Effects on Plasma Lipids and Fatty Acid Composition of Very Low Fat Diets Enriched with Fish Or Kangaroo Meat

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The effects of very low fat diets (<7% energy) enriched with different sources of long chain (C20 and C22) polyunsaturated fatty acids (PUFA) on plasma lipid levels and plasma fatty acid composition were studied in 13 healthy volunteers. Three diets provided 500 g/day of tropical Australian fish (rich in arachidonic acid and docosahexaenoic acid), southern Australian fish (rich in docosahexaenoic acid) or kangaroo meat (rich in linoleic and arachidonic acids). The fourth diet was vegetarian, similarly low in fat but containing no 20- and 22-carbon PUFA. Subjects ate their normal or usual diets on weeks 1 and 4 and the very low fat diets in weeks 2 and 3. Weighed food intake records were kept, and weeks 2, 3 and 4 were designed to be isoenergetic with week 1.

Plasma cholesterol levels fell significantly on all diets within one week. There were reductions in both low density (LDL) and high density lipoprotein (HDL) cholesterol levels, with effects on HDL cholesterol being more consistent. There were no consistent or significant effects on total triglyceride levels despite the high carbohydrate content of the diets. On all diets the percentage of linoleic acid fell in the plasma phospholipid and cholesteryl ester fractions, while the percentage of palmitic acid in the phospholipids and cholesteryl esters and palmitoleic acid in the cholesteryl ester fraction rose on all diets. The percentage of arachidonic acid rose in the phospholipid and cholesteryl esters on the two diets that were good sources of this fatty acid (tropical fish and kangaroo meat). The percentage of docosahexaenoic acid also rose on the two diets that were the richest sources of this fatty acid (the fish diets), and the percentage of eicosapentaenoic acid rose in the phospholipid and cholesteryl esters in proportion to the dietary level of this fatty acid (southern fish > kangaroo > tropical fish). The changes in fatty acid composition were almost completely reversed within seven days of returning to the usual higher fat diets.

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We have previously shown that two types of diets traditionally eaten by aborigines from the northern coastal regions of Australia are associated with a threefold increase in the proportion of arachidonic acid in plasma lipids (1,2). The first was a diet derived almost exclusively from tropical seafood (1), which we have subsequently analyzed and found to be a rich source of arachidonic acid (1,3). The second diet was one in which kangaroo meat and freshwater fish (linoleic and arachidonic acid-rich) were the major sources of fat (2). Both diets were very low in fat despite being rich in fish and red meat (2,4). Plasma triglycerides fell on both diets, and bleeding time increased (1,2). Plasma cholesterol levels, which were not high initially, were not significantly affected by the diets, even when they were rich in lean red meat.

The aim of the present study was to follow up these original observations by examining under controlled conditions the effects on plasma lipoprotein lipids and plasma fatty acid composition of four diets similarly low in fat but containing quite different polyunsaturated fatty acids (PUFA): three contained either 500 g/day of tropical fish (rich in arachidonic and docosahexaenoic acids), southern Australian fish (rich in docosahexaenoic acid) or kangaroo meat (rich in linoleic and arachidonic acids). The fourth diet was vegetarian, with a similarly low fat content and polyunsaturated/saturated ratio but containing no long chain (>20 carbon) PUFA.

METHODS

Thirteen healthy, weight-stable subjects (seven women and six men) participated in these studies. They had a mean age of 31.3 ± 2.8 years and a mean body mass index of 21.2 ± 0.5 kg/m^2. Each subject participated in 1–4 diet studies with a break of at least three months between diets. The protocol was approved by the Ethics Committee of the Royal Melbourne Hospital, Victoria, Australia.

Diets. The four experimental diets were designed to meet two objectives, namely, to provide a minimum of fat (<7% of total energy intake) and to be isoenergetic such that no loss of body weight occurred over the 2-wk period. An experienced research dietitian instructed each subject individually to eat a wide variety of suitable foods to ensure the nutritional adequacy and maximize the palatability of each diet. Subjects were familiar with all food items apart from the fish and kangaroo meat that were supplied. An extensive list of recipes was provided with each diet. Allowable foods included skim milk and other nonfat dairy products. Grains and cereal products such as bread and pasta were encouraged. All vegetables and fruits except avocado and olives were allowed. Legumes, except soybeans, were also encouraged. Sugars, jams, soft drinks and candies were used as a source of energy. Fats, oils, nuts, meat and fish (other than those supplied) as well as commercial foods with added fat were excluded from the diet. Due to the high bulk and low energy density of the experimental diets, subjects with a high basal energy requirement (>2500 kcal/day) found it difficult to maintain their energy intake. In an attempt to overcome this problem, high energy supplementary drinks (high carbohydrate, no fat, 250 kcal each) were recommended. Subjects were advised to have one drink after breakfast and another before retiring at night. Every subject was provided with a set of kitchen scales and standard food-record sheets. All food and beverages consumed over each 2-wk diet period plus the pre- and

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Abbreviations: PUFA, polyunsaturated fatty acids; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein.

TABLE 1

Sample 2000-kcal Low Fat Menus

3 Sample breakfasts (common to all four diets)

300 g oatmeal porridge	200 ml skim milk	200 ml orange juice
100 ml skim milk	200 g baked beans (homemade)	60 g very low fat muesli (homemade)
2 tsp sugar	2 slices wholemeal toast	150 ml skim milk
2 medium peaches		2 slices wholemeal toast
1 slice wholemeal toast		15 g nonfat cottage cheese
10 g marmalade jam		10 g yeast extract

Tropical fish	Southern fish	Kangaroo	Vegetarian
Midday meal			
250 g Barramundi, poached with green peppercorns, capers and Dijon mustard 150 g tossed salad (lettuce, onion, tomato, cucumber, radishes, peppers, large pear)	250 g Rock Ling, poached in mango sauce with dry white wine and nonfat yogurt 150 g baked potato 70 g pumpkin, steamed 60 g spinach, steamed	250 g Kangaroo in curry sauce (no oil) 100 g nonfat yogurt 200 g steamed rice ½ cantaloupe	4 slices wholemeal bread 2 tsp prepared mustard 80 g salad vegetables (tomato, mung beans, grated carrot, beetroot) 1 medium banana
Evening meal			
250 g Barramundi, chow-mein style (1 T. sherry, 1 T. soy sauce) 150 g mixed vegetables 200 g rice noodles	250 g Nanagai, grilled with lemon juice 250 ml split pea soup 1 wholemeal bread roll tossed salad (oil-free dressing)	250 g kangaroo shasliks 100 g steamed potato 70 g broccoli 60 g carrot 200 g fruit salad 100 g nonfat yogurt	1 bowl minestrone soup (no oil) 2 cups cooked spaghetti 180 g tomato and vegetable sauce 150 g tossed salad, no dressing
Snacks (in addition to above meals three snacks were eaten each day)			
(e.g.) 2 slices of bread or 1 crumpet with jam or honey	small banana plus 200 ml nonfat yogurt	400 ml carbonated soft drink or 250 ml high energy supplement drink	2 Rye Vita crackers with 20 g nonfat cottage cheese 30 g tomato plus 40 g dried apricots

postcontrol weeks were recorded. Typical 2000-kcal menus for each diets are presented in Table 1. The breakfasts and most snacks were interchangeable. Dietary analyses were performed using the Microdiet Computer Software package based on the British Food Tables (5). The fat content of the tropical fish, southern fish and kangaroo meat was determined by analysis in our laboratory (3,6).

The daily intake of saturated and monounsaturated fatty acids and linoleic acid were calculated using the Microdiet data base. The levels of the 20- and 22-carbon PUFA were calculated from our own data of the total lipid content and fatty acid composition of a variety of foods.

Experimental protocol. Each diet study ran for four weeks. Fasting blood samples were taken before the study began and at weekly intervals after for the measurement of plasma lipoprotein lipid and fatty acid compositions. During the first week the subjects remained on their usual diets, weighing and recording food intake. They began the experimental diets during the second and third weeks and returned to their usual diets for the fourth week. Weighed food-intake records were maintained throughout the four weeks. Body weights were also monitored regularly.

Lipoprotein lipid analysis. Concentrations of cholesterol and triglyceride in fasting plasma were measured enzymatically after enzymatic hydrolysis on a Cobas-B10 Centrifugal Analyser using commercially available kits (Cholesterol Enzymatic Merckotest, E. Merck, Darmstadt, FRG; Triglyceride Rapid Test, Roche, Basle, Switzerland). The normal range for cholesterol concentrations in fasting plasma is 3.5–6.0 mmol/l and for triglycerides is 0.5–2.0 mmol/l. Very low density lipoproteins (VLDL) were separated by 16-hr ultracentrifugation of plasma at 40,000 rev/min in a Beckman L-50 Ultracentrifuge. High density lipoproteins (HDL) were separated within 2 hr of blood collection from other plasma lipoproteins that had been precipitated by heparin-manganese chloride. Lipids in low density lipoproteins (LDL) were calculated from the difference between whole plasma and VLDL and HDL (7). Cholesterol concentrations were determined for all three lipoprotein fractions. Triglyceride concentrations were measured in whole plasma and VLDL only.

Plasma fatty acid analysis. Fasting blood samples were collected in heparinized tubes, and the lipids were extracted from the plasma by chloroform/methanol extraction (8). Internal standards of cholesterol heptadecanoate (Nu-Chek-Prep, Elysian, MN) and di-hepta-decanoylphosphatidylcholine (Sigma Chemical Co., St. Louis, MO) were added to the plasma samples prior to lipid extrac-

tion. The lipid extracts were separated by thin layer chromatography (1,2), and the fatty acid methyl esters of the cholesteryl ester and phospholipid fractions were formed as described previously (9). The fatty acid methyl esters were separated using an 8 m × 0.22 mm ID fused silica bonded phase (BP-20) capillary column (SGE, Melbourne, Australia). The gas liquid chromatograph was equipped with flame ionization detectors and was programmed from 100 to 190 C at 8 C/min with a helium carrier gas flow rate of 50 cm/sec. Standard methyl esters (Nu-Chek-Prep) were routinely chromatographed to determine the identity of the fatty acid methyl esters and to determine the detector response to the different esters. Quantitative response factors were determined (using Nu-Chek-Prep standards) and were used in the calculation of the data. The capillary column provided adequate separation of the following critical methyl esters: 18:3n-3 from 20:0 and 20:1; 20:5n-3 from 22:0 and 22:1; 20:4n-6 from 20:3n-6 and 20:3n-3.

Statistical analyses. The paired t-test was used to compare results within a study, and the unpaired t-test to compare results between studies. All results are expressed as mean ± SEM; significance was taken as $p < 0.05$.

TABLE 2

Dietary Composition Before, During and After the Four Experimental Diets (Mean ± SEM)

Diet	Energy (KJ)	% of total energy				Dietary fiber (g)	Cholesterol (mg)	P/S ratio[a]
		Protein	Carbohydrate	Fat	Ethanol			
Baseline	9188 ± 292	14.7 ± 0.4	43.5 ± 0.8	36.3 ± 0.9	5.5 ± 0.5	24.7 ± 1.4	277 ± 13	0.45 ± 0.04
Southern fish (10)	7871 ± 373***	27.9 ± 1.3***	59.2 ± 1.1***	5.9 ± 0.4***	7.0 ± 0.9	31.7 ± 3.8**	266 ± 4	1.78 ± 0.06***
Tropical fish (11)	7512 ± 431**	29.9 ± 1.3***	56.3 ± 1.7***	7.2 ± 0.6***	6.6 ± 1.0	28.4 ± 2.2	263 ± 5	1.18 ± 0.07***
Kangaroo meat (10)	8651 ± 545	28.7 ± 1.2***	57.8 ± 2.2***	6.9 ± 0.4***	6.6 ± 1.0	34.6 ± 3.5***	299 ± 4	1.33 ± 0.08***
Vegetarian (7)	7553 ± 885**	15.0 ± 0.6***	71.3 ± 1.9***	6.6 ± 0.6	7.1 ± 1.2	43.4 ± 4.9**	13 ± 2***	1.42 ± 0.18***
Postdiet	9632 ± 301*	14.5 ± 0.4	44.5 ± 0.9	35.7 ± 1.0	5.3 ± 0.5	23.4 ± 1.1	325 ± 22*	0.42 ± 0.03

Paired t-test comparing baseline diet 1 with the other five diets. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

[a]P/S: polyunsaturated/saturated.

TABLE 3

Cholesterol Concentration in Fasting Whole Plasma and in Lipoprotein Fractions During the Dietary Studies (mmol/1, mean ± SEM)

Diet	Baseline (day 0)	Diet (day 7)	Diet (day 14)	Post diet (day 21)
Total plasma cholesterol				
Southern fish (10)	4.54 ± 0.22	3.83 ± 0.12***	3.51 ± 0.12***	4.20 ± 0.12
Tropical fish (11)	4.75 ± 0.27	4.08 ± 0.19*	3.86 ± 0.22*	4.13 ± 0.16
Kangaroo (10)	5.22 ± 0.43	4.42 ± 0.41**	4.17 ± 0.42**	4.86 ± 0.37
Vegetarian (7)	4.54 ± 0.32	3.59 ± 0.25	3.44 ± 0.27**	4.12 ± 0.36
VLDL cholesterol				
Southern fish (10)	0.12 ± 0.02	0.09 ± 0.05	0.11 ± 0.04	0.10 ± 0.03
Tropical fish (11)	0.17 ± 0.03	0.18 ± 0.03	0.14 ± 0.03	0.17 ± 0.03
Kangaroo (8)	0.23 ± 0.05	0.31 ± 0.09	0.27 ± 0.09	0.22 ± 0.06
Vegetarian (7)	0.16 ± 0.03	0.21 ± 0.05	0.17 ± 0.03	0.13 ± 0.05
LDL cholesterol				
Southern fish (10)	3.17 ± 0.21	2.79 ± 0.12***	2.50 ± 0.15***	3.00 ± 0.13
Tropical fish (8)	3.13 ± 0.24	2.75 ± 0.14	2.57 ± 0.22	2.60 ± 0.14*
Kangaroo (8)	3.63 ± 0.51	3.07 ± 0.39*	3.05 ± 0.43**	3.22 ± 0.25
Vegetarian (7)	2.91 ± 0.28	2.29 ± 0.22	2.38 ± 0.26	2.88 ± 0.34
HDL cholesterol				
Southern fish (10)	1.26 ± 0.13	0.98 ± 0.10***	0.93 ± 0.08**	1.16 ± 0.08
Tropical fish (8)	1.53 ± 0.14	1.20 ± 0.14**	1.21 ± 0.19*	1.42 ± 0.13
Kangaroo (8)	1.48 ± 0.09	1.21 ± 0.08*	1.19 ± 0.06*	1.58 ± 0.20
Vegetarian (7)	1.35 ± 0.13	1.08 ± 0.13**	0.89 ± 0.12***	1.11 ± 0.10

Statistically significant differences from baseline day 0: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

TABLE 4

Ratio of Cholesterol in Low Density to High Density Lipoprotein Lipid Fractions During the Dietary Studies (Mean ± SEM)

Diet	Day 0	Day 7	Day 14	Day 21
Southern fish (10)	2.78 ± 0.32	3.19 ± 0.45	2.96 ± 0.49	2.75 ± 0.31
Tropical fish (8)	2.21 ± 0.21	2.40 ± 0.17	2.22 ± 0.34	1.96 ± 0.19
Kangaroo (8)	2.38 ± 0.25	2.55 ± 0.30	2.54 ± 0.26	2.15 ± 0.18
Vegetarian (7)	2.35 ± 0.29	2.39 ± 0.41	3.09 ± 0.71	2.73 ± 0.44

RESULTS

The changes in dietary composition during the dietary studies are shown in Table 2. Although there was a trend for energy intake to fall on all four diets, it was not statistically significant. However, on all diets except the kangaroo diet, energy intake increased significantly when the baseline diet was resumed, although there were no significant differences between the two baseline periods (weeks 1 and 4). The marked fall in proportion of energy derived from fat on all diets was compensated for by increased carbohydrate intake in the vegetarian diet and increased protein and carbohydrate intakes on the other three diets containing lean meat or fish. Cholesterol intake was extremely low on the vegetarian diet, but unchanged on the other three diets relative to the baseline periods. Dietary fiber increased on all diets, the effect being most marked with the vegetarian diet. The P/S ratio increased from 0.4 on the baseline diet to over 1 on the vegetarian, tropical fish and kangaroo diets and to almost 2 on the southern fish diet.

Fasting cholesterol fell 19–24% over the 2-wk period, with the most pronounced fall occurring in the first week of the diet (Table 3). The effect of the diets on cholesterol levels appeared to be equally rapidly reversible on all diets, and levels had risen significantly within one week of resuming the baseline diet for all diets except tropical fish. The fall in total cholesterol on these diets was due to reductions in both HDL and LDL cholesterol, with effects on HDL cholesterol being more consistent. The HDL-cholesterol levels fell significantly on all four diets and rose significantly within one week of resuming the baseline diet. LDL-cholesterol levels behaved in a similar fashion for all diets except tropical fish, where the fall on the diet was less pronounced (and not statistically significant) and more attenuated in that it remained low one week after resuming the baseline diet. The ratios of LDL/HDL cholesterol over the study period are shown in Table 4. Although there was a trend to higher ratios during all four diets, there were no statistically significant effects.

Triglyceride levels in fasting plasma and VLDL were not affected by the diets (Table 5). There were trends toward higher triglyceride levels on all diets; however, they were not statistically significant. The exception was on the tropical fish diet, where there was a significant increase in triglyceride levels after one week that disappeared by two weeks.

The fatty acid composition of the foods used in supplementing the low fat diets is given in Table 6. Kangaroo meat and tropical and southern Australian fish are all low in fat with a high proportion of PUFA. Kangaroo meat contains predominantly n-6 PUFA, with linoleic and arachidonic acids being the most abundant. However, it is important to note that kangaroo meat contains significant amounts of n-3 PUFA (linolenic acid and its more polyunsaturated derivatives). Tropical fish are rich in both the n-6 and n-3 PUFA, with arachidonic and docosahexaenoic acids being the most abundant. Southern Australian fish, in contrast, contain predominantly the n-3 PUFA, with docosahexaenoic acid being the most abundant.

TABLE 5

Triglyceride Concentrations in Fasting Total Plasma and VLDL During the Dietary Studies (mmol/l, mean ± SEM)

Diet	Baseline (day 0)	Diet (day 7)	Diet (day 14)	Postdiet (day 21)
Total plasma triglycerides				
Southern fish (10)	0.72 ± 0.04	0.71 ± 0.06	0.76 ± 0.04	0.62 ± 0.05
Tropical fish (11)	0.77 ± 0.10	0.89 ± 0.07*	0.84 ± 0.05	0.74 ± 0.05
Kangaroo (10)	0.85 ± 0.10	1.21 ± 0.24	1.16 ± 0.24	0.96 ± 0.13
Vegetarian (7)	0.75 ± 0.10	0.92 ± 0.10	0.94 ± 0.13	0.81 ± 0.13
VLDL triglycerides				
Southern fish (10)	0.16 ± 0.02	0.14 ± 0.05	0.16 ± 0.07	0.14 ± 0.02
Tropical fish (10)	0.25 ± 0.04	0.31 ± 0.04	0.20 ± 0.03	0.25 ± 0.04
Kangaroo (10)	0.31 ± 0.07	0.52 ± 0.17	0.46 ± 0.16	0.43 ± 0.09
Vegetarian (7)	0.24 ± 0.06	0.32 ± 0.05	0.33 ± 0.06	0.27 ± 0.05

* $p < 0.05$ (paired t-test comparing baseline diet with the other diets).

The estimated daily dietary intake of saturated, monounsaturated and the different PUFA is shown in Table 7. In the baseline periods, the intake of saturated, monounsaturated and linoleic acids was 31, 30 and 13 g/day, respectively. In all experimental periods, the levels of each of these three groups of fatty acids declined to about 2–3 g/day. The baseline diet contained 20- and 22-carbon PUFA of both the n-6 and n-3 types with arachidonic and docosahexaenoic being the most significant. The vegetarian diet contained no 20- and 22-carbon PUFA, whereas the other three low fat diets were particularly enriched in the long chain PUFA derived from the main foods characteristic of each diet; southern fish with eicosapentaenoic and docosahexaenoic acids, tropical fish with arachidonic and docosahexaenoic acids and kangaroo meat with arachidonic, eicosapentaenoic and docosapentaenoic acids.

The changes in fatty acid composition of plasma phospholipids and cholesteryl esters after two weeks on the four experimental diets is presented in Table 8. Some changes appeared to be in response to the low fat diet per se, since they occurred in all four diets, while other changes (particularly those pertaining to the long chain PUFA) were quite diet-specific. In both phospholipids and cholesteryl esters, the proportion of linoleic acid fell, while that of palmitic and palmitoleic acid rose during all diets and returned to baseline values within one week of resuming the normal diet. The changes observed in the C20 and C22 PUFA reflected the fatty acid composition of the major dietary components; the proportion of arachidonic acid increased on the two diets that were good sources of this PUFA (tropical fish and kangaroo meat); the proportion of docosahexaenoic acid rose in both plasma lipid fractions on the two diets that were good sources of this PUFA (southern fish and tropical fish); and eicosapentaenoic acid increased most markedly on the southern fish diet. The quantitatively largest changes in fatty acid composition were the fall in linoleic acid and the increases in arachidonic and docosahexaenoic acids. The fatty acid changes from day 0 to day 14 were almost completely reversed within 7 days after subjects resumed their normal diets.

DISCUSSION

In this study of the effects on lipoprotein lipids and fatty acid composition in fasting plasma during four very low fat diets enriched with different long chain PUFA, some effects (consistently observed on all four diets) appeared to be simply attributable to the low dietary fat, while others (specific to the different diets) were attributable to differences in long chain PUFA composition.

The changes in lipoprotein composition in response to all four diets were similar. There was a rapid reduction in total cholesterol within one week and a slower return toward baseline values one week after resuming the normal diet. The fall in total cholesterol was due to falls in both LDL- and HDL-cholesterol levels, with the effects being more consistent on HDL cholesterol. Indeed, there was a trend to increased LDL/HDL cholesterol ratios on all four diets, although it was not statistically significant. These results are consistent with the observations of

TABLE 6

Fatty Acid Composition of the Major Sources of Long Chain Polyunsaturated Fatty Acids in the Low Fat Diets (% Total Fatty Acids of the Fresh, Raw Food)

	Kangaroo meat	Tropical fish	Southern Australian fish
14:0	1[a]	2[b]	2[c]
16:0	15	22	18
16:1	2	4	3
18:0	12	9	6
18:1	22	15	13
18:2n-6	17	5	1
18:3n-3	5	1	—
20:1	—	—	2
20:3n-6	1	1	1
20:4n-6	7	11	4
20:5n-3	2	2	7
22:4n-6	—	2	1
22:5n-6	—	3	1
22:5n-3	2	2	2
22:6n-3	1	10	31
Total lipid	1.4%	1.0%	0.7%

[a]Other components include 16- and 18-carbon aldehydes, 17:0, 17:1, 20:2, 22:4.

[b]Other components include 16- and 18-carbon aldehydes, 15:0, 17:0, 20:1, 20:2, 22:0.

[c]Other components include 15:0, 17:0, 17:1, 18:3, 18:4, 20:2.

TABLE 7

Estimated Fatty Acid Intakes (g/day) of Baseline and Low Fat Diets

			Polyunsaturated				
Diet	Saturated	Monounsaturated	18:2n-6	18:3n-3	20:4n-6	20:5n-3	22:6n-3
Baseline diet	30.7[a]	29.5	11.42	0.86	0.07[b]	0.04	0.09
Southern fish	2.1	2.4	2.64	0.24	0.14	0.25	1.08
Tropical fish	2.5	2.3	2.40	0.26	0.56	0.11	0.51
Kangaroo meat	3.2	3.7	3.55	0.58	0.49	0.14	0.07
Vegetarian	2.2	2.4	3.14	0.37	0	0	0

[a]Values from the baseline and postdiet periods (weeks 1 and 4).

[b]Values for the 20- and 22-carbon polyunsaturated fatty acids calculated from total lipid content and fatty acid composition of foods used in this study.

TABLE 8

Fatty Acid Composition of Plasma Phospholipids and Cholesteryl Esters Before, During and After Dietary Studies (Percentage of Total Fatty Acids, Mean ± SEM, Number of Subjects in Parentheses)

	Study day[a]	16:0	16:1	18:0	18:1	18:2n-6	20:3n-6	20:4n-6	20:5n-3	22:5n-3	22:6n-3
Phospholipids											
Southern fish	0	28.2 ± 1.0	0.9 ± 0.1	12.6 ± 0.6	12.2 ± 0.5	25.7 ± 1.0	2.8 ± 0.2	10.8 ± 0.8	1.0 ± 0.1	0.9 ± 0.1	4.0 ± 0.3
(10)	14	32.5 ± 1.4**	1.3 ± 0.2	10.8 ± 0.6**	11.0 ± 0.3*	16.1 ± 0.7***	2.3 ± 0.2	12.0 ± 0.7	2.8 ± 0.3***	1.2 ± 0.1*	9.1 ± 0.4***
	21	29.4 ± 0.9***	0.9 ± 0.1	12.4 ± 0.7**	12.0 ± 0.5*	25.1 ± 0.8***	2.5 ± 0.2	9.2 ± 0.5**	1.4 ± 0.1***	0.9 ± 0.1***	5.4 ± 0.2***
Tropical fish	0	29.6 ± 1.0	1.1 ± 0.2	12.5 ± 0.4	11.7 ± 0.3	25.7 ± 1.2	2.9 ± 0.2	9.8 ± 0.7	0.8 ± 0.1	1.1 ± 0.1	3.9 ± 0.3
(9)	14	32.7 ± 1.4*	1.7 ± 0.3*	10.1 ± 0.7***	11.4 ± 0.7	15.5 ± 0.6***	3.0 ± 0.3	15.7 ± 0.1***	1.2 ± 0.1*	1.2 ± 0.1	6.6 ± 0.5***
	21	30.7 ± 1.3	1.0 ± 0.2**	11.8 ± 0.6***	11.2 ± 0.4	25.8 ± 0.9***	2.5 ± 0.2***	10.4 ± 0.6***	0.8 ± 0.1**	0.8 ± 0.1***	4.3 ± 0.4***
Kangaroo	0	28.8 ± 1.0	1.0 ± 0.1	12.1 ± 0.6	11.5 ± 0.6	26.4 ± 1.7	3.0 ± 0.2	10.8 ± 0.8	1.1 ± 0.1	1.1 ± 0.1	3.5 ± 0.4
(8)	14	33.9 ± 1.4***	1.7 ± 0.3*	10.6 ± 0.6***	11.0 ± 0.6	16.3 ± 1.1***	3.6 ± 0.3*	15.1 ± 0.8***	1.4 ± 0.1*	1.5 ± 0.1***	4.0 ± 0.5
	21	29.7 ± 1.0*	1.1 ± 0.2*	12.1 ± 0.8	12.0 ± 0.7	23.5 ± 1.2***	3.3 ± 0.2*	12.4 ± 0.6***	1.4 ± 0.2	1.4 ± 0.1	3.8 ± 0.3
Vegetarian	0	29.4 ± 0.7	1.4 ± 0.1	12.4 ± 0.4	12.8 ± 0.8	25.7 ± 0.7	2.9 ± 0.2	9.2 ± 0.6	0.9 ± 0.1	0.8 ± 0.1	3.4 ± 0.3
(10)	14	34.8 ± 1.7**	2.0 ± 0.2*	11.2 ± 0.5*	12.8 ± 0.7	20.1 ± 1.5**	3.4 ± 0.4	9.7 ± 0.6	0.6 ± 0.1**	0.8 ± 0.1	3.8 ± 0.3
	21	30.5 ± 0.6*	1.6 ± 0.2*	12.4 ± 0.5*	12.9 ± 0.7	24.1 ± 1.0**	2.8 ± 0.2	9.0 ± 0.7	1.4 ± 0.3*	0.9 ± 0.1	3.2 ± 0.2
Cholesteryl esters											
Southern fish	0	11.5 ± 0.6	3.3 ± 0.3	0.9 ± 0.1	18.1 ± 0.9	56.6 ± 1.4	0.7 ± 0.1	6.3 ± 0.6	0.9 ± 0.1		0.9 ± 0.1
(10)	14	15.4 ± 0.7***	5.1 ± 0.6**	1.2 ± 0.4	21.4 ± 1.0*	40.2 ± 2.1***	0.9 ± 0.2	9.9 ± 0.7***	3.1 ± 0.5***		2.0 ± 0.1***
	21	12.1 ± 0.5***	3.2 ± 0.4**	0.9 ± 0.1	18.7 ± 1.0*	49.2 ± 6.3	1.4 ± 0.7	6.3 ± 0.5***	1.1 ± 0.1**		1.3 ± 0.1**
Tropical fish	0	12.6 ± 0.8	3.8 ± 0.4	1.1 ± 0.1	17.9 ± 0.8	55.7 ± 1.8	0.7 ± 0.1	6.2 ± 0.8	0.8 ± 0.1		0.7 ± 0.1
(9)	14	15.1 ± 0.8**	6.8 ± 0.9**	0.9 ± 0.1	21.1 ± 0.9**	40.4 ± 1.5***	1.0 ± 0.1*	11.3 ± 0.9***	1.0 ± 0.1		1.6 ± 0.3**
	21	11.9 ± 0.6***	3.8 ± 0.5***	1.0 ± 0.1	17.4 ± 1.0*	55.4 ± 1.9***	0.6 ± 0.1*	7.3 ± 0.7***	0.7 ± 0.1**		1.1 ± 0.2**
Kangaroo	0	12.7 ± 0.8	3.8 ± 0.2	1.0 ± 0.2	19.2 ± 0.9	53.7 ± 1.8	0.7 ± 0.1	6.7 ± 0.7	0.9 ± 0.1		0.7 ± 0.1
(8)	14	16.8 ± 1.5**	6.5 ± 0.7***	1.1 ± 0.1	20.9 ± 0.8	39.3 ± 2.1***	1.2 ± 0.1***	10.8 ± 0.1***	1.3 ± 0.1***		1.3 ± 0.3*
	21	13.2 ± 0.8**	4.5 ± 0.4***	1.0 ± 0.1	19.2 ± 1.0	50.3 ± 2.1***	0.9 ± 0.1*	8.1 ± 0.6**	1.1 ± 0.1		0.8 ± 0.1
Vegetarian	0	12.3 ± 0.5	5.4 ± 0.8	1.2 ± 0.1	16.8 ± 1.1	55.6 ± 1.7	0.6 ± 0.1	5.4 ± 0.6	0.6 ± 0.1		0.7 ± 0.1
(10)	14	13.9 ± 0.4*	7.6 ± 1.2**	1.3 ± 0.3	20.8 ± 1.5**	46.4 ± 2.5***	0.8 ± 0.1	6.7 ± 0.7**	0.5 ± 0.1		1.1 ± 0.3
	21	12.0 ± 0.5*	5.6 ± 1.0*	1.5 ± 0.1	18.8 ± 1.0	53.1 ± 1.8**	0.7 ± 0.1	5.5 ± 0.4*	0.8 ± 0.1*		1.0 ± 0.3

Statistical comparisons (relative to the value above): *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

[a]Baseline, day 0; diet, day 14; postdiet, day 21.

others showing reductions in both LDL and HDL cholesterol on diets that either are low in fat (10) or have a high P/S ratio (11). It appears that monounsaturated fatty acids (oleic acid as in olive oil) are unique in specifically lowering LDL cholesterol while leaving HDL cholesterol relatively unaffected (12). In the present study, consistent reductions in plasma cholesterol occurred rapidly in normo-cholesterolemic subjects consuming low fat diets with quite different cholesterol intakes (11–316 mg/day) and appear to be explained entirely by the very low fat contents.

High carbohydrate intakes are associated with increased triglyceride and reduced HDL-cholesterol concentrations (13). In the present study, all diets were associated with consistent falls in HDL-cholesterol, but there were no significant effects on triglycerides. In view of the well-documented triglyceride-lowering effect of fish and n-3 PUFA (14), it may be significant that the greatest trends toward increased triglycerides (not statistically significant) were observed on the two diets that did not contain fish (kangaroo and vegetarian). The relatively high dietary fiber intake (from fruit and vegetables) may have also helped prevent the rise in triglycerides (15).

Another consistent finding on all diets was the substantial reduction in the percentage of linoleic acid in both the cholesteryl ester and phospholipid fractions and the increase in the palmitic acid percentage in the phospholipid and cholesteryl ester fractions and palmitoleic acid percentage in the cholesteryl ester fraction. The reductions in linoleate were evident in all subjects within seven days of starting the low fat diets and were even more marked by 14 days. Following the resumption of the normal high fat diets the percentage of linoleate returned to the prediet values. The linoleic acid content of all the low fat diets was considerably reduced compared with the normal high fat diet values, and this presumably acounts for the reduction in the percentage of linoleic acid in the plasma lipids. Some workers have estimated that the dietary requirements of linoleic acid in man are greater than the values found on the present low fat diets (16); however, there was no evidence of any significant amounts of 20:3n-9 in the plasma in these studies.

There were other changes in plasma lipid fatty acid profiles in this study that were quite diet-dependent. The three diets containing 20- and 22-carbon PUFA showed a marked alteration of fatty acid profiles, with an obvious positive relationship between the presence of particular dietary 20- and 22-carbon PUFA and the increase in the percentage of those PUFA in the phospholipid and cholesteryl ester fractions. There was no change in the percentage of 20- and 22-carbon PUFA on the vegetarian diet, suggesting that the increase of these PUFA in the plasma lipids on the other low fat diets was due to their presence in the diet.

Despite very low levels of the dietary 20- and 22-carbon PUFA (0.3–1.1 g per day), there was a significant increase in the proportion of these fatty acids in the plasma lipids. The data also show that there is a marked difference in the incorporation of dietary 18-carbon PUFA and dietary 20-and 22-carbon PUFA in man. This is particularly evident on the kangaroo diet, where the level of 18:2n-6 was twice that of 20:4n-6 in the meat, yet the percentage of 20:4n-6 rose and that of 18:2n-6 fell in the plasma lipids. This difference has been noted previously in our field studies (2). Differences between the incorporation of dietary 20:4n-6 and 22:6n-3 relative to their respective 18-carbon precursors have been noted previously in experimental animals (17–20) and also more recently in man for 20:5n-3 and 22:6n-3 compared with 18:3n-3 (21).

Since the predominant PUFA in the Western diet are 18-carbon PUFA (22), it has been assumed that most of the tissue 20- and 22-carbon PUFA originate from the conversion of the 18-carbon EFA in the liver. However, there has been some discussion as to the relative importance of dietary long chain PUFA, since it has been argued that the conversion process of 18- to 22-carbon PUFA in man is an inefficient process (23–25). The present results using very low fat diets show that small amounts of dietary 20- and 22-carbon PUFA (0.3–1.1 g/day) can have a significant effect on the plasma fatty acid composition in man.

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