The influence of a fish oil high in docosahexaenoic acid on plasma lipoprotein and vitamin E concentrations and haemostatic function in healthy male volunteers

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(*Received 20 June 1991 – Accepted 1 August 1991*)

Nine healthy male subjects consumed a daily fish oil supplement providing 2·1 g docosahexaenoic acid (22:6 *n*-3; DHA) and 0·8 g eicosapentaenoic acid (20:5 *n*-3; EPA) for 6 weeks. The proportion of EPA and DHA in plasma, erythrocytes, leucocytes and platelet phospholipids was increased by the supplement. Plasma concentration of triacylglycerol and very-low-density-lipoprotein-cholesterol were lowered and those of high-density-lipotrotein (HDL)- and HDL_2-cholesterol and apoprotein B were increased. Platelet aggregation and thromboxane B_2 production induced by collagen were partially inhibited. Both systolic and diastolic blood pressure fell during treatment and rose following withdrawal of the supplement. Statistically significant reductions in erythrocyte counts, packed cell volume and haemoglobin and increases in total leucocyte and monocyte counts occurred with the supplement. Plasma α-tocopherol concentrations fell below the normal range during the period of supplementation. It is suggested that future studies consider components other than EPA in fish oil. Further studies are needed to investigate the extent to which fish oil increases the requirement for antioxidant nutrients.

Dietary fat: Coronary risk factors: Vitamin E: Polyunsaturated fatty acids: Fish oil

Oily fish are rich sources of eicosapentaenoic acid (20:5 *n*-3; EPA) and docosahexaenoic acid (22:6 *n*-3; DHA). DHA is the major *n*-3 fatty acid in mackerel, tuna and salmon, whereas in pilchard, sardine, anchovy and herring EPA predominates (Ackman, 1982). A large number of studies have investigated the influence of fish oils on plasma lipids and platelet function (Harris, 1989). A reduction in plasma triacylglycerol and very-low-density-lipoprotein (VLDL) concentrations has been a consistent finding. Increases in high-density-lipoprotein (HDL)-cholesterol and apoprotein B have been reported in some but not all studies. Studies of platelet function have yielded more variable results (Goodnight, 1986). The majority of these studies have used oils in which EPA predominates, such as MaxEPA (Seven Seas Ltd, Hull), salmon oil or cod-liver oil. Cod-liver oil contains slightly more DHA than EPA but also has substantial amounts of vitamins A and D and $C_{20:22}$ monounsaturated fatty acids. Salmon oil contains more DHA than EPA but is high in $C_{20:22}$ monounsaturated fatty acids. McLennan *et al.* (1990) found favourable alterations in platelet function and susceptibility to cardiac arrhythmia in animals fed on tuna oil in which DHA is predominant. A few studies (Von Schacky & Weber, 1985; Fischer *et al.* 1987) have investigated the effects of ethyl esters of DHA in man but the duration of these studies, less than 1 week, and the number of subjects studied were small owing to the high cost of the DHA. No studies have examined the effects in man resulting from the consumption of a fish oil in which DHA is predominant but which is low in vitamins A and D and $C_{20:22}$ monoenes such as tuna oil. From a practical standpoint it is important to

know the nutritional effects resulting from the consumption of such oils. We report the effects of such a supplement on plasma lipids, platelet function and vitamin E status in healthy volunteers.

MATERIALS AND METHODS

Nine healthy male volunteers (aged 22–35 years) were recruited from amongst the staff and student population of King's College (Kensington Campus). Informed written consent was obtained from each subject. The protocol of the study was approved by the College Ethical Committee. The study consisted of a baseline period of 1 week, a 6-week treatment period and a follow-up 20 weeks after withdrawal of the supplement.

The subjects refrained from taking any medication, particularly non-steroidal anti-inflammatory drugs such as aspirin and anti-histamines, for at least 10 d before each blood sample was to be taken and throughout the supplementation periods. They were also asked not to drink alcohol 3 d before each blood sample. The subjects were asked not to change their diets. Subjects were questioned regarding their dietary habits but no formal weighed food inventory was made. Heights and weights (wearing minimal indoor clothing) were recorded using a beam balance.

Blood pressure was measured in the recumbent position using a random-zero sphygmomanometer (Hawksley and Sons, Lancing, Sussex): the mean value from two cuff inflations was taken. Venous blood samples (60 ml) were collected between 08.00 and 10.00 hours with minimal venous occlusion after an overnight fast from 22.00 hours the previous evening. Two sets of baseline measurements were carried out, separated by a 1-week interval. Subjects were provided with a known number of opaque soft gelatin capsules each containing 1 g of a fish oil rich in DHA (Seven Seas Ltd, Marfleet, Hull). The oil contained dodecyl gallate (100 mg/kg), a permitted antioxidant, and additional vitamin E (2 mg/g) to guard against lipid peroxidation. According to the manufacturers the peroxide value of the oil was 1·6 mmol/kg, the iodine value 202·4, unsaponifiable matter 6·8 g/kg, refractive index 1·4833, vitamin A < 15 mg/kg and polychlorinated biphenyls 2·6 mg/kg; the following trace metals: arsenic, cadmium, selenium, mercury and lead were below 1 mg/kg. The oil was analysed by gas–liquid chromatography after methylation with sodium methoxide. Subjects were instructed to take ten capsules daily with meals for 6 weeks. Further fasting blood samples were obtained and blood pressure measurements made at 3 and 6 weeks (two samples on consecutive days) and 20 weeks following withdrawal of the supplement.

Platelet aggregation induced by collagen (1 and 10 μg/ml) was measured in citrated platelet-rich plasma (PRP) adjusted to 250000 platelets/μl as previously described (Haines *et al.* 1986) before and after 6 weeks of supplementation and 20 weeks following withdrawal of the supplement. The lag phase before aggregation commenced and the rate and extent of aggregation were measured. Thromboxane B_2 (TXB_2) concentrations were measured in aggregated PRP 4 min after addition of the aggregating agent: a portion of aggregated PRP was pipetted into imidazole buffer, rapidly frozen and stored at $-22°$ until analysis by radioimmunoassay (Haines *et al.* 1986). The samples for each subject for TXB_2 concentrations were determined in the same assay to reduce between-assay variation. Full blood counts were determined on a Coulter counter by the Haematology Department at the Central Middlesex Hospital at baseline, after supplementation and 20 weeks following withdrawal of the supplement.

Platelet, erythrocyte (Haines *et al.* 1986) and leucocyte suspensions (Baron & Ahmed, 1969) were prepared. As a relatively large blood sample was needed for the preparation of leucocytes, they were only prepared on one occasion at baseline, after 6 weeks of supplementation and following withdrawal of the supplement. Phospholipid fractions were

Table 1. *Fatty acid composition of the fish-oil supplement*

Fatty acid	wt %
14:0	4·2
16:0	17·7
16:1	5·7
18:0	4·1
18:1	18·0
18:2 *n*-6	1·5
18:3 *n*-3	2·2
18:4 *n*-3	1·6
22:1	1·3
20:4 *n*-6	1·4
20:5 *n*-3	8·5
22:4 *n*-6	0·86
22:5 *n*-3	1·4
22:6 *n*-3	22·0
Others	9·54

isolated by chromatography on silica Sep-Pak cartridges (Christie, 1982). The fatty acid methyl esters were prepared by transesterification with sodium methoxide in methanol (Christie, 1982) and analysed by capillary gas–liquid chromatography using a 25 m CpSil88 column (Chromopac, UK) fitted with an injection splitter using hydrogen as a carrier gas on a Pye Model 204 Chromatograph connected to a Shimadzu CR1B integrator.

Plasma was separated from fasting blood samples anticoagulated with K EDTA (1 mg/ml) within 3 h of collection. VLDL was isolated by ultracentrifugation (Terpstra *et al.* 1981). HDL and HDL_3 fractions were prepared by sequential precipitation (Gidez *et al.* 1982). HDL and HDL_3 fractions were treated with sodium bicarbonate before measurement of cholesterol (Bachorik *et al.* 1984). Cholesterol and triacylglycerol concentrations were measured using commercially available enzymic assays (Boehringer Mannheim, Lewes). Apolipoprotein B concentrations were measured by radial immunodiffusion (Lutalo-Bosa *et al.* 1985); samples from baseline and supplementation periods were measured in the same assay but apoprotein B was not measured in the samples following withdrawal of the supplement. Low-density-lipoprotein (LDL)-cholesterol concentration was calculated as total cholesterol less VLDL- and HDL-cholesterol concentrations. The same batch of quality-control sera (Precilip, Boehringer Mannheim and OTCA Behring) was used throughout the study and the values obtained for cholesterol and triacylglycerol measurements were inside 98·5–102·5% of the stated values.

Plasma tocopherol concentrations were determined in plasma stored at −20° by high-performance liquid chromatography on a C_{18} reversed-phase column 5 μm Spherisorb with methanol–water (96:4, by vol.) as mobile phase with detection at 292 nm using retinyl acetate as an internal standard (Driskell *et al.* 1982). Samples from the same subject were analysed consecutively in order to minimize between-run variation.

Statistical analysis was carried out using the repeated measures analysis of variance module of the SPSS statistical package (SPSS/PC$^+$, 1986).

RESULTS

Three of the nine subjects participating in the study were smokers; none of the subjects admitted taking any non-steroidal anti-inflammatory drugs for 2 weeks before or during the study. One subject never consumed fish, three rarely ate fish more than twice monthly,

Table 2. *Changes in the fatty acid composition (wt % total fatty acids) of plasma, platelet, leucocyte and erythrocyte phospholipids in nine male subjects before, during (Rx) and 20 weeks after (post-Rx) treatment with fish-oil supplement**

(Mean values with their standard errors)

Fatty acid		Baseline		3 weeks Rx		6 weeks Rx		Post-Rx		Within-subject SD	Statistical significance
		Mean	SE	Mean	SE	Mean	SE	Mean	SE		
18:2 *n*-6	Plasma	24·5	0·72	21·1	0·92	20·9	1·07	22·8	1·05	2·57	$P < 0·01$
	Leucocytes	10·2	0·27	—	—	9·2	0·38	10·7	1·36	2·37	$P < 0·05$
	Platelets	6·2	0·28	6·3	0·31	6·1	0·35	6·8	0·54	0·75	NS
	Erythrocytes	12·9	0·43	11·7	0·41	12·6	0·91	12·4	0·62	1·32	NS
20:3 *n*-6	Plasma	2·6	0·19	1·37	0·22	1·42	0·11	3·3	0·19	0·47	$P < 0·01$
	Leucocytes	1·9	0·16	—	—	1·8	0·26	2·6	0·20	0·50	NS
	Platelets	1·4	0·10	1·2	0·10	1·3	0·08	1·6	0·16	0·22	NS
	Erythrocytes	1·7	0·16	1·5	0·08	1·3	0·08	1·6	0·12	0·26	$P < 0·05$
20:4 *n*-6	Plasma	8·2	0·54	8·9	0·54	8·3	0·48	9·2	0·77	1·45	NS
	Leucocytes	17·2	0·10	—	—	18·8	0·35	21·5	1·28	2·78	$P < 0·05$
	Platelets	24·7	0·85	22·5	0·45	22·5	0·40	24·0	0·64	1·64	$P < 0·01$
	Erythrocytes	14·8	0·49	14·2	0·42	12·8	0·67	12·7	0·38	2·29	$P < 0·01$
20:5 *n*-3	Plasma	1·1	0·16	4·3	0·25	4·2	0·19	1·5	0·17	0·51	$P < 0·01$
	Leucocytes	1·2	0·18	—	—	3·0	0·32	1·1	0·18	0·65	$P < 0·01$
	Platelets	1·3	0·20	2·1	0·12	2·5	0·18	0·7	0·09	0·50	$P < 0·01$
	Erythrocytes	1·2	0·10	2·5	0·09	2·6	0·26	1·2	0·11	0·42	$P < 0·01$
22:6 *n*-3	Plasma	2·2	0·25	5·4	0·30	5·6	0·33	4·8	0·38	0·57	$P < 0·01$
	Leucocytes	1·9	0·20	—	—	3·2	0·33	3·0	0·27	0·69	$P < 0·01$
	Platelets	1·3	0·13	2·4	0·10	2·9	0·09	1·5	0·14	0·36	$P < 0·01$
	Erythrocytes	4·5	0·43	5·0	0·20	5·2	0·51	3·5	0·23	1·09	$P < 0·01$

NS, not significant.

* For details, see p. 164 and Table 1.

Table 3. *Plasma lipoprotein concentrations in nine male subjects before, during (Rx) and 20 weeks after (post-Rx) treatment with fish-oil supplement**

(Mean values with their standard errors)

	Baseline		3 weeks Rx		6 weeks Rx		Post-Rx		Within-subject SD	Statistical significance
	Mean	SE	Mean	SE	Mean	SE	Mean	SE		
Total cholesterol (mmol/l)	4·77	0·165	5·02	0·155	4·71	0·151	4·54	0·215	0·378	NS
VLDL-cholesterol (mmol/l)	0·24	0·048	0·11	0·020	0·12	0·035	0·21	0·041	0·118	$P < 0·01$
LDL-cholesterol (mmol/l)	2·99	0·217	2·97	0·207	2·82	0·216	2·89	0·184	0·628	NS
HDL-cholesterol (mmol/l)	1·41	0·084	1·74	0·127	1·50	0·074	1·47	0·097	0·104	$P < 0·01$
HDL_2-cholesterol (mmol/l)	0·11	0·061	0·29	0·062	0·29	0·042	0·20	0·041	0·176	$P < 0·01$
HDL_3-cholesterol (mmol/l)	1·38	0·072	1·39	0·127	1·21	0·067	1·26	0·083	0·201	NS
Apoprotein *B* (mg/l)	970	65	1080	37	1110	53	—	—	118	$P < 0·01$
Triacylglycerols (mmol/l)	0·75	0·068	0·51	0·055	0·57	0·050	0·79	0·090	0·129	$P < 0·01$
Plasma α-tocopherol (μmol/l)	20·0	3·00	7·2	1·16	10·9	1·16	18·3	2·10	7·69	$P < 0·01$
Body-wt (kg)	68·5	1·89	—	—	69·5	1·78	69·4	1·76	1·42	NS

NS, not significant; VLDL, very-low-density-lipoprotein; LDL, low-density-lipoprotein; HDL, high-density-lipoprotein.

* For details, see p. 164 and Table 1.

e 4. *Platelet aggregation, thromboxane formation and blood pressure in nine male subjects before, during (Rx) and 20 weeks after (post-Rx) treatment with fish-oil supplement**

(Mean values with their standard errors)

		Baseline		6 weeks Rx		Post-Rx		Within-subject SD	Statistical significance
		Mean	SE	Mean	SE	Mean	SE		
Lag phase for platelet aggregation (s) induced by:	1 μg collagen	72	6·9	100	10·0	66	7·9	21·6	$P < 0·05$
	10 μg collagen	41	2·9	51	4·1	43	4·5	9·6	NS
Thromboxane B_2 formation (ng/10^8 platelets) induced by:	1 μg collagen	5·8	1·29	2·2	0·65	4·5	0·63	1·7	$P < 0·05$
	10 μg collagen	105	19·1	61	12·3	80	12·6	25·1	$P < 0·05$
Blood pressure (mm Hg):									
Systolic		116	3·9	113	5·3	118	4·1	4·2	$P < 0·01$
Diastolic		71	1·7	68	2·6	71	2·6	3·8	$P < 0·05$

NS, not significant.
* For details, see p. 164 and Table 1.

ble 5. *Blood counts in eight male subjects before, during (Rx) and 20 weeks after (post-Rx) treatment with fish-oil supplement**

(Mean values with their standard errors)

	Baseline		6 weeks Rx		Post-Rx		Within-subject SD	Statistical significance
	Mean	SE	Mean	SE	Mean	SE		
Packed cell volume (l/l)	0·46	0·005	0·44	0·006	0·48	0·006	0·020	$P < 0·01$
Erythrocytes (10^{12}/l)	5·1	0·06	4·8	0·06	5·2	0·09	0·21	$P < 0·01$
Haemoglobin (g/l)	159	2·2	149	2·2	159	3·3	5·7	$P < 0·05$
MCV (fl)	91·3	0·51	90·6	0·53	91·3	0·49	0·48	$P < 0·01$
Leucocytes (10^9/l)	4·8	0·29	5·9	0·33	5·2	0·35	0·95	$P < 0·05$
Monocytes (10^9/l)	0·36	0·030	0·53	0·010	0·38	0·030	0·070	$P < 0·01$
Lymphocytes (10^9/l)	1·6	0·13	2·0	0·10	1·8	0·12	0·40	NS
Neutrophils (10^9/l)	2·7	0·22	3·1	0·26	2·8	0·22	0·56	NS
Platelets (10^9/l)	226	14·0	203	15·2	251	23·5	44·9	NS

NS, not significant; MCV, mean corpuscular volume.
* For details, see p. 164 and Table 1.

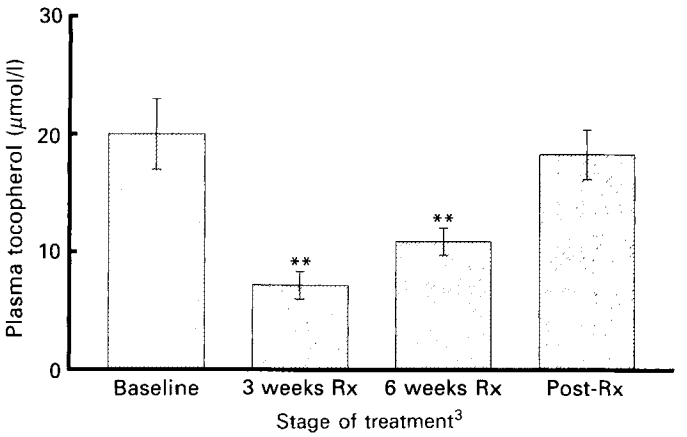

Fig. 1. Plasma vitamin E (α-tocopherol) concentrations before, during (Rx) and 20 weeks after (post-Rx) treatment with fish-oil supplement. Values are means with their standard errors represented by vertical bars. Mean values were significantly different from baseline values: ** $P < 0.01$. For details of supplement, see p. 164 and Table 1.

three usually ate fish once weekly and two subjects ate fish twice weekly or more. The subjects reported no change in their eating or drinking habits over the period of the study. Compliance to the supplement was excellent as judged by capsule count.

The fatty acid composition of the supplement is shown in Table 1. The daily supplement provided intakes of 2·1 g DHA and 0·8 g EPA. Table 2 shows the main changes in plasma, erythrocyte, platelet and leucocyte phospholipids with the supplement. The proportion of linoleic acid (18:2 *n*-6) in plasma ($P < 0.01$) and leucocyte phospholipids ($P < 0.05$) fell with the supplement but changed little in erythrocytes and platelets. The proportion of dihomo-γ-linolenic acid (20:3 *n*-6) fell significantly in plasma ($P < 0.01$) and erythrocytes ($P < 0.05$) but not in the other phospholipid fractions. The proportion of arachidonic acid (20:4 *n*-6) was significantly lower in erythrocytes and platelets ($P < 0.01$) during supplementation and increased significantly in leucocytes post-treatment ($P < 0.01$). The proportion of EPA and DHA was significantly increased ($P < 0.01$) in all fractions ($P < 0.01$) with the supplement. The increment in EPA was greater in the plasma phospholipids than in other phospholipid fractions.

Total cholesterol concentrations were not affected by the supplement. Plasma triacylglycerol and VLDL-cholesterol concentrations were significantly decreased ($P < 0.01$) and those of HDL-cholesterol, HDL_2-cholesterol and apoprotein B were significantly increased ($P < 0.01$) by the supplement (Table 3). Body-weight remained constant throughout the study.

Systolic and diastolic blood pressure decreased significantly after treatment ($P < 0.05$ and $P < 0.01$ respectively) and returned to baseline values post-treatment (Table 4). The lag phase for platelet aggregation induced by 1 μg collagen was significantly increased by the supplement ($P < 0.05$); none of the other measures of platelet aggregation was significantly altered (values not shown). TXB_2 production induced by 1 and 10 μg collagen was significantly lower after treatment.

One blood sample for full blood counts was clotted when received by the hospital carrying out the blood counts. As this sample was from the supplement period, the blood counts for this subject have not been included in the statistical analysis. The supplement led to significant increases in leucocyte ($P = 0.05$) and monocyte ($P < 0.01$) counts and a significant decrease in erythrocyte count ($P < 0.01$), haemoglobin concentration ($P <$

0·05), mean corpuscular volume ($P < 0·01$) and packed cell volume ($P < 0·01$), although levels remained inside laboratory normal ranges (Table 5). Blood counts returned to baseline values 20 weeks following withdrawal of the supplement. Plasma α-tocopherol concentrations fell markedly ($P < 0·01$) during supplementation but returned to baseline values 20 weeks following withdrawal of the supplement (Fig. 1).

DISCUSSION

The primary aim of the present study was to investigate the effects of a fish oil rich in DHA on blood lipids and haemostatic function rather than to examine the specific effects of DHA. The relative proportions of DHA and EPA in the supplement are similar to those found in several species of oily fish such as mackerel, tuna and salmon. The design of the present study can be criticized because it did not include a control group. However, two baseline measurements and follow-up measurements after withdrawal of the supplement as well as replicate measurements (for blood pressure and plasma lipids) inside the supplementation period were made. Care was also taken to minimize day-to-day variation in methods by the use of quality-control serums and by analysing the samples in batches.

As expected, the supplement led to changes in the fatty acid composition of blood phospholipids. The increase in the proportion of EPA was reversed on withdrawal of the supplement but the increase in the proportion of DHA tended to persist in plasma and leucocyte phospholipids. This finding is in agreement with earlier studies using cod-liver oil (Sanders *et al.* 1981). In a study carried out in the same laboratory employing similar methods using a fish-oil concentrate providing 1·8 g EPA/d for 3 weeks with eight male subjects, the proportion of EPA in plasma phospholipids increased from 1·1(SE 0·10) wt% to 5·1(SE 0·48) wt% after 3 weeks (T. A. B. Sanders & S. Rana., unpublished results). The amount of EPA provided by the supplement in the present study was considerably less (0·8 g/d) than this amount yet there was a substantial increase in the proportion of EPA in plasma phospholipids. It has been proposed that DHA is retroconverted to EPA (Von Schacky & Weber, 1985). Evidence supporting this has been an increased production of prostacyclin PGI_3 following acute administration of the ethyl esters of DHA (Fischer *et al.* 1987). However, the proportion of EPA found in the platelets of our subjects was similar to that predicted (2·1 wt%) from dose-response studies with MaxEPA (Sanders & Roshanai, 1983).

The supplement decreased the proportions of *n*-6 polyunsaturated fatty acids in blood phospholipids. In plasma and leucocyte phospholipids the proportions of linoleic acid and dihomo-γ-linolenic acid were decreased, whereas in erythrocyte and platelet phospholipids, the proportion of arachidonic acid (20:4 *n*-6) was decreased. This disparity may reflect differing specificities of the various acyl transferases.

The supplement led to an increase in the lag phase before platelet aggregation induced by 1 μg collagen. An increase in this variable indicates inhibition of the release of aggregatory substances such as TXB_2 and PGH_2. We made a similar observation in insulin-dependent diabetics given 15 g MaxEPA/d providing 2·5 g EPA and 1·8 g DHA (Haines *et al.* 1986). Both DHA and EPA are reversible inhibitors of cyclo-oxygenase, the key enzyme regulating prostaglandin synthesis (Corey *et al.* 1983). Although our results showing reduced TXB_2 formation are consistent with inhibition of cyclo-oxygenase, decreased TXB_2 formation could also result from a reduction in the amount of arachidonic acid released.

Slight falls in both systolic and diastolic blood pressure were observed after ingestion of the DHA concentrate, and as blood pressure returned to baseline levels following withdrawal of the supplement, it is tempting to conclude that the supplement lowered blood

pressure. Singer *et al.* (1985) reported that the consumption of mackerel (in which DHA predominates) led to a reduction of blood pressure but herring (in which EPA predominates) did not. Knapp & Fitzgerald (1989) reported that 40 g MaxEPA but not 10 g MaxEPA lowered blood pressure in hypertensive subjects. However, others (Mortensen *et al.* 1983; Rogers *et al.* 1987) in larger double-blind controlled trials have noted a reduction in blood pressure with 10 g MaxEPA providing 1·8 g EPA and 1·2 g DHA. A reduction in blood pressure might result from changes in vascular reactivity. It has been found recently that individuals who eat fish regularly have improved vascular compliance (Wahlquist *et al.* 1989). Bonaa *et al.* (1990) have reported that a fish-oil concentrate containing both EPA and DHA lowered blood pressure in subjects who seldom consumed fish. In order to confirm whether DHA lowers blood pressure it would be necessary to use a double-blind protocol.

Total plasma cholesterol concentrations were not lowered by the supplement. However, total HDL- and HDL_2-cholesterol concentrations increased with treatment. Abbey *et al.* (1990) found that fish oil (MaxEPA) but not linseed oil raised HDL_2-cholesterol in mildly hypercholesterolaemic subjects and that this change was accompanied by a decrease in lipid transfer protein activity. Sanders *et al.* (1989) were unable to note any change in HDL-cholesterol in subjects receiving a fish oil rich in EPA providing 1·8 g EPA/d. Consequently, this might suggest that DHA is responsible for the increase in HDL-cholesterol seen with some fish-oil supplements or that higher doses of *n*-3 fatty acids are required to produce this effect.

Plasma triacylglycerol and VLDL-cholesterol concentrations were lowered by the supplement. Both EPA and DHA decrease plasma triacylglycerol concentrations in the rat, and in vitro studies on human liver cell lines have shown that EPA and DHA are reversible inhibitors of hepatic triacyglycerol synthesis and secretion (Harris, 1989). Although LDL-cholesterol concentrations were unchanged, the concentration of apoprotein B was increased. This would suggest that either there is an increased conversion of VLDL to LDL or that the rate of removal of LDL was decreased. We have previously shown (Sullivan *et al.* 1986) that smaller VLDL particles are produced following fish-oil ingestion. It is well known that small VLDL particles are converted more efficiently to LDL than large particles (Kesaniemi *et al.* 1985). The recent report by Harris *et al.* (1990) showing an increased catabolic rate of VLDL following fish-oil treatment would be consistent with this observation.

Charnock *et al.* (1987) noted that rats fed on tuna oil developed yellow fat disease, which is characterized by lipofuscin deposition and inflammation of adipose tissue (steatitis), a decreased packed cell volume and an increased leucocyte count (Danse & Verschuren, 1978). The changes in blood counts reported in the present study have not been reported in subjects receiving cod-liver oil (Sanders *et al.* 1981) or MaxEPA (Mortensen *et al.* 1983; Haines *et al.* 1986; Rogers *et al.* 1987). However, van Houwelingen *et al.* (1987) also noted a fall in haemoglobin concentration in volunteers fed on a mackerel paste.

In view of these changes in haemoglobin concentrations and blood counts we measured plasma tocopherol concentrations in the subjects. These were markedly decreased by the supplement below the level of 5 mg/l (11·6 μmol/l), which is regarded as the lower limit of normality (Underwood, 1976). These changes occurred even though the oil contained additional vitamin E and synthetic antioxidants and had a low peroxide value. Vitamin E is carried mainly on the LDL (Bjorneboe *et al.* 1990). It is unlikely, therefore, that the lower levels of vitamin E can be explained by changes in lipid levels. Another possibility is that the treatment led to an expansion of plasma volume. However, there was no reduction in leucocyte or platelet count. It is, of course, possible that lipid peroxidation occurred in the capsule during storage. Studies using MaxEPA have been unable to demonstrate

reductions in plasma vitamin E concentrations below the normal range. Knapp *et al.* (1985) could find no change in plasma tocopherol concentrations in patients receiving 40 g MaxEPA/d for 6 months. Bjorneboe *et al.* (1988) noted a small but statistically significant decrease in plasma tocopherol concentrations in patients with atopic eczema receiving 10 g MaxEPA/d. We have measured plasma tocopherol concentrations in hypertriacylglycerolaemic patients who had received 10–20 g MaxEPA/d for 2 years and found them to be inside the normal range (T. A. B. Sanders and R. Saynor, unpublished results). Further studies are needed to investigate the extent to which fish-oil supplements or oily fish consumption increase the requirement for antioxidant nutrients. This may be of importance as it has been proposed that oxidative modification of LDL may lead to foam cell formation and atherogenesis (Steinberg *et al.* 1989).

It should be borne in mind that fish oil or oily fish may contain materials other than EPA that may influence the risk of cardiovascular disease, both in the saponifiable and unsaponifiable fractions. In conclusion, our results demonstrate that the consumption of a fish-oil supplement rich in DHA has a number of effects that might be of significance with regard to the development of atherosclerosis and coronary heart disease. Future studies need to consider the effects of constituents of fish oil other than EPA.

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Printed in Great Britain