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Effect on blood lipids and haemostasis of a supplement of cod-liver oil, rich in eicosapentaenoic and docosahexaenoic acids, in healthy young men

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Summary

1. Twelve healthy male subjects took a daily supplement of 20 ml of cod-liver oil for 6 weeks. This provided 1·8 g of eicosapentaenoic acid (20:5ω3) and 2·2 g of docosahexaenoic acid (22:6ω3). The effects of the supplement on blood lipids, haemostatic variables, bleeding time and plasma vitamin A and carotene were studied. In seven subjects platelet aggregation induced by adenosine 5′-pyrophosphate (ADP) was also studied.

2. The proportions of 20:5ω3 and 22:6ω3 in platelet and erythrocyte phosphoglycerides were substantially increased by the supplement mainly at the expense of ω6 polyunsaturated fatty acids.

3. Mean plasma triglyceride concentrations were reduced and those of high-density-lipoprotein (HDL) cholesterol were increased by the supplement.

4. The mean bleeding time was significantly prolonged after 3 weeks of taking the supplement, but had returned to the presupplementation value 5 weeks after withdrawal of the supplement.

5. The maximum estimated response to platelet aggregation induced by ADP was increased by the supplement.

6. The mean levels of antithrombin III (immunological) and blood pressure were lower at the end of the period of supplementation and remained so 5 weeks after withdrawal of the supplement. No significant changes in other variables were noted.

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Introduction

It has been suggested that the polyunsaturated fatty acids found in fatty fish might be of value in the prevention of coronary heart disease [1].

There are two series of essential fatty acids, the ω6 and ω3, derived from linoleic (18:2ω6) and linolenic (18:3ω3) acids respectively. Neither linoleic nor linolenic acids can be synthesized *de novo* in animal tissues, but both can undergo further desaturation and chain elongation to form C_{20-22} derivatives [2]. Moreover, no interconversion occurs between the ω6 and ω3 series. It has been argued that the C_{20} derivatives from the two series may have opposing roles in haemostasis [1].

Linoleic acid is an essential nutrient [2]: its derivatives eicosatrienoic (20:3ω6) and arachidonic (20:4ω6) acids are components of cell membranes and are precursors of prostaglandins and leucotrienes. Although proof that linolenic acid is essential is still lacking, it derivatives seem to be important: eicosapentaenoic acid (20:5ω3) is the precursor of a family of prostaglandins and leucotrienes; docosahexaenoic acid (22:6ω3) is the major C_{22} polyunsaturated fatty acid in human brain [3].

Most edible vegetable oils and margarines 'high in polyunsaturates' contain far more linoleic than linolenic acids. A high intake of these fats suppresses the conversion of linolenic into

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eicosapentaenoic and docosahexaenoic acids [4–6]. However, eicosapentaenoic and docosahexaenoic acids can also be obtained preformed by eating fatty fish. Diets containing large amounts of mackerel lead to a reduction in plasma triglyceride concentrations, an increase in high-density-lipoprotein (HDL) cholesterol concentrations [7] and the inhibition of platelet aggregation induced by submaximal doses of collagen [8]. Such diets are unlikely to be acceptable so we decided to study the effects of a fish-oil supplement on blood lipids and haemostatic function. Cod-liver oil was used because it is a readily available source of eicosapentaenoic and docosahexaenoic acids, it has been used for more than 50 years as a supplementary source of vitamins A and D, and animal experiments [9] suggest it has a marked influence on haemostatic function.

Methods

Twelve healthy male volunteer subjects were recruited from among the staff and students of Queen Elizabeth College, London. Their mean age was 23 years (range 19–31 years). They had a mean weight of 66 kg (SEM 2·9), a mean height of 173 cm (SEM 1·6) and a mean weight/height^2 of 21·8 kg/m^2 (SEM 0·58). Consent was obtained from individuals and from the College Ethical Committee for all procedures. Subjects took no medication from 2 weeks before the start until the end of the study. Two sets of baseline measurements were carried out, separated by a 1 week interval. They were then instructed to take 5 ml of cod-liver oil four times daily with meals for 6 weeks. Fresh bottles of oil were issued each week and the oil consumption of all the subjects was monitored. The oil (British Cod-Liver Oils, Marfleet, Hull, U.K.) was from the same batch and was analysed for fatty acids by gas–liquid chromatography after methylation with sodium methoxide [10]: 20 ml of the oil contained 8000 i.u. of vitamin A, 800 i.u. of vitamin D and 20 i.u. of vitamin E, 2 mg of propyl gallate and 1 mg of citric acid, according to the manufacturers. Two sets of measurements were made at the end of week 6 of supplementation. Five weeks after the subjects had stopped taking the oil a final set of measurements was made.

Heights and weights of the subjects were recorded without shoes and wearing minimum indoor clothing. Blood pressure was measured in duplicate with a random zero sphygmomanometer on each occasion that blood samples were taken. Subjects fasted overnight from 22.00 hours and venous blood samples (50 ml) were drawn with minimum venous occlusion between 09.00 and 11.00 hours the following morning. Bleeding times were measured with the Simplate device (General Diagnostics, Warner–Lambert Ltd, Eastleigh, Hants, U.K.) each time a blood sample was taken and at weekly intervals when the subjects were receiving the cod-liver oil. This device produces two standard incisions (1 mm in depth and 5 mm in length) on the anterior surface of the forearm during application of a constant congesting pressure of 40 mmHg to the upper arm with a sphygmomanometer cuff. Bleeding time was taken as the time from the incision until blood ceased to appear on a filter paper applied to the edge of the incision every 30 s.

Plasma cholesterol (CHOD/PAP method, Boehringer Manheim, Lewes, Sussex, U.K.), HDL cholesterol [11], triglycerides (fully enzymatic method, Boehringer Manheim), vitamin A and carotene [12] were measured on blood collected into ethylenediaminetetra-acetate (EDTA) (1 mg/ml). Platelet-rich plasma for lipid analyses was isolated from 20 ml of blood collected into EDTA (1 mg/ml) by centrifugation at 120 *g* for 20 min at 4°C and by washing three times with three 5 ml portions of ice-cold EDTA solution (0·1 mmol/l) containing sodium chloride (8·9 g/l). Platelet lipids were extracted twice from the washed platelet button with a total of 12 ml of chloroform/methanol (1:1, v/v) containing 50 mg of the antioxidant butylated hydroxytoluene/l of extracting solvent. Methyl esters were prepared from the lipid extracts dissolved in 0·3 ml of hexane by reaction with 0·1 ml of sodium methoxide (2 mol/l) in dry methanol for 5 min at room temperature, followed by the addition of 2·5 ml of hexane and 0·5 g of anhydrous calcium chloride. The solution containing the methyl esters was filtered and solvents were removed under a jet of nitrogen. The dry methyl esters were taken up in 50–100 μl of hexane and aliquots were analysed by gas–liquid chromatography.

Erythrocyte lipids were prepared from washed erythrocyte suspensions as described previously [4] and methyl esters were prepared and analysed by gas–liquid chromatography as for platelets. Preliminary studies showed that the separation of total phospholipids from the total lipid extracts by thin-layer chromatography (t.l.c.) on plates coated with a 0·5 mm layer of silica gel G and developed in hexane/diethyl ether (4:1, v/v, containing 500 mg of butylated hydroxytoluene/l) followed by elution and methylation of the phosphoglycerides and analysis by gas–liquid chromatography yielded similar results to those obtained above for platelets and erythrocytes.

Therefore, these values represent total platelet and erythrocyte phosphoglyceride fatty acids respectively.

Gas–liquid chromatographic analyses were made on 1·8 mm × 4 mm internal diameter glass columns packed with 10 g of Silar 10C/100 g Gas Chrom Q (100–120 mesh) (obtained through Field Instruments, Richmond, Surrey, U.K.) on a Pye Series 204 gas chromatograph equipped with flame ionization detectors; oven temperature 200°C, injection temperature 210°C, detector temperature 260°C, nitrogen carrier gas flow rate 40 ml/min. Chromatograms were quantified with a DP 88 integrator (W. G. Pye, Cambridge, U.K.) and methyl esters were identified with standards obtained from the Sigma Chemical Co. (Poole, Dorset, U.K.) and Nu-Chek Prep (Elysian, MN, U.S.A.) and through the calculation of separation factors and log plots [13]. Analyses were confirmed on a less-polar column packed with 10 g of Silar 5C/100 g of Chromosorb W HP (80–100 mesh) (Applied Science Laboratories, PA, U.S.A.) operated at 218°C.

Platelet counts were made on a Coulter Thrombocounter and leucocyte and erythrocyte counts were made on a Coulter model S counter (Coulter Instruments, Luton, Beds.). Dilute clot-lysis time [14], factor II [15], factor VII [16], factor VIII (clotting) [17], factor VIII (immunological) [18], factor X [19], antithrombin III (immunological) [18] and clottable fibrinogen [20] were measured in the Coagulation Laboratory, MRC Epidemiology and Medical Care Unit, Northwick Park Hospital. Except for dilute clot-lysis time and plasma fibrinogen assays, which were done on the fresh samples, all of these tests were carried out on samples that had been stored in liquid nitrogen; all these samples were run in one batch so as to reduce between-run variations. The same standard (Immuno, Vienna, batch R 826 S) was used throughout the study.

Platelet aggregation was studied as described by Born [21] in seven of the subjects before and after 6 weeks of taking the cod-liver oil. Platelet-rich plasma was obtained from blood collected into a solution of 31·3 g of trisodium citrate/l of water (1 part/9 parts of blood) after centrifugation (120 *g* for 10 min) at 22°C within 1 h of collecting the blood. Platelet-poor plasma was prepared by centrifuging the remaining citrated blood at 1500 *g* for 15 min at 22°C. Platelet-rich plasma was adjusted to 250 000 platelets/μl with autologous platelet-poor plasma. Platelet aggregation was measured in Payton dual-channel aggregometers (Payton Associates Ltd, Scarborough, Ontario, Canada) commencing 1 h and being completed within 3 h after the taking of the blood sample. Portions (0·5 ml) of the adjusted platelet-rich plasma were stirred at 1000 rev./min at 37°C. Transmitted light was adjusted to 90% for platelet-poor plasma and to 10% for the adjusted platelet-rich plasma. A test for the development of spontaneous aggregation was made by following for 10 min the light transmittance trace of a portion of adjusted platelet-rich plasma. Platelet aggregation induced by ADP was then studied. Portions of adjusted platelet-rich plasma (0·5 ml) were incubated at 37°C for 4 min without stirring, then 1 min with stirring to give a steady baseline after which time doses of ADP in 5 μl of sodium chloride solution (150 mmol/l: saline) were added and the response was recorded. ADP was added to give final concentrations in platelet-rich plasma of 50, 25, 5, 2·5, 2, 1·5, 1, 0·5, 0·25 and 0·1 μmol/l. The minimum dose to result in secondary aggregation was noted, and a dose–response curve for primary aggregation was plotted by computer. The response was taken as the initial maximum rate of primary aggregation after the addition of ADP (measured as the rate of change in light transmittance in arbitrary units/min) and this was plotted against log ADP concentration. This produced a sigmoid curve from which three parameters were measured: the estimated maximum response; the ED_{50} (the dose of ADP giving half this maximum response); the 'scaled slope' (the slope of the steepest part of the sigmoid divided by the maximum response), indicating the rate of increase of aggregation with ADP dose at the ED_{50}.

Statistical analyses were performed by analysis of variance for comparison of the results obtained before, during and after supplementation. Two sample comparisons were made with a paired-sample *t*-test.

Results

Table 1 shows the fatty acid composition of the cod-liver oil fed to the subjects. Allowing for its density and non-fatty acid components, it was calculated that 20 ml of the oil provided 1·8 g of eicosapentaenoic acid (20:5ω3) and 2·2 g of docosahexaenoic acid (22:6ω3).

After 6 weeks of taking the oil, the proportions of 20:5ω3 and 22:6ω3 were substantially increased (both $P < 0{\cdot}01$) in both platelet and erythrocyte phosphoglycerides mainly at the expense of ω6 polyunsaturated fatty acids (Tables 2, 3). Five weeks after the supplement had been withdrawn, the fatty acid composition of the platelet phosphoglycerides had almost returned to its initial pattern whereas that of the erythrocytes remained altered.

TABLE 1. *Fatty acid composition of the cod-liver oil fed to the subjects*

Fatty acids	Total fatty acids by weight (%)
14:0	3·7
16:0	10·7
16:1	8·7
18:0	2·7
18:1	22·4
18:2ω6	0·7
18:3ω3	0·5
20:0	1·3
20:1ω11	11·5
20:5ω3	10·3
22:1ω11	7·0
22:5ω3	1·7
22:6ω3	12·5

Plasma total cholesterol, vitamin A and carotene concentrations were not significantly altered, but HDL cholesterol concentrations were increased and triglyceride concentrations were reduced by the supplement ($P < 0·01$ and $<0·02$ respectively). After withdrawing the supplement, both HDL cholesterol and plasma triglyceride concentrations assumed their initial values (Table 4).

Bleeding time was prolonged after 3 weeks of the cod-liver oil supplement ($P < 0·01$) and remained so until the end of the period of supplementation (Fig. 1). The mean bleeding time approached its initial value 5 weeks after the withdrawal of the supplement. The estimated maximum response to platelet aggregation in-

TABLE 2. *Platelet phosphoglyceride fatty acid composition in 12 male subjects before, during and after taking a daily 20 ml supplement of cod-liver oil*

Results are means ± SEM.

Fatty acid	Total fatty acids by weight (%)			*P*
	Before supplement	During supplement	After supplement	
16:0	13·6 ± 0·71	13·5 ± 0·69	13·1 ± 0·71	N.S.
16:1 + 17:0	1·0 ± 0·14	1·1 ± 0·07	1·0 ± 0·06	N.S.
18:0	20·6 ± 0·44	20·0 ± 0·27	20·0 ± 0·30	N.S.
18:1	17·5 ± 0·49	17·5 ± 0·48	16·9 ± 0·37	N.S.
18:2ω6 + 20:0*	7·3 ± 0·34	7·2 ± 0·33	8·1 ± 0·47	N.S.
20:1	1·0 ± 0·06	1·6 ± 0·12	1·2 ± 0·06	<0·01
20:2 + 22:0	0·3 ± 0·03	0·3 ± 0·03	0·3 ± 0·03	N.S.
20:3ω6 + 22:1†	2·1 ± 0·15	1·9 ± 0·05	2·2 ± 0·13	N.S.
20:4ω6	28·8 ± 0·82	24·4 ± 0·62	27·2 ± 0·40	<0·01
20:5ω3	0·6 ± 0·04	3·2 ± 0·27	1·1 ± 0·19	<0·01
22:4ω6	2·3 ± 0·12	1·3 ± 0·12	2·1 ± 0·15	<0·01
22:5ω6	0·15 ± 0·02	0·09 ± 0·020	0·15 ± 0·019	N.S.
22:5ω3	3·0 ± 0·41	3·2 ± 0·20	2·8 ± 0·11	N.S.
22:6ω3	2·6 ± 0·28	4·6 ± 0·25	3·2 ± 0·21	<0·01

* Mainly 18:2ω6.
† Mainly 20:3ω6.

TABLE 3. *Erythrocyte phosphoglyceride fatty acid composition in 12 male subjects before, during and after taking a daily supplement of 20 ml of cod-liver oil*

Results are means ± SEM

Fatty acid	Total fatty acids by weight (%)			*P*
	Before supplement	During supplement	After supplement	
16:0	19·0 ± 0·26	18·6 ± 0·62	17·5 ± 0·74	N.S.
16:1 + 17:0	1·4 ± 0·18	0·8 ± 0·03	0·7 ± 0·05	<0·01
18:0	16·1 ± 0·37	16·9 ± 0·15	17·1 ± 0·22	<0·05
18:1	16·4 ± 0·34	14·9 ± 0·34	15·0 ± 0·35	<0·01
18:2ω6 + 20:0*	12·7 ± 0·40	11·1 ± 0·41	12·6 ± 0·43	<0·01
20:1	0·6 ± 0·11	0·5 ± 0·03	0·5 ± 0·06	N.S.
20:2 + 22:0	0·3 ± 0·04	0·2 ± 0·01	0·2 ± 0·01	N.S.
20:3ω6 + 22:1†	1·8 ± 0·09	1·5 ± 0·06	1·8 ± 0·01	<0·01
20:4ω6	17·1 ± 0·47	15·1 ± 0·29	15·1 ± 0·41	<0·01
20:5ω3	1·1 ± 0·12	3·7 ± 0·20	2·9 ± 0·21	<0·01
22:4ω6	3·2 ± 0·19	2·8 ± 0·17	2·6 ± 0·19	<0·01
22:5ω6	0·2 ± 0·04	0·3 ± 0·04	0·3 ± 0·04	N.S.
22:5ω3	3·7 ± 0·15	4·6 ± 0·25	4·7 ± 0·20	<0·01
22:6ω3	6·0 ± 0·29	8·6 ± 0·21	8·8 ± 0·29	<0·01

* Mainly 18:2ω6.
† Mainly 20:3ω6.

TABLE 4. *Plasma vitamin A, carotene, triglyceride, and total and HDL cholesterol concentrations in 12 male subjects before, during and after taking a daily 20 ml supplement of cod-liver oil*

Results are means ± SEM.

	Before supplement	During supplement	After supplement	*P*
Vitamin A (μmol/l)	1·26 ± 0·093	1·19 ± 0·063	—	N.S.
Carotene (μmol/l)	2·09 ± 0·222	1·94 ± 0·207	—	N.S.
Total cholesterol (mmol/l)	4·32 ± 0·296	4·13 ± 0·195	3·85 ± 0·179	N.S.
HDL cholesterol (mmol/l)	1·35 ± 0·065	1·48 ± 0·06	1·30 ± 0·094	<0·01
Triglycerides (mmol/l)	0·89 ± 0·116	0·69 ± 0·105	0·94 ± 0·139	<0·02

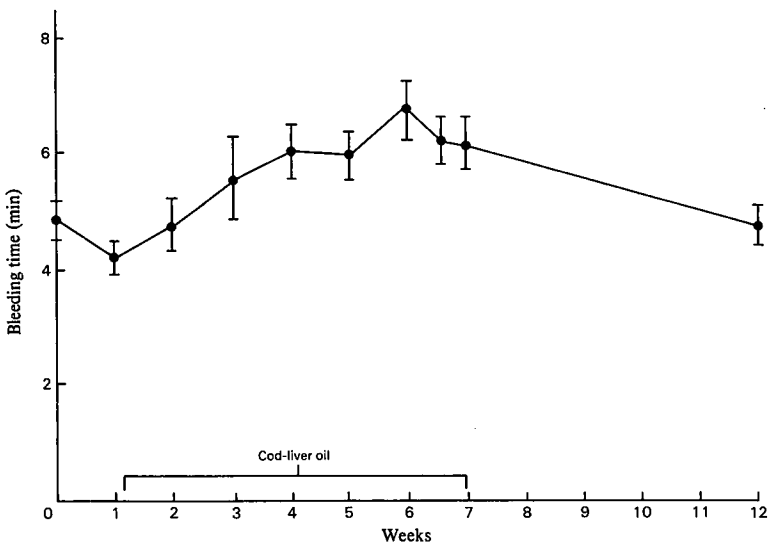

FIG. 1. Change in bleeding time with cod-liver oil supplement in 12 male subjects. The points represent means ± SEM.

TABLE 5. *ADP-induced aggregation of platelets in seven male subjects before and after 6 weeks of supplementation with cod-liver oil*

Results are means ± SEM. Significance of difference between means: * $P < 0·01$.

	Before supplement	After supplement
Estimated max. response (Δ light transmittance in arbitrary units/min)	75·5 ± 3·88	93·4 ± 3·97*
Scale slope (Δ light transmittance per minute per log ADP concn.)	2·52 ± 0·267	3·37 ± 0·357
ED_{50} (ADP 10^{-7} mol/l)	19·7 ± 3·53	19·9 ± 2·91
Min. dose of ADP required for secondary aggregation (10^{-7})	61·0 ± 24·6	42·1 ± 7·3

duced by ADP (Table 5) was greater after the cod-liver oil supplement ($P < 0·01$), but the minimum dose required to promote secondary aggregation was not altered significantly. No subject showed any spontaneous aggregation either before or after the supplement.

The level of antithrombin III (immunological) and both systolic and diastolic blood pressure were substantially lower after the subjects had taken the supplement for 6 weeks and remained so after its withdrawal (all $P < 0·01$; Table 6). There was no evidence of changes in leucocyte and erythrocyte counts, dilute clot-lysis time, plasma fibrinogen, factors II, VII, VIII (clotting), VIII (immunological) and X and body weight.

Discussion

The changes in platelet and erythrocyte phosphoglyceride fatty acid composition confirm that the subjects were taking the supplement. The increase in the proportions of eicosapentaenoic ($20:5\omega3$) and docosahexaenoic ($22:6\omega3$) acids were surprisingly large. Similar changes have been reported in the platelet lipids of subjects fed 500–800 g of mackerel/day for 1 week [8]. In

TABLE 6. *Haemostatic variables, blood pressures and body weights in 12 male subjects before, during and after taking a daily 20 ml supplement of cod-liver oil*

Results are means ± SEM.

Variable	Before supplement	During supplement	After supplement	*P*
Erythrocyte count (10^{12}/l)	4·97 ± 0·085	4·83 ± 0·060	4·80 ± 0·077	N.S.
Leucocyte count (10^9/l)	6·1 ± 0·26	6·2 ± 0·21	6·4 ± 0·45	N.S.
Platelet count (10^9/l)	251 ± 10·8	245 ± 10·3	245 ± 12·9	N.S.
Bleeding time (*min*)	4·5 ± 0·25	6·3 ± 0·30	4·9 ± 0·31	<0·01
Dilute clot-lysis time (h)	8·7 ± 0·9	8·2 ± 1·6	8·1 ± 1·7	N.S.
Fibrinogen (g/l)	2·46 ± 0·130	2·28 ± 0·084	2·27 ± 0·092	N.S.
Factor II (%)	97 ± 4·0	92 ± 3·7	88 ± 6·3	N.S.
Factor VII (%)	79 ± 2·7	82 ± 3·3	74 ± 5·6	N.S.
Factor VIII clotting (%)	114 ± 7·3	121 ± 11·8	97 ± 14·6	N.S.
Factor VIII immunological (%)	78 ± 7·6	78 ± 6·3	80 ± 15·8	N.S.
Factor X (%)	92 ± 3·7	94 ± 3·2	88 ± 2·7	N.S.
Antithrombin III (%)	126 ± 4·6	106 ± 3·0	101 ± 6·2	<0·01
Systolic BP (mmHg)	124 ± 2·4	111 ± 1·6	115 ± 2·8	<0·01
Diastolic BP (mmHg)	81 ± 1·5	69 ± 1·2	71 ± 2·2	<0·01
Weight (kg)	65·7 ± 2·01	66·2 ± 1·91	66·8 ± 2·9	N.S.

this latter study it was estimated that their daily intake of 20:5ω3 was 7–11 g, approximately five times that of our subjects. This either implies that smaller amounts of eicosapentaenoic acid consumed over a long period have an equally marked effect on platelet lipids or that Siess *et al.* [8] overestimated the amount of eicosapentaenoic acid provided by the mackerel. The latter seems likely as the fat content of mackerel varies greatly by season [22]. Moreover, we have observed similar changes in platelet phosphoglycerides in subjects after consuming a fish-oil supplement containing 2·5 g of eicosapentaenoic acid/day for 2 weeks [6]. Farquhar & Ahrens [23] estimated that it takes 4–5 weeks to alter the fatty acid composition of erythrocyte lipids. However, we have noted a large increase in eicosapentaenoic acid mainly at the expense of linoleic acid (18:2ω6) in the erythrocyte lipids of subjects fed a fish-oil supplement after 1 week (T. A. B. Sanders & K. M. Younger, unpublished work). The fatty acid composition of the platelets reverted to the presupplement level 5 weeks after withdrawing the supplement, whereas that of the erythrocytes remained altered. This difference probably reflects the higher rate of turnover of platelets compared with erythrocytes.

Bleeding time is believed to measure the combined reaction of platelets and the vessel wall. It is believed to be controlled by metabolites of arachidonic acid (20:4ω6) with opposing effects: thromboxane, TxA_2, which is produced in the platelets, is a potent aggregating agent; prostacyclin, PGI_2, which is produced in the vascular endothelium, inhibits platelet aggregation [24]. It is possible, therefore, that the prolonged bleeding time observed after 3 weeks of the cod-liver oil supplement was caused by a change in the balance between thromboxane and prostacyclin in favour of prostacyclin.

As the proportion of arachidonic acid (20:4ω6) in the platelet lipids was only slightly lower on the cod-liver oil, it cannot be concluded that the prolonged bleeding time was caused by a shortage of substrate for TxA_2 formation. Dyerberg *et al.* [1] suggested that the high intake of eicosapentaenoic acid by Greenland Eskimos was responsible for their protracted bleeding times. It was suggested that eicosapentaenoic acid was converted into an inactive thromboxane, TxA_3, and an active prostacyclin, PGI_3. However, more recent studies have shown TxA_3 to be weakly pro-aggregatory and that eicosapentaenoic acid is a poor substrate for cyclo-oxygenase [25]. Nevertheless, eicosapentaenoic acid does inhibit TxA_2 formation and platelet aggregation induced by low doses of collagen *in vitro* [25, 26]. Seiss *et al.* [8] also found evidence to suggest that TxA_2 formation and platelet aggregation induced by 1 μg of collagen, but not by 10 μg of collagen or ADP, were inhibited in his subjects who were fed mackerel. The estimated maximum response to ADP was increased by the cod-liver oil supplement. This parameter of platelet aggregation was not measured in other studies of the effects of eicosapentaenoic acid. We do not yet know what pathophysiological importance, if any, should be attributed to the change in the estimated maximum response.

The supplement resulted in lower levels of antithrombin III measured by immunological assay. Lower levels have been reported in a group of vegans and vegetarians compared with non-vegetarians [27]. Vegans and vegetarians also tend to have high intakes of polyunsaturated fatty acids, mainly as linoleic acid [4]. The level of

antithrombin III (immunological) may be affected by diet and this deserves further study. Although individuals with congenital antithrombin III deficiency have a high risk of venous and arterial thrombosis [28], a low level may reflect a reduced requirement for this protective mechanism. Patients who have had coronary heart disease or who are at high risk tend to have higher levels than those at low risk [29, 30].

Although both systolic and diastolic blood pressures fell after taking the supplement and remained lower after its withdrawal, it cannot be concluded that this change was caused by the supplement. It is well known that lower values are obtained on repeated measurement of blood pressure as the subjects become habituated to the measurement. A double-blind cross-over study would be necessary to determine whether cod-liver oil affects blood pressure.

Our finding that plasma vitamin A and total cholesterol concentrations were unaffected at this level of supplementation is in accord with the earlier studies of Josephs [31] and Kingsbury, Aylott, Morgan & Emmerson [32]. The rise in plasma HDL cholesterol and fall in plasma triglyceride concentrations were probably not fortuitous or seasonal as they were reversed when the supplement was withdrawn. This could not be attributed to changes in body weight, which was not significantly altered by the supplement. Moreover, similar changes have been reported in monks who substituted 200 g of mackerel for 150 g of Gouda cheese in their daily diet for 3 weeks [7] and these changes were attributed to the docosahexaenoic and eicosapentaenoic acids provided by the mackerel. This amount of mackerel, depending on the time of year it was caught, would provide 2·2 g of eicosapentaenoic acid (range 0·7–2·6 g) and 3·6 g of docosahexaenoic acid (range 1·1–4·5 g) according to the tables of Southgate & Paul [22]; amounts similar to those provided by our cod-liver oil supplement. All our subjects had plasma lipid concentrations inside the 'normal' range. The effect of a fish-oil supplement on subjects with hypertriglyceridaemia deserves study.

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