetic (16·28 MJ/kg) and contained the same quantities of lipids, vitamins, minerals and fibre. Animals were maintained in wire-bottomed cages at constant temperature (24°) and humidity (60 %) with a 12 h light cycle (07.00–19.00 hours). They ate

Table 1. *Diet compositions (g/kg diet)*

	COC	COd	SAC	SAd
Milk casein*	200	20	200	20
DL-Methionine†	3	3	3	3
Maize starch‡	587	767	587	767
Sucrose	50	50	50	50
Fibre (agar-agar)*	50	50	50	50
Mineral mix§	40	40	40	40
Vitamin mix‖	20	20	20	20
Coconut oil¶	50	50	0	0
Salmon oil**	0	0	50	50

The diets were isoenergetic (16·80 MJ/kg) and given in powdered form.
* Prolabo, Paris, France.
† Merck, Darmstadt, Germany.
‡ Ets Louis François, Saint Maur les Fossés, France.
§ UAR 205 B (Villemoisson, 91360 Epinay/Orge, France). Mineral mix provided the following (g/kg diet): Ca 4, K 2·4, Na 1·6, Mg 0·4, Fe 0·12, Mn 0·032, Cu 0·005, Zn 0·018, Co 0·00004, I 0·00002.
‖ UAR 200 (Villemoisson). Vitamin mix provided the following (mg/kg diet): thiamin 40, riboflavin 30, nicotinic acid 140, pyridoxine 20, pyridoxal 300, cyanocobalamin 0·1, ascorbic acid 1600, α-tocopherol 340, menadione 80, calcium pantothenate 200, choline 2720, pteroylmonoglutamic acid 10, *p*-aminobenzoic acid 100, biotin 0·6, retinol 12, cholecalciferol 0·125.
¶ Astra Calvé, Asniere, France.
** Gattefossé, St Priest, France.

and drank *ad lib.* We followed the general guidelines for the care and use of laboratory animals (Council of European Communities, 1986).

Blood samples

On day 28 of the experiment, after an overnight fast, rats were anaesthetized with sodium pentobarbital (60 mg/kg body weight) before blood collection by abdominal puncture. Serum was obtained by low-speed centrifugation and preserved with 0·26 mmol Na_2-EDTA/l and 3 mmol NaN_3/l.

Total lipoprotein (density < 1·21 kg/l) isolation. The density of the serum was adjusted to 1·21 kg/l by addition of crystalline KBr (0·322 kg/l; Havel *et al.* 1955). Lipoproteins of density (*d*) < 1·21 kg/l were isolated from about 2 ml of each serum sample by a single centrifugation flotation (Model L 8-55 ultracentrifuge, 50 Ti rotor; Beckman Instruments, Palo Alto, CA, USA). All centrifugations were performed at 122000 ***g*** at 15° for 48 h. Lipoproteins were dialysed against 0·15 M NaCl and 1 mM Na_2EDTA, pH 7·4 at 4° in Spectra/Por 2 dialysis tubing (Spectrum Medical Industries, Los Angeles, CA, USA).

Isolation of lipoprotein fractions. Three fractions (VLDL, low-density-lipoprotein–high-density-lipoprotein ($LDL–HDL_1$), HDL_{2-3}) were isolated by a single-spin discontinuous gradient (Redgrave *et al.* 1975, modified by Meghelli-Bouchenak *et al.* 1989*a*). Each discontinuous gradient was constructed at ambient temperature in a thin-walled polyallomer tube (13·5 mm diameter × 89 mm length) of the Beckman SW41-Ti swinging bucket rotor. The centrifugation was performed at 274000 ***g*** at 15° in a Beckman L5-50 ultracentrifuge. VLDL portions (2 ml) were taken with a syringe at the top of the tube. Then the content of the tube was displaced by a KBr solution of *d* 1·346 kg/l at the bottom of the tube and collected from the surface. During the displacement, this effluent was monitored by measurement of absorption at 280 nm in a Beckman spectrophotometer (Model 35) to isolate the $LDL–HDL_1$ and HDL_{2-3} peaks.

Table 2. *The effects of control (C) or protein-deficient (d) diets containing coconut (CO) or salmon oil (SA) on protein (g/l) and lipid contents (mmol/l) of serum, very-low-density lipoprotein (VLDL), low-density lipoprotein – high-density lipoprotein$_1$ (LDL–HDL$_1$) and HDL$_{2-3}$ fractions of growing rats**

(Values are means for six rats with their pooled standard errors)

						Statistical significance (*P*) of effect of		
Diet...	COC	COd	SAC	SAd	SEM	oil type (CO *v.* SA)	level of protein (C *v.* d)	Interaction (oil type × level of protein)
Protein								
:Serum	58·9	45·8	68·9	47·7	1·76	0·003	< 0·001	0·032
VLDL	0·26	0·17	0·21	0·14	0·02	0·059	< 0·001	< 0·622
LDL–HDL$_1$	0·08	0·06	0·15	0·10	0·05	< 0·001	< 0·001	0·007
HDL$_{2-3}$	0·71	0·56	1·05	0·74	0·06	< 0·001	< 0·001	0·554
Phospholipids								
:Serum	44·8	43·9	35·2	33·9	2·78	0·002	0·693	> 0·90
VLDL	5·36	3·06	3·77	3·06	0·38	0·049	< 0·001	0·049
LDL–HDL$_1$	4·84	4·84	5·49	5·81	0·59	0·185	0·790	0·790
HDL$_{2-3}$	17·3	21·8	16·8	24·5	1·08	0·319	< 0·001	0·154
Triacylglycerols								
:Serum	1·65	0·89	1·11	0·49	0·05	< 0·001	< 0·001	0·176
VLDL	1·14	0·60	0·50	0·24	0·05	< 0·001	< 0·001	0·011
LDL–HDL$_1$	nd	nd	nd	nd	—	—	—	—
HDL$_{2-3}$	nd	nd	nd	nd	—	—	—	—
Unesterified cholesterol								
:Serum	0·68	0·70	0·50	0·48	0·06	0·003	> 0·99	0·744
VLDL	0·14	0·19	0·07	0·02	0·01	< 0·001	> 0·99	< 0·001
LDL–HDL$_1$	0·16	0·08	0·26	0·04	0·02	0·149	< 0·001	0·002
HDL$_{2-3}$	0·37	0·48	0·32	0·38	0·03	0·021	0·010	0·416
Esterified cholesterol								
:Serum	2·85	2·80	2·02	1·61	0·11	< 0·001	0·049	0·118
VLDL	0·35	0·38	0·37	0·13	0·04	0·009	0·016	0·003
LDL–HDL$_1$	0·28	0·09	0·34	0·19	0·03	0·004	< 0·001	< 0·001
HDL$_{2-3}$	1·41	0·68	0·77	0·64	0·05	< 0·001	< 0·001	< 0·001
Total cholesterol								
:Serum	3·55	3·51	2·54	2·10	0·23	< 0·001	0·310	0·394
VLDL	0·50	0·58	0·44	0·16	0·05	< 0·001	0·059	0·001
LDL–HDL$_1$	0·44	0·17	0·60	0·23	0·04	0·012	< 0·001	0·225
HDL$_{2-3}$	1·78	1·17	1·10	1·02	0·07	< 0·001	< 0·001	0·001

COC, 200 g casein + 50 g CO/kg; COd, 20 g casein + 50 g CO/kg; SAC, 200 g casein + 50 g SA/kg; SAd, 20 g casein + 50 g SA/kg; nd, not determined.

* For details of diets and procedures, see Table 1 and pp. 376–379.

Purified fractions of VLDL ($d < 1{\cdot}006$), LDL–HDL$_1$ ($1{\cdot}006 < d < 1{\cdot}06$) and HDL$_{2-3}$ ($1{\cdot}06 < d < 1{\cdot}15$) were dialysed as described previously.

Chemical analyses

Liver, serum and lipoprotein total lipids were extracted according to the method of Folch *et al.* (1957). The amount of total lipid per g liver was determined gravimetrically from a subsample. Phospholipid (PL) and TG fractions were isolated by TLC (Stahl *et al.* 1956). PL and TG fractions were methylated, and then fatty acid analysis of PL and TG fractions

Table 3. *The effects of control (C) or protein-deficient (d) diets containing coconut (CO) or salmon oil (SA) on major fatty acid compositions of serum triacylglycerols (mg/100 mg fatty acids) of growing rats**

(Values are means for six rats with their pooled standard errors)

						Statistical significance (*P*) of effect of		
Diet...	COC	COd	SAC	SAd	SEM	oil type (CO *v.* SA)	level of protein (C *v.* d)	Interaction (oil type × level of protein)
Total saturated	32·5	42·3	22·0	37·0	2·85	0·012	< 0·001	0·374
Total monounsaturated	54·4	47·4	27·9	24·4	3·42	< 0·001	0·139	0·615
20:3*n*-9	1·9	2·9	0·9	1·0	0·28	< 0·001	0·064	0·124
18:2*n*-6	5·8	3·0	5·7	5·8	0·44	< 0·001	< 0·001	0·003
20:4*n*-6	3·0	2·1	0·7	1·9	0·29	< 0·001	0·616	0·001
22:5*n*-6	0·8	1·8	0·6	1·2	0·23	0·098	0·001	0·396
Total *n*-6	9·6	6·9	7·0	8·9	0·89	0·738	0·658	0·018
18:3*n*-3	nd	nd	1·6	0·5	0·07	—	< 0·001	—
20:5*n*-3	tr	tr	18·3	5·9	1·82	—	< 0·001	—
22:5*n*-3	nd	nd	3·9	3·2	0·55	—	0·270	—
22:6*n*-3	tr	tr	17·9	18·8	1·91	—	0·720	—
Total *n*-3	tr	tr	41·7	28·4	4·12	—	0·009	—
P:S	2·02	1·35	3·52	1·69	0·29	0·004	< 0·001	0·064
20:4*n*-6/18:2*n*-6	0·51	0·70	0·12	0·32	0·04	< 0·001	< 0·001	0·90
20:3*n*-9/20:4*n*-6	0·63	1·38	1·28	0·52	0·07	0·15	> 0·90	< 0·001

COC, 200 g casein + 50 g CO/kg; COd, 20 g casein + 50 g CO/kg; SAC, 200 g casein + 50 g SA/kg; SAd, 20 g casein + 50 g SA/kg; nd, not detected; tr, trace (< 0·1); P:S, polyunsaturated:saturated.

* For details of diets and procedures, see Table 1 and pp. 376–379.

was performed by GLC (Slover & Lanza, 1979), using a Becker Gas Chromatograph Packard 417, equipped with a glass column, length 39 m, internal diameter 0·3 mm, stationary phase carbowax 20M, H_2 flow-rate 6 ml/min, inlet heater 202°, detector temperature 240° (Becker Instruments, Downers Grove, IL, USA), with 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). The average molecular weight of fatty acids was determined, allowing amounts of serum and VLDL TG to be calculated. The solutions used for lipid extraction and TLC contained 2,6 di-*tert*-butyl-*p*-cresol (50 mg/l) as an antioxidant, and the lipid extracts were stored under N_2 gas in the dark at −20° to prevent peroxidation of unsaturated fatty acids.

Total cholesterol (TC) determination was performed by GLC (Gambert *et al.* 1979) and unesterified cholesterol was measured according to the method of Gambert *et al.* (1982). PL were quantified by the measurement of P (Bartlett, 1958). Protein contents were measured (Lowry *et al.* 1951) using bovine serum albumin as standard.

Electrophoretic evaluation of VLDL and HDL apolipoproteins

After partial delipidation, VLDL and HDL_{2-3} apolipoproteins were estimated using SDS–PAGE (2·5–> 20%) by the method of Meghelli-Bouchenak *et al.* (1989*a*). Electrophoresis was performed in a LKB 2001-001 vertical electrophoresis unit (LKB Produkte, Bromme, Sweden) at 4°, for 18 h with 20 mA/gel slab. Gels were then stained with Coomassie brilliant blue G 250.

Table 4. *The effects of control (C) or protein-deficient (d) diets containing coconut (CO) or salmon oil (SA) on major fatty acid compositions of very-low-density lipoprotein (VLDL) triacylglycerols (mg/100 mg fatty acids) of growing rats**

(Values are means for six rats with their pooled standard errors)

						Statistical significance (*P*) of effect of		
Diet...	COC	COd	SAC	SAd	SEM	oil type (CO *v.* SA)	level of protein (C *v.* d)	Interaction (oil type × level of protein)
Total saturated	44·6	55·6	34·0	43·2	2·70	< 0·001	< 0·001	0·746
Total monounsaturated	43·6	35·2	23·3	21·9	2·82	< 0·001	0·098	0·23
20:3*n*-9	0·9	1·2	0·6	0·9	0·23	0·208	0·208	> 0·99
18:2*n*-6	5·2	2·5	2·4	2·2	0·38	< 0·001	< 0·001	0·003
20:4*n*-6	4·2	2·6	2·7	2·6	0·30	0·242	0·010	0·212
22:5*n*-6	0·9	2·0	nd	nd	0·29	—	0·003	—
Total *n*-6	10·3	7·1	5·1	4·8	0·81	< 0·001	0·043	0·088
18:3*n*-3	nd	nd	2·5	1·0	0·22	—	< 0·001	—
20:5*n*-3	nd	nd	15·2	10·4	1·10	—	0·001	—
22:5*n*-3	nd	nd	4·8	2·7	0·35	—	< 0·001	—
22:6*n*-3	nd	nd	12·9	15·0	0·76	—	0·020	—
Total *n*-3	nd	nd	35·4	29·1	2·40	—	0·025	—
P:S	1·22	0·78	1·89	1·31	0·15	< 0·001	< 0·001	0·65
20:4*n*-6/18:2*n*-6	0·80	1·04	1·12	1·18	0·07	< 0·001	0·044	0·215
20:3*n*-9/20:4*n*-6	0·21	0·46	0·22	0·34	0·05	0·284	0·001	0·208

COC, 200 g casein + 50 g CO/kg; COd, 20 g casein + 50 g CO/kg; SAC, 200 g casein + 50 g SA/kg; SAd, 20 g casein + 50 g SA/kg; nd, not detected; P:S, polyunsaturated:saturated.

* For details of diets and procedures, see Table 1 and pp. 376–379.

Destained gels were scanned at 600 nm with a densitometer (Model Profil 26; Sebia, Issy les Moulineaux, France). Apolipoproteins were determined semiquantitatively with the densitometer tracings. To estimate the concentration of each apolipoprotein, the percentage of the area relative to each apolipoprotein was multiplied by the total apolipoprotein content of each serum sample. Staining affinity of each peptide was not determined individually; however, when 50–200 mg total protein was applied, the chromogenicity of each major band varied linearly with the amount of total protein applied to the gel. Apolipoprotein samples for the four groups were subjected to electrophoresis in parallel for each lipoprotein fraction. Results were expressed as arbitrary units (AU).

Statistical analysis

Results were reported as arithmetical means for each group with their pooled standard errors (SEM). The significance of differences were determined by Student's *t* test for unpaired values. The differences were considered significant at $P < 0{\cdot}05$.

RESULTS

Body weights and food intakes

After 28 d on a protein-deficient diet, rats developed fatty livers. The total lipid contents (mg/g liver) were: 145 (SEM 13), 104 (SEM 11), 68 (SEM 1) and 58 (SEM 4), for the COd, SAd, COC and SAC groups respectively. The values for the protein-deficient groups were significantly greater than those for protein-sufficient groups ($P < 0{\cdot}001$). In spite of similar

Table 5. *The effects of control (C) or protein-deficient (d) diets containing coconut (CO) or salmon oil (SA) on major fatty acid compositions of serum phospholipids (mg/100 mg fatty acids) of growing rats**

(Values are means for six rats with their pooled standard errors)

						Statistical significance (*P*) of effect of		
Diet...	COC	COd	SAC	SAd	SEM	oil type (CO *v.* SA)	level of protein (C *v.* d)	Interaction (oil type × level of protein)
Total saturated	48·7	67·3	26·7	49·1	3·24	< 0·001	< 0·001	0·561
Total monounsaturated	30·3	17·2	18·9	17·2	2·30	0·022	0·004	0·022
20:3*n*-9	4·6	6·7	0·8	1·6	0·53	< 0·001	0·012	0·232
18:2*n*-6	5·3	3·4	5·9	3·7	0·37	0·236	< 0·001	0·686
20:4*n*-6	5·8	2·4	11·2	5·6	0·92	< 0·001	< 0·001	0·247
22:5*n*-6	1·4	2·2	1·5	nd	0·13	< 0·001	0·014	0·001
Total *n*-6	13·4	8·0	18·6	9·3	1·30	0·021	< 0·001	0·149
18:3*n*-3	nd	nd	3·0	0·9	0·25	—	< 0·001	—
20:5*n*-3	nd	nd	6·3	6·8	0·65	—	0·550	—
22:5*n*-3	nd	nd	4·4	1·4	0·41	—	< 0·001	—
22:6*n*-3	1·7	nd	19·2	13·2	0·59	—	< 0·001	—
Total *n*-3	1·7	nd	32·9	22·3	1·64	—	< 0·001	—
P:S	1·02	0·49	2·66	1·02	0·25	< 0·001	< 0·001	0·038
20:4*n*-6/18:2*n*-6	0·91	0·70	1·89	1·51	0·11	< 0·001	0·014	0·45
20:3*n*-9/20:4*n*-6	0·86	2·79	0·07	0·28	0·13	< 0·001	< 0·001	< 0·001

COC, 200 g casein + 50 g CO/kg; COd, 20 g casein + 50 g CO/kg; SAC, 200 g casein + 50 g SA/kg; SAd, 20 g casein + 50 g SA/kg; nd, not detected; P:S, polyunsaturated:saturated.

* For details of diets and procedures, see Table 1 and pp. 376–379.

daily food and energy intakes/kg body weight (76 (SD 5) g and 1243 (SD 85) kJ respectively), body weights of both protein-deficient groups (SAd and COd) were only 43 and 44% of their respective control groups. Final body weights were similar in both control groups (289 (SD 9) g).

Serum and lipoprotein fractions

Protein. Protein contents of serum and total apolipoproteins of all lipoprotein fractions were significantly reduced in rats fed on low-protein diets, particularly with coconut oil (except for VLDL), compared with those fed on 200 g casein/kg diets (Table 2).

Total cholesterol. Protein-deficient diets lowered LDL–HDL_1 TC values in both protein-deficient groups, HDL_{2-3} TC in the COd group and VLDL TC in the SAd group, relative to their respective control groups (Table 2). This fall concerned the two fractions (esterified and unesterified). In addition, serum and VLDL TC concentrations were depressed (essentially due to the esterified fraction) in the SAd group compared with the SAC group. Consumption of coconut oil compared with that of salmon oil enhanced the TC (esterified and unesterified) concentrations in serum and HDL_{2-3}.

Phospholipids. VLDL PL concentrations were depressed in the COd group, and HDL_{2-3} PL concentrations were raised in both protein-deficient groups, compared with their respective controls (Table 2). Feeding salmon oil diets resulted in lower concentrations of serum and VLDL PL than feeding coconut oil diets.

Triacylglycerol. Serum and VLDL TG concentrations were depressed by low-protein diets relative to rats given 200 g casein/kg diets (Table 2). Furthermore, these values were lower in rats given salmon oil diets compared with rats given coconut oil diets.

Table 6. *The effects of control (C) or protein-deficient (d) diets containing coconut (CO) or salmon oil (SA) on major fatty acid compositions of very-low-density lipoprotein (VLDL) phospholipids (mg/100 mg fatty acids) of growing rats**

(Values are means for six rats with their pooled standard errors)

Diet...	COC	COd	SAC	SAd	SEM	Statistical significance (P) of effect of: oil type (CO v. SA)	level of protein (C v. d)	Interaction (oil type × level of protein)
Total saturated	47·6	52·8	34·2	41·5	3·87	0·004	0·123	0·79
Total monounsaturated	32·1	24·3	14·7	18·3	1·29	< 0·001	0·118	< 0·001
20:3*n*-9	5·8	8·7	nd	nd	0·57	—	< 0·001	—
18:2*n*-6	6·9	4·3	8·9	5·9	0·82	0·040	0·005	0·812
20:4*n*-6	4·9	3·7	12·7	5·3	0·70	< 0·001	< 0·001	< 0·001
22:5*n*-6	2·2	4·6	nd	nd	0·45	—	< 0·001	—
Total *n*-6	14·0	12·6	21·6	11·2	1·63	0·072	0·001	0·012
18:3*n*-3	nd	nd	1·0	0·6	0·10	—	0·002	—
20:5*n*-3	nd	nd	13·0	9·1	0·36	—	< 0·001	—
22:5*n*-3	nd	nd	3·7	1·3	0·45	—	< 0·001	—
22:6*n*-3	nd	nd	11·2	15·8	0·95	—	< 0·001	—
Total *n*-3	nd	nd	28·9	26·8	2·25	—	0·380	—
P:S	1·09	0·86	1·90	1·35	0·14	< 0·001	0·011	0·267
20:4*n*-6/18:2*n*-6	0·71	0·86	1·42	0·89	0·09	< 0·001	0·047	0·001
20:3*n*-9/20:4*n*-6	1·18	2·35	—	—	0·22	—	< 0·001	—

COC, 200 g casein + 50 g CO/kg; COd, 20 g casein + 50 g CO/kg; SAC, 200 g casein + 50 g SA/kg; SAd, 20 g casein + 50 g SA/kg; nd, not detected; P:S, polyunsaturated:saturated.

* For details of diets and procedures, see Table 1 and pp. 376–379.

Fatty acid composition (g/100 g total fatty acids) of serum and lipoproteins

Serum and VLDL triacylglycerols. Protein-deficient diets diminished the polyunsaturated:saturated fatty acid (P:S) value in serum and VLDL TG (Tables 3 and 4). The fatty acid 22:5*n*-6 was not detected in VLDL TG of the SAd group but was raised in serum of both protein-deficient groups. The 20:4*n*-6:18:2*n*-6 value in serum and the 20:3*n*-9:20:4*n*-6 value (Holman's (1960) EFA deficiency index) in VLDL were enhanced by protein-deficient diets. In addition, the COd group, relative to the COC group, had lower linoleic and arachidonic acids, and thereby total *n*-6 in both serum and VLDL, whereas the SAd group, relative to the SAC group, had lower 20:5*n*-3 and total *n*-3 fatty acids in serum and VLDL, but enhanced 20:4*n*-6 and total *n*-6 fatty acids in serum, and 22:6*n*-3 in VLDL.

Compared with coconut oil diets, salmon oil diets raised total *n*-3 fatty acids, the 20:3*n*-9:20:4*n*-6 and P:S values, whereas they diminished total saturated, monounsaturated, 20:4*n*-6 and total *n*-6 fatty acids in serum and VLDL TG fractions. In addition, rats fed on salmon oil diets relative to those fed on coconut oil diets had higher 20:4*n*-6:18:2*n*-6 ratios in VLDL TG, unlike the levels obtained in serum TG. Furthermore *n*-3 fatty acids were generally not detected with coconut oil diets.

Serum, VLDL and HDL_{2-3} phospholipids. Low-protein diets enhanced total saturated and the 20:3*n*-9:20:4*n*-6 value and diminished 18:2*n*-6, 20:4*n*-6, total *n*-6 fatty acids and the P:S value in serum and VLDL PL fractions (Tables 5 and 6). In addition, in the COd group, relative to the COC group, 22:5*n*-6 was generally higher in overall PL studied (serum, VLDL, HDL_{2-3}) whereas, in the SAd group, relative to the SAC group, 22:6*n*-3 was enhanced in VLDL- and HDL_{2-3}-PL (Tables 5–7). Compared with salmon oil diets, coconut oil diets increased total saturated, monounsaturated, 20:3*n*-9 and the 20:3*n*-

Table 7. *The effects of control (C) or protein-deficient (d) diets containing coconut (CO) or salmon oil (SA) on major fatty acid compositions of high-density lipoprotein (HDL) phospholipids (mg/100 mg fatty acids) of growing rats**

(Values are means for six rats with their standard errors)

Diet...	COC	COd	SAC	SAd	SEM	Statistical significance (*P*) of effect of: oil type (CO *v.* SA)	level of protein (C *v.* d)	Interaction (oil type × level of protein)
Total saturated	48·0	58·5	32·3	39·1	3·53	< 0·001	0·023	0·608
Total monounsaturated	31·1	19·9	20·9	17·5	1·59	< 0·001	< 0·001	0·023
20:3*n*-9	10·1	12·4	tr	tr	1·12	—	0·067	—
18:2*n*-6	3·5	1·7	6·5	5·3	0·56	< 0·001	0·014	0·601
20:4*n*-6	4·3	3·3	13·0	15·3	0·73	< 0·001	0·384	0·035
22:5*n*-6	2·2	3·8	nd	nd	0·20	—	< 0·001	—
Total *n*-6	10·0	8·8	19·5	20·6	1·35	< 0·001	> 0·90	0·405
18:3*n*-3	nd	nd	2·0	tr	—	—	—	—
20:5*n*-3	nd	nd	12·5	7·8	0·29	—	< 0·001	—
22:5*n*-3	nd	nd	2·7	2·9	0·25	—	0·420	—
22:6*n*-3	nd	nd	9·2	11·6	0·51	—	< 0·001	—
Total *n*-3	nd	nd	26·4	22·3	1·05	—	0·003	—
P:S	1·06	0·70	2·06	1·54	0·21	< 0·001	0·048	0·708
20:4*n*-6/18:2*n*-6	1·22	1·94	2·00	2·88	0·22	< 0·001	0·001	0·722
20:3*n*-9/20:4*n*-6	2·34	3·75	—	—	0·55	—	0·028	—

COC, 200 g casein + 50 g CO/kg; COd, 20 g casein + 50 g CO/kg; SAC, 200 g casein + 50 g SA/kg; SAd, 20 g casein + 50 g SA/kg; nd, not detected; tr, trace (< 0·1); P:S, polyunsaturated:saturated.

* For details of diets and procedures, see Table 1 and pp. 376–379.

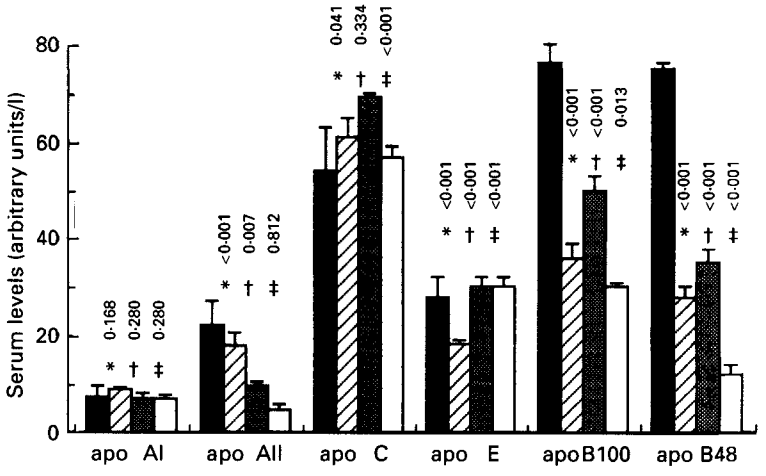

Fig. 1. Very-low-density lipoprotein (VLDL)-apolipoprotein (apo) distribution in young rats fed on protein-deficient diets with coconut or salmon oils for 28 d. Values are means with their standard errors represented by vertical bars for six rats. (■), 200 g casein + 50 g coconut oil/kg; (▨), 20 g casein + 50 g coconut oil/kg; (▩), 200 g casein + 50 g salmon oil/kg; (□), 20 g casein + 50 g salmon oil/kg. Statistical significance (*P*) is given for the effects of: * oil type (coconut *v.* salmon), † level of protein (200 g *v.* 20 g), ‡ interaction (oil type × level of protein).

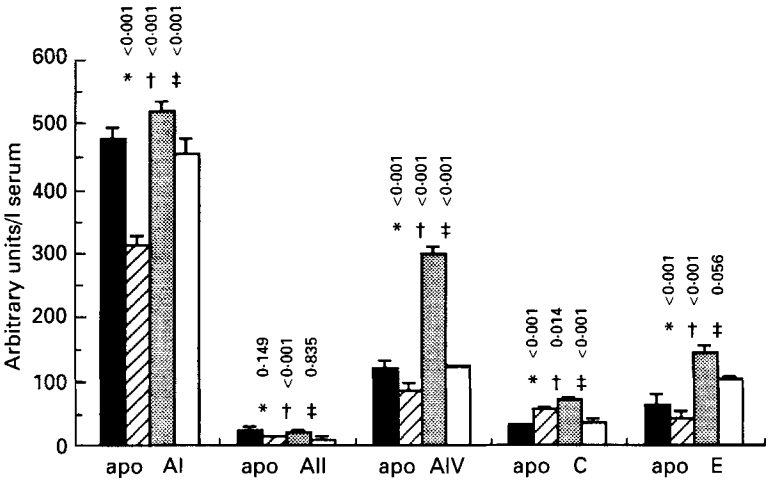

Fig. 2. High-density lipoprotein$_{2-3}$ (HDL_{2-3})-apolipoprotein (apo) distribution in young rats fed on protein-deficient diets with coconut or salmon oils for 28 d. Values are means with their standard errors represented by vertical bars for six rats. (■), 200 g casein + 50 g coconut oil/kg; (▨), 20 g casein + 50 g coconut oil/kg; (▩), 200 g casein + 50 g salmon oil/kg; (□), 20 g casein + 50 g salmon oil/kg. Statistical significance (*P*) is given for the effects of: * oil type (coconut *v.* salmon), † level of protein (200 g *v.* 20 g), ‡ interaction (oil type × level of protein).

9:20:4*n*-6 value but decreased 20:4*n*-6, total *n*-6 and *n*-3 fatty acids, the P:S and the 20:4*n*-6:18:2*n*-6 values in overall PL (serum, VLDL, HDL_{2-3}).

VLDL-apolipoprotein composition

Apo B_{100}, apo B_{48} and apo A_{IV} were depressed in rats fed on low-protein diets (Fig. 1). Compared with their respective controls, the COd group had lower apo E and the SAd group lower apo C. Apo A_I was not affected by protein-deficient diets. Salmon oil diets resulted in lower apo B_{100}, apo B_{48} and apo A_{IV} and higher apo C values than those obtained with coconut oil diets.

HDL_{2-3}-apolipoprotein composition

Total apolipoproteins of HDL_{2-3} were higher in the SAC group compared with those of the COC group (Fig. 2). Protein-deficient diets depressed these values in both protein deficient groups. However, compared with their respective controls, apo A_{II} in the SAd group and apo E and apo C in the COd group were not affected by protein deficiency. Furthermore, in the SAd group, apo A_{IV}, C and E values were generally depressed and apo A_I was less affected, compared with those in the COd group.

DISCUSSION

Fatty liver occurs in children suffering from kwashiorkor. This liver lipid accumulation (mainly TG) is attributed by Flores *et al.* (1970) and Meghelli-Bouchenak *et al.* (1989*b*) to the defect of hepatic TG exportation by VLDL. The presence of fatty liver and similarity between the skin lesions of kwashiorkor and those described in experimental EFA deficiency lead to the hypothesis that protein and EFA deficiencies may both be involved in chronic malnutrition. Our results demonstrate a similar association between protein deficiency and fatty liver in a rat model.

Protein deficiency has a strong influence on the PUFA pattern of heart, liver and serum (Hill & Holman, 1980). PUFA are mainly rapidly incorporated into structural lipids or stored in adipose tissues and are not very useful for oxidation. The Triene:tetraene ratio, 20:3*n*-9:20:4*n*-6 (limit of normality < 0·2), introduced by Holman (1960) to assess EFA

deficiency was too simplistic, and serum PUFA profile, which reveals disproportion between *n*-6, *n*-3, and *n*-9 PUFA, is preferred as an indicator of EFA status. In the present work, to compare dietary variables, data are only considered with respect to proportions (relative percentages by weight) and, therefore, do not provide actual amounts of fatty acids. The reason for this is that the properties of cell membranes are influenced by the proportions of various types of fatty acids in their lipids, and because serum PL reflect the EFA status of tissue lipids (Holman, 1986).

In our study protein malnutrition decreased the protein content of serum and lipoproteins, yet the values obtained with salmon oil diets were higher than those obtained with hydrogenated coconut oil diets, except for VLDL (due to apo B_{100} and apo B_{48}). Serum and VLDL TG contents were lowered with protein-deficient diets but more when salmon oil was associated. These results for proteins and TG are consistent with those in the literature, but those for TC and PL are inconsistent. In our study the PL contents of VLDL were diminished but those of HDL_{2-3} were enhanced in rats fed on low-protein diets. These rats had a lower LDL–HDL_1 TC content. Furthermore, in the SAd group, serum and VLDL TC, and in the COd group HDL_{2-3} TC were lower than in the SAC and COC groups respectively. Koleztko *et al.* (1986) have reported that total amounts of PUFA as well as the P:S value were severely reduced in plasma PL and cholesteryl esters in malnourished Nigerian children. In the overall lipid fractions studied we also found a decreased P:S value with protein-deficient diets, but the values in the presence of salmon oil were higher than those obtained with hydrogenated coconut oil. Similarly, Holman's (1960) index was enhanced for both protein-deficient groups in VLDL TG but only with hydrogenated coconut oil in overall PL. Koleztko *et al.* (1986) reported an elevated 20:3*n*-9:20:4*n*-6 value in the serum TG fraction, but not in PL and cholesteryl esters in malnourished children.

Fatty acid profiles of serum PL showed a fall in linoleic, linolenic and arachidonic acids and consequently depressed total *n*-6 and *n*-3 fatty acids with both protein-deficient diets. In VLDL and HDL_{2-3} PL fractions, the same results were observed in the COd group, while in the SAd group, linoleic, arachidonic and total *n*-6 fatty acids were not reduced. These changes showed that the 20:4*n*-6:18:2*n*-6 ratio is not the best reflection of Δ6 desaturase activities. Mercuri *et al.* (1979) and De Tomas *et al.* (1980) have shown that partial protein deprivation is associated with decreased arachidonate:linoleate values in PL from rat liver and have postulated that 18:2*n*-6 accumulation was due to impaired chain elongation or desaturation or both in protein-deficient rats. However, Gerson & Wong (1978) showed that the incubation *in vitro* of liver microsomes of rats fed on a protein-free diet for 7 weeks revealed unchanged chain elongation and desaturation enzyme activities. Narce *et al.* (1988) showed that hepatic microsomal Δ6- and Δ5-desaturase activities are not strictly paralleled by changes in the altered fatty acid composition of liver total lipids.

It is well known that 18:3*n*-3, as 20:5*n*-3 (Garg *et al.* 1988), competes with 18:2*n*-6 and 18:1*n*-9 for Δ6 desaturase. The competitive activities of the three substrates for Δ6 desaturation are *n*-3 > *n*-6 > *n*-9. In the present study the arachidonate:linoleate value, though subject to a wide variation, showed, for example, increased values in the COd group (except for serum PL), yet we did not find 18:2*n*-6 accumulation in protein-deficient groups as observed by De Tomas *et al.* (1980). The variations in this ratio with protein deficiency were the result of both arachidonic and linoleic acid decreases, but for each fatty acid the fall was not the same. On the other hand, when 18:2*n*-6 was diminished by protein-deficient diets it was accompanied by a rise in 20:3*n*-9 (particularly in the COd group and in serum PL of the SAd group), or in total monounsaturated fatty acids (in VLDL PL of the SAd group), even though salmon oil supplies a little of both arachidonic and linoleic acids (1·1 and 4·0 g/100 g respectively). For these reasons we conclude that Δ6 and Δ5 desaturase activities may be reduced only a little in our protein-deficient rats. In support of this view,

we have shown in a previous study with the same experimental protocol that there was no correlation between microsomal desaturation rate and microsome PL profiles, and that the PUFA incorporated into microsomal membranes have no effect on desaturases (Ulmann *et al.* 1992).

Fatty acid profiles of VLDL and HDL_{2-3} PL fractions showed a greater proportion of 22:5*n*-6 and 22:6*n*-3 in the COd and SAd groups respectively. These increments could be attributable to higher hepatic microsomal Δ4 desaturase activity or to a lower utilization of these fatty acids by other tissues (Δ4 desaturase activity involves the desaturation of 22:5*n*-3 to 22:6*n*-3 or 22:4*n*-6 to 22:5*n*-6). This activity increases with *n*-3 fatty acid deficiency in rat liver (Hagve *et al.* 1984).

Total VLDL apolipoproteins are reduced by protein deficiency, probably on account of a decrease in synthesis as observed by Yagasaki & Kametaka (1984) and Meghelli-Bouchenak *et al.* (1987). Results obtained in previous reports of rats subjected to 20 g casein or 50 g gluten and 50 g sunflower oil/kg diet showed that the reduced synthesis of liver VLDL apolipoproteins, indeed apo B_{100}, apo B_{48} and apo C which are the main apolipoproteins synthesized by liver in rats, is a cause of impaired hepatic TG export, which results in fatty liver (Meghelli-Bouchenak *et al.* 1989*b*). The fall in total apolipoproteins of VLDL and HDL_{2-3} induced by protein deficiency in this present study was less pronounced (and mainly due to apo B_{100} and apo B_{48}) than in previous work (Bouziane *et al.* 1992). Furthermore, we have shown in a previous report that protein deficiency reduced the half-lives of VLDL apolipoproteins and increased their clearance from VLDL, probably owing to an increase in peripheral uptake of different organs but not to a higher uptake by liver (Meghelli-Bouchenak *et al.* 1991). Apo B_{100} and apo B_{48} in both protein-deficient groups, apo A_I and apo E in the COd group and apo A_{IV} in the SAd group were the most affected by protein deficiency. With salmon oil diets, apo C, apo A_I, apo E and apo A_{IV} values were greater than those obtained with coconut oil diets.

In conclusion, giving protein-deficient diets to rats for a relatively short period of 28 d led to a reduced P:S ratio in serum phospholipids even in the presence of apparently adequate levels of dietary PUFA, suggesting an impairment in 'availability' of PUFA. One consequence may be a modification of membrane viscosity and the functions of integral proteins (Léger *et al.* 1987), while another could be decreased availability of eicosanoids, since arachidonic and eicosapentaenoic acids are precursors of these important metabolic regulators. This may provide an explanation for the link between protein malnutrition and EFA deficiency with their common clinical features of loss of hair, dry scaly dermatitis, tendency to superinfection and increased water permeability. It may be prudent to include adequate amounts of EFA in the rehabilitation diets of infants suffering from kwashiorkor.

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