Effect of varying the ratio of n−6 to n−3 fatty acids by increasing the dietary intake of α-linolenic acid, eicosapentaenoic and docosahexaenoic acid, or both on fibrinogen and clotting factors VII and XII in persons aged 45–70 y: the OPTILIP Study[1–3]

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ABSTRACT

Background: Elevated fibrinogen, activated factor XII (FXIIa), and factor VII coagulant activity (FVIIc) are associated with higher risk of fatal ischemic heart disease. This study tested the hypothesis that lowering the dietary ratio of n−6 to n−3 polyunsaturated fatty acids (n−6:n−3) would modify these risk factors in older men and women.

Objective: The objective of the study was to measure fasting hemostatic risk factors and postprandial changes in activated FVII (FVIIa) concentrations after a 6-mo alteration in dietary n−6:n−3.

Design: In a randomized, parallel design in 258 subjects aged 45–70 y, we compared 4 diets providing 6% of energy as polyunsaturated fatty acids at an n−6:n−3 between 5:1 and 3:1 with a control diet that had an n−6:n−3 of 10:1. The diets were enriched in α-linolenic acid, eicosapentaenoic (EPA) and docosahexaenoic (DHA) acid, or both.

Results: Fasting and 3-h plasma triacylglycerol concentrations were 11.1% and 7.2% lower with the diet that had an n−6:n−3 of ≈3:1 and that was enriched with EPA and DHA than with the other diets. Fasting fibrinogen, FXIIa, FVIIc, FVIIa, and FVII antigen and postprandial FVIIa were not influenced by the diets. Avoiding foods high in fat the day before measurement decreased FVIIc and FVIIa by 8% and 19.2%, respectively. A test meal containing 50 g fat resulted in a mean 47% (95% CI: 42%, 52%) increase in FVIIa 6 h later, but the response did not differ by n−6:n−3.

Conclusion: Decreasing the n−6:n−3 to ≈3:1 by increasing the intake of EPA and DHA lowers fasting and postprandial plasma triacylglycerol concentrations in older persons but does not influence hemostatic risk factors. *Am J Clin Nutr* 2006;84:513–22.

KEY WORDS Factor VII, factor XII, n−3 fatty acids, ratio of n−6 to n−3 fatty acids, triacylglycerol, nonesterified fatty acids, fibrinogen

INTRODUCTION

The role of hemostatic function in determining the risk of acute coronary syndromes is now well recognized (1). The Northwick Park Heart Study (2) and the Prospective Cardiovascular Munster Study (3) found high fibrinogen concentrations and factor VII coagulant activity (FVIIc) to be associated with a higher risk of fatal ischemic heart disease (IHD) than were low concentrations and activities. Fasting FVIIc is positively associated (4) with plasma cholesterol and triacylglycerol concentrations. FVIIc and fibrinogen increase with age, especially in postmenopausal women, and are positively associated with obesity (5, 6). Controlled feeding studies found that total fat intake acutely influenced FVIIc (7), and a subsequent cross-sectional study found FVIIc activity to be related to recent fat intake in middle-aged men (8). FVIIc is dependent on the concentration of both FVII zymogen and activated FVII (FVIIa). Factor VII zymogen concentration, which is usually measured as FVII antigen (FVIIag), is partly determined by genetic factors, particularly the FVII R353Q polymorphism (9), but it is known to increase with age. FVIIa accounts for ≈1–2% of circulating FVII, but this proportion can increase after a high intake of fat. It was shown in middle-aged men and women that FVIIc increased by ≈10% after a standardized test meal containing 50 g fat and that this increase was attributable to an ≈35% increase in FVIIa concentration, whereas there was no increase in FVIIag (10). The mechanism by which postprandial lipemia increases FVII activation is uncertain, but it may have to do with the activation of the contact system of coagulation by lipid exchange reactions in the postprandial period (11, 12). The contact system can activate FVII via the activation of factor XII (FXII), and activated FXII (FXIIa) was found in the Northwick Park Heart Study to be associated

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with a greater risk of IHD (13). The lipolysis of triacylglycerol-rich lipoproteins has been shown to activate FXII (14). Consequently, changes in postprandial triacylglycerol and nonesterified fatty acid (NEFA) concentrations could influence the extent to which FXII is activated.

Increasing the intake of n−3 fatty acids may decrease the risk of fatal IHD (15, 16), particularly in older persons, who are those most at risk. The Quantification of the Optimal n−6/n−3 Ratio in the UK Diet (OPTILIP) Study was designed to assess the effects of lowering the ratio of dietary n−6:n−3 on cardiovascular disease (CVD) risk factors in older persons. This objective was achieved by using a food-based intervention that involved increasing the intake of α-linolenic acid (ALA; 18:3n−3), an n−3 long-chain polyunsaturated fatty acid (LC-PUFA) [notably, eicosapentaenoic acid (EPA; 20:5n−3) or docosahexaenoic acid (DHA; 22:6n−3)], or both in relation to the intake of linoleic acid (LA). The results for insulin sensitivity and lipoproteins are reported elsewhere (BA Griffin, et al, unpublished observations, 2006). This report presents results for fasting fibrinogen, FXIIa, and FVII and the postprandial increase in FVIIa in response to a standardized high-fat meal.

SUBJECTS AND METHODS

Subjects

Men and postmenopausal women aged 45–70 y were primarily recruited from general practices participating in the UK Medical Research Council General Practice Research Framework in the towns of Camberley and North Mymms. An additional 29 subjects were recruited from among the staff of King's College London and its associated hospitals. Exclusion criteria were body mass index (BMI; kg/m^2) <20 or >35; fasting serum cholesterol > 8 mmol/L or triacylglycerol > 6.0 mmol/L; abnormal liver function or hematologic tests; a clinical history of cholestatic liver disease, pancreatitis, diabetes mellitus, or myocardial infarction; and current use of anticoagulants other than aspirin. In the younger women, postmenopausal status—defined as a span of ≥1 y since menstruation—was confirmed by measurement of the serum concentration of follicle-stimulating hormone. Subjects taking blood pressure– or lipid-lowering medication were eligible if their medication regimens were stable. Suitable subjects were identified from general practice records and were invited by letter to take part in the study. Subjects from King's College London were invited by a broadcast e-mail to take part in the study. To further assess eligibility for the study, we asked subjects to complete a health and food questionnaire identical to that used in the European Prospective Investigation in Cancer study (17) and to attend a screening clinic. Blood samples were collected after an overnight fast for liver function tests, follicle-stimulating hormone, lipids, plasma glucose, insulin, and routine hematologic tests. Blood pressure (Omron 705CP; Omron Healthcare Inc, Milton Keynes, United Kingdom) was recorded with an automated sphygmomanometer, height without shoes and weight in minimum indoor clothing were measured with a stadiometer and beam balance, respectively. Eligible subjects were then requested to make a 24-h urine collection for the measurement of urinary cotinine and microalbumin concentrations and to complete a 7-d dietary record before beginning the study. The subjects made another urine collection and completed a second dietary record at the end of the study.

The study participants received a modest financial reimbursement for their participation in the study. They were provided at regular intervals with some foods [yellow fat spreads (ie, butter, margarine, and low-fat spreads), oil, and fish] for the dietary intervention period.

Participants gave written informed consent. The study protocol was reviewed and approved by the Human Research Ethics Committee of King's College London and the research ethics committees of the East and North Hertfordshire Hospitals and North West Surrey.

Study design

The study used a randomized parallel design comparing 4 dietary treatments with a control; the duration of the intervention was 6 mo. The 4 dietary treatments were the n−3 LC-PUFA diet, the high linolenate diet, the n−3 LC-PUFA + linolenate diet, and the moderate linolenate diet. The baseline characteristics of the subjects by allocation to dietary treatments are shown in **Table 1**. Subjects were studied in 3 cohorts over a 3-y period. The diets were designed to keep the intake of saturated and monounsaturated fatty acids constant and to provide ≈6% of energy from PUFAs with an n−6:n−3 of 10:1 (control diet), 5:1, or 3:1 when the n−3 fatty acids were provided predominantly as either ALA, n−3 LC-PUFAs (mainly EPA and DHA), or both.

The dietary calculations were based on the consumption of 20 g fat as yellow fat spreads providing 16 g fat/d and 16 g as cooking oil/d. The subjects were provided with oils and spreads for the duration of the study. The LA and ALA contents of the spreads were 39.6 and 0.5 g/100 g in the control diet, 16.9 and 5.0 g/100 g in the high linolenate diet, and 4.7 and 0.8 g/100 g in the moderate linolenate diet. The spreads used in the n−3 LC-PUFA–enriched diets contained 11.8 g/100 g LA, 5.3 g/100 g ALA, and 2.0 g/100g n−3 LC-PUFA. Subjects following the control and n−3 LC-PUFA–enriched diets were provided with high-oleic sunflower oil (LA, 10.6 g/100 g; ALA, 0.3 g/100 g), and those following the high linolenate, n−3 + linolenate, and moderate linolenate diets were provided with rapeseed (canola) oil (LA, 19.7 g/100 g; ALA, 8.9 g/100 g). The spreads were manufactured for the study by Unilever Research (Vlaardingen, Netherlands), and the vegetable oils were purchased in a single batch from Anglia Oils (Hull, United Kingdom).

In addition to the 0.4 g n−3 LC-PUFA/d provided by the spread, subjects allocated to the higher intakes of n−3 LC-PUFA (the n−3 LC-PUFA and n−3 + linolenate diets) were advised to increase their consumption of oily fish, particularly salmon, which is estimated to supply 0.9 g n−3 LC-PUFA/d. These subjects were provided with tinned salmon (John West Ltd, Liverpool, United Kingdom) and salmon pâté (Arctic Fjord; Mills DA, Oslo, Norway) and asked to consume 2 portions of oily fish a week. The tinned salmon and salmon pâté had an n−3 LC-PUFA of 1.6 g/100 g. As an incentive, the subjects were also reimbursed the cost of any other oily fish consumed. The groups not receiving advice to increase their intake of n−3 LC-PUFA were provided with 2 small cans of tuna/wk; the tuna was canned in olive oil in the control diet and in the high linolenate diet and was canned in high-linoleic sunflower oil in the moderate linolenate diet (John West Ltd). The drained canned tuna had an n−3 LC-PUFA content of 0.1 g/100 g. Otherwise, the subjects maintained their habitual diets.

TABLE 1
Baseline characteristics of the subjects according to allocation to dietary treatment[1]

	Control diet (n = 44)	n−3 LCP diet (n = 61)	High linolenate diet (n = 53)	n−3 LC-PUFA + linolenate diet (n = 51)	Moderate linolenate diet (n = 49)
Age (y)	58 (46–70)[2]	62 (46–70)	60 (46–70)	61 (46–68)	61 (45–70)
Male [n (%)]	30 (68)	35 (57)	32 (60)	24 (47)	31 (63)
Female [n (%)]	14 (32)	26 (43)	21 (40)	27 (53)	18 (37)
Current smokers [n (%)][3]	7 (16)	8 (13)	7 (13)	9 (18)	11 (22)
Current HRT user [n (%)][4]	6 (43)	11 (42)	9 (43)	9 (18)	6 (12)
Impaired fasting glucose [n (%)][5]	12 (25)	11 (18)	15 (28)	11 (22)	14 (28)
Fasting insulin >70 pmol/L [n (%)]	14 (32)	20 (32)	21 (39)	18 (35)	20 (41)
Metabolic syndrome[6] [n (%)]	12 (27)	17 (28)	20 (38)	7 (14)	16 (33)
BMI (kg/m^2)	26.4 ± 2.9[7]	25.6 ± 3.3	26.2 ± 3.6	25.9 ± 3.2	26.4 ± 3.4
Systolic BP (mm Hg)	126.2 ± 19.3	127.2 ± 21.0	125.7 ± 20.8	131.2 ± 19.2	128.3 ± 15.9
Diastolic BP (mm Hg)	75.6 ± 10.7	75.8 ± 9.5	74.2 ± 12.1	77.6 ± 10.6	76.0 ± 10.4
Serum cholesterol (mmol/L)	5.6 ± 0.9	5.6 ± 0.8	5.7 ± 1.0	5.6 ± 0.9	5.6 ± 0.9
Serum LDL (mmol/L)	3.3 ± 0.9	3.4 ± 0.8	3.5 ± 0.9	3.5 ± 0.8	3.5 ± 0.9
Serum HDL (mmol/L)	1.5 ± 0.4	1.5 ± 0.5	1.4 ± 0.4	1.5 ± 0.4	1.4 ± 0.4
Serum triacylglycerol (mmol/L)	1.2 ± 0.8	1.2 ± 0.7	1.4 ± 0.8	1.1 ± 0.6	1.3 ± 0.8
10-y risk of IHD (%)[8]	12.9 ± 7.6	15.1 ± 8.1	15.6 ± 8.7	16.9 ± 8.0	17.2 ± 8.2

[1] LC-PUFA, long-chain polyunsaturated fatty acid; HRT, hormone (estrogen) replacement therapy; BP, blood pressure; IHD, ischemic heart disease. No significant differences were found between groups.
[2] $\bar{x}$; range in parentheses (all such values).
[3] Smoking status was confirmed by urinary cotinine measurements.
[4] In female subjects only.
[5] Glucose >6.1 and < 7.0 mmol/L.
[6] Impaired fasting glucose > 6.1 mmol/L or fasting insulin >70 pmol/L and ≥ 2 of the following: BP >130/85, dyslipidemia (HDL <1.05 mmol/L for men to <1.29 mmol/L for women or serum triacyglycerol >1.7 mmol/L), obesity (BMI >30 or waist-hip ratio >0.9 for men and 0.85 for women), or microalbuminuria >3.5 mg/μmol Cr.
[7] Geometric $\bar{x}$ ± SD (all such values).
[8] Based on an algorithm cited from the Framingham Study (18).

Subjects were asked to make 7-d weighed food intake records at baseline and toward the end of the study. During the preexperiment period, the subjects were issued scales and were instructed how to weigh and record in a food diary all food and drink consumed for 7 d; this record was to function as a measurement of their nutrient intake at baseline. On completion of the diary, an investigator checked the food record with each volunteer to clarify that the information was comprehensible. Dietary energy and nutrient intakes were calculated from the UK Nutrient Data Base (The Stationery Office, Norwich, United Kingdom) and by using the US Food Composition Table (SR14; US Department of Agriculture, Washington, DC), the latter of which gives more detailed composition of fish and meat products. These data were supplemented with information provided by food retailers and by chemical analyses of the foods provided.

Fasting venous blood samples were collected twice at baseline and twice at the end of the study for measurement of blood lipids and clotting factors. An additional fasting blood sample for erythrocyte lipids was obtained after 3 mo of dietary treatment to assess compliance to the dietary advice. Subjects were asked to fast from 2200, and blood samples were collected between 0800 and 1000 on the next day. The second blood samples at baseline and follow-up were collected after subjects were advised to avoid foods high in fat on the previous day and to abstain from strenuous exercise; subjects were given a list of foods to avoid and were provided with a frozen low-fat (<10 g fat) evening meal to facilitate compliance with the dietary advice. After the second blood samples at baseline and follow-up were drawn, the subjects consumed a test meal and then further blood samples were obtained at 2, 3, and 6 h after the meal. The test meal consisted of a muffin (85 g) and a strawberry-flavored milkshake (350 mL) that provided 50 g fat (14.8 g saturated fatty acids, 28.2 g oleic acid, 3.4 g LA, and 0.2 g ALA), 17 g protein, and 75 g carbohydrate within 15 min, which was followed by a 200-mL glass of water. The following ingredients were used to make the muffin: 30 g test high-oleic sunflower oil, 10 g wheat flour, 5 g cornstarch, 5 g cocoa powder, 15 g sugar, 20 mL skimmed milk, 2 g pasteurized egg white, 2 g vanilla essence, and 2 g baking powder. The muffins were baked in batches and stored frozen for up to 6 mo. The milkshake consisted of 100 mL light cream (18% butter fat), 220 mL skimmed milk, 30 g liquid glucose polymer (Polycal; Nutricia Clinical, Trowbridge, United Kingdom), and 18 g milkshake mix (Nesquik; Nestlé, Croydon, United Kingdom).

After the 3-h blood sample was drawn, subjects were provided with a standardized low-fat meal (1.7 MJ) consisting of a piece of fruit, a low-fat yogurt (<1g fat), and a glass of water. Subjects were advised to avoid any strenuous activity throughout the study period but were allowed to leave the clinic to return to home or work between blood samples.

Laboratory methods

Blood was drawn into Vacutainer tubes (Becton Dickinson, Oxford, United Kingdom) by the application of minimal compression when necessary for display of the vein. For measurement of fibrinogen, FXIIa, FVIIc, FVIIag, and FVIIa, blood was

TABLE 2

Dietary intakes at baseline and after a 6-mo dietary intervention containing additional n−3 fatty acids as linolenic acid or n−3 long-chain polyunsaturated fatty acids (LC-PUFAs) or both[1]

	Control diet (n = 38)		n−3 LC-PUFA diet (n = 58)		High linolenate diet (n = 51)		n−3 LC-PUFA + linolenate diet (n = 47)		Moderate linolenate diet (n = 40)	
	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up
Energy (MJ/d)	9.02 ± 1.84	8.99 ± 1.95	8.38 ± 1.83	8.70 ± 1.94	8.83 ± 1.83	8.76 ± 1.94	9.02 ± 1.85	9.19 ± 1.96	8.89 ± 1.84	8.73 ± 1.95
Protein (% of energy)	16.1 ± 2.9	16.4 ± 3.1	16.5 ± 2.9	16.3 ± 3.1	16.0 ± 2.9	16.4 ± 3.1	16.8 ± 2.9	16.5 ± 3.1	16.3 ± 2.9	16.4 ± 3.1
Carbohydrate (% of energy)[2]	43.5 ± 6.7	41.0 ± 6.7	44.5 ± 6.8	41.3 ± 6.8	45.8 ± 6.6	42.3 ± 6.7	45.5 ± 6.7	42.6 ± 6.7	44.3 ± 6.6	41.8 ± 6.7
Fiber (g/d)	21.5 ± 7.9	21.1 ± 7.5	21.6 ± 7.9	21.5 ± 7.5	21.8 ± 7.9	20.0 ± 7.5	22.7 ± 8.0	22.4 ± 7.5	20.6 ± 7.9	19.4 ± 7.5
Alcohol (g/d)	14.8 ± 16.6	16.1 ± 17.0	17.2 ± 16.5	15.9 ± 17.0	16.7 ± 16.5	16.8 ± 17.0	19.5 ± 16.7	18.2 ± 17.1	15.6 ± 16.5	14.6 ± 17.0
Fat (% of energy)[2]	35.7 ± 5.8	37.4 ± 5.9	32.6 ± 5.9	36.5 ± 6.0	32.8 ± 5.8	36.1 ± 5.9	32.0 ± 5.8	35.5 ± 5.9	34.3 ± 5.8	37.0 ± 5.9
SFA (% of energy)[2]	13.7 ± 3.3	12.2 ± 2.6	11.7 ± 3.3	11.5 ± 2.6	11.8 ± 3.3	11.5 ± 2.6	12.1 ± 3.3	11.2 ± 2.6	12.8 ± 3.3	11.9 ± 2.6
MUFA (% of energy)[2]	11.8 ± 2.3	14.4 ± 2.7	11.0 ± 2.4	13.4 ± 2.9	11.9 ± 2.5	14.2 ± 3.0	11.3 ± 2.4	14.4 ± 2.8	11.8 ± 2.2	15.1 ± 2.7
PUFA (% of energy)[3]	6.0 ± 1.7	8.2 ± 1.8[a]	5.7 ± 1.7	6.4 ± 1.8[b]	5.7 ± 1.7	6.8 ± 1.8[b]	5.6 ± 1.7	6.6 ± 1.8[b]	5.9 ± 1.7	6.9 ± 1.8[b]
n−6 (% of energy)[4]	4.7 ± 1.6	7.0 ± 1.6[a]	4.3 ± 1.6	4.4 ± 1.6[b]	4.4 ± 1.6	5.1 ± 1.6[b,c]	4.5 ± 1.6	4.2 ± 1.6[b]	4.7 ± 1.6	5.4 ± 1.6[c]
n−3 (% of energy)[4]	0.8 ± 0.3	0.7 ± 0.5[a]	0.8 ± 0.3	1.6 ± 0.5[b]	0.7 ± 0.3	1.2 ± 0.5[c]	0.6 ± 0.3	1.8 ± 0.5[b]	0.7 ± 0.3	0.9 ± 0.5[a,c]
Linoleic (% of energy)[4]	4.7 ± 1.6	7.0 ± 1.6[a]	4.3 ± 1.6	4.4 ± 1.6[b]	4.4 ± 1.6	5.1 ± 1.6[b,c]	4.5 ± 1.6	4.2 ± 1.6[b]	4.7 ± 1.6	5.4 ± 1.6[c]
Linolenic (% of energy)[4]	0.6 ± 0.2	0.5 ± 0.4[a]	0.5 ± 0.2	1.0 ± 0.4[b,c]	0.5 ± 0.2	1.1 ± 0.4[c,d]	0.5 ± 0.2	1.2 ± 0.4[d]	0.5 ± 0.2	0.7 ± 0.4[a,e]
n−3 LC-PUFA (% of energy)[4]	0.18 ± 0.23	0.18 ± 0.25[a]	0.27 ± 0.23	0.67 ± 0.25[b]	0.20 ± 0.23	0.16 ± 0.25[a]	0.14 ± 0.23	0.68 ± 0.25[b]	0.21 ± 0.23	0.19 ± 0.25[a]
n−6:n−3[4]	7.1 ± 4.4	11.4 ± 2.3[a]	6.7 ± 4.3	2.9 ± 2.3[b]	6.8 ± 4.3	4.4 ± 2.3[c]	8.3 ± 4.4	2.4 ± 2.3[b]	8.0 ± 4.3	6.6 ± 2.3[d]

[1] All values are $\bar{x}$ ± SD (adjusted for sex). SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. There were no significant diet × sex interactions (analysis of covariance) . Values in a row with different superscript letters are significantly different, $P < 0.05$ (Bonferroni adjustment for comparisons).

[2] Overall effect of follow-up versus baseline, $P = 0.0001$.

[3,4] Time × treatment interaction: [3]$P = 0.023$, [4]$P = 0.00001$.

collected into trisodium citrate. Only an atraumatic venipuncture was accepted. Blood (4.5 mL) was collected in 0.5 mL of 0.105 mol trisodium citrate/L (Vacutainer 367691; Becton Dickinson) and kept at room temperature until completion of centrifugation (1500 × g for 15 min at room temperature). Plasma was pipetted in 0.25-mL aliquots in polypropylene cryovials and rapidly frozen and stored at −70 °C until assayed. Blood for lipid analyses was collected into EDTA (Vacutainer 17644; Becton Dickinson), and plasma was separated by centrifugation at 1500 × g for 15 min at 4 °C and stored at −40 °C until analyzed. The remaining packed cells were washed with saline, the white blood cells were removed, and lipid extracts were prepared and stored in solvents containing 50 mg butylated hydroxytoluene/L at −20 °C until analyzed.

Plasma concentrations of triacylglycerol and NEFAs were measured by enzymatic assays and with the use of an autoanalyzer (ACE; Alfa Wassermann, Woerden, Netherlands). Reagents were obtained from Randox Laboratories (Crumlin, United Kingdom). Erythrocyte phosphoglyceride fatty acid composition was measured by using capillary gas liquid chromatography (18). Fibrinogen, FVIIc, FVIIag, and FVIIa were measured as described previously (10); FXIIa was measured by using an enzyme-linked immunosorbent capture assay with a monoclonal antibody to FXIIa (Axis-Shield Diagnostics Ltd, Kimbolton, United Kingdom); samples from the same subject were analyzed in the same run to minimize between-assay variations. Urinary cotinine was measured by using an enzyme-linked immunosorbent assay with horseradish peroxidase–labeled cotinine (Cozart Diagnostics, Abingdon, United Kingdom). Urinary microalbumin was measured by using an immunoturbidometric assay, and creatinine concentrations were measured by using the Jaffé reaction on an Advia 1650 analyzer (Bayer Diagnostics, Newbury, United Kingdom).

Statistical analysis

Data were log normalized before statistical analysis, and outliers (±3 SD) were excluded from the analysis to stabilize the variance. The number of outliers for fibrinogen, FVIIc, FVIIag, and FVIIa were 3, 4, 2, and 3, respectively. Data for plasma triacylglycerol and NEFAs were analyzed as log values by using repeated-measures analysis of variance. Changes after treatment were analyzed as the difference in log-transformed data and are expressed as the percentage increase or decrease (95% CI); probabilities were adjusted for multiple comparisons. When appropriate, probabilities were adjusted by analysis of covariance for smoking, BMI, age, and sex. Data for FXIIa could not be transformed to a normal distribution, and they were analyzed by using the nonparametric Kruskal-Wallis test.

RESULTS

We screened a total of 354 subjects and excluded 46 subjects because they did not meet the inclusion criteria. The remaining subjects were randomly assigned to 1 of 5 diets; subjects living together were allocated to the same treatment. Forty-four subjects withdrew from the study for personal reasons (eg, family commitments or a change in work circumstances) or because of an inability or unwillingness to comply with the requirements of the study; 4 subjects withdrew for reasons of ill health (cancer, 2; mental illness, 1; orthopedic surgery, 1). A total of 258 subjects (88 from Camberley, 141 from North Mymms, and 29 from King's College London) satisfactorily completed the 6-mo dietary intervention. The dietary intakes of the subjects at baseline and after receiving the dietary advice are shown in **Table 2**. Most (76%) of the subjects had serum cholesterol > 5.0 mmol/L, 16% had low HDL (<1.0 mmol/L for men and < 1.2 mmol/L for women), 41% had blood pressure > 140/90 mm Hg, and 16% were smokers (and their habit remained stable throughout the study as assessed by urinary cotinine excretion). Only 11% of the subjects had none of these risk factors for IHD; 43% had 1 risk factor, 34% had 2 risk factors, and 11% had ≥3 risk factors. The 10-y risk of IHD according to the Framingham Study score cited in an Adult Treatment Panel III Study (19) was 17.5% in the men and 18.5% in the women, and it did not differ significantly according to allocation to treatment. According to World Health Organization criteria cited by Zimmet et al (20), 28% of the subjects were classified as having the metabolic syndrome. The intake of alcohol was not normally distributed; most of the subjects were nondrinkers or occasional drinkers. However, 39% of the men and 12% of the women ($n = 54$) consumed amounts of alcohol above the UK recommended safe limits (210 mL/wk for women and 280 mL/wk for men). In most of these subjects, serum γ-glutamyl transferase activity was within the normal range (<55 units/L). However, 5 of these 54 subjects had mild elevations of γ-glutamyl transferase (<120 units/L) but no other abnormalities on their liver function tests.

Of the 258 subjects in the trial, 234 satisfactorily completed both baseline and endpoint weighed 7-d intake records. PUFAs provided ≈5.8% of the dietary energy and the n−6:n−3 in the diet was 8:1, which was slightly lower than anticipated. After subjects received the dietary advice, their intake of PUFAs was 1% higher than the 6% of energy that was planned. Intakes of n−3 LC-PUFA and LA were close to those planned, but the intake of ALA in the rest of the diet (ie, other than from fish, yellow spreads, and oils) was slightly higher than anticipated. Compared with baseline, estimates of total fat intakes as a proportion of the dietary energy were greater (3.1% of energy; 95% CI: 2.4%, 3.8%; $P < 0.000001$), and the proportion of carbohydrate was correspondingly lower (3.0% of energy; 95% CI: 2.4, 3.6; $P < 0.00001$) after the dietary advice, but the percentages did not differ between treatments. Intakes of saturated fatty acids were significantly lower at follow-up than at baseline (−0.7% of energy; 95% CI: 1.1, −0.4; $P < 0.0001$), and those of monounsaturated fatty acids were significantly higher (2.7% of energy; 95% CI: 2.4, 3.1; $P < 0.0001$), but the diet × follow-up interactions were not significant. The diet × follow-up interaction was statistically significant ($P = 0.023$) for the proportion of energy derived from PUFAs when intake was greater in the control group than in the other dietary groups. This difference resulted from a higher intake of LA in the control group than in the other groups. The proportion of energy derived from n−3 fatty acids did not differ significantly from baseline in the control group, but it was significantly higher than baseline in all of the dietary intervention groups. The ALA in the 2 groups receiving additional n−3 LC-PUFA differed significantly between the 2 groups and also between each of those groups and the control group, but not between the 2 groups and the high linolenate diet group.

Analysis of the erythrocyte phosphoglycerides showed a significant increase in the proportion of EPA and DHA with the n−3 + LC-PUFA diet but not with the diets containing only additional LA (**Figure 1**). The proportion of EPA in erythrocyte lipids decreased slightly from baseline in the control group. Overall body weight increased slightly but significantly over the study period ($\bar{x}$: 0.8 kg; 95% CI: 0.5, 1.1; $P < 0.0001$), but the

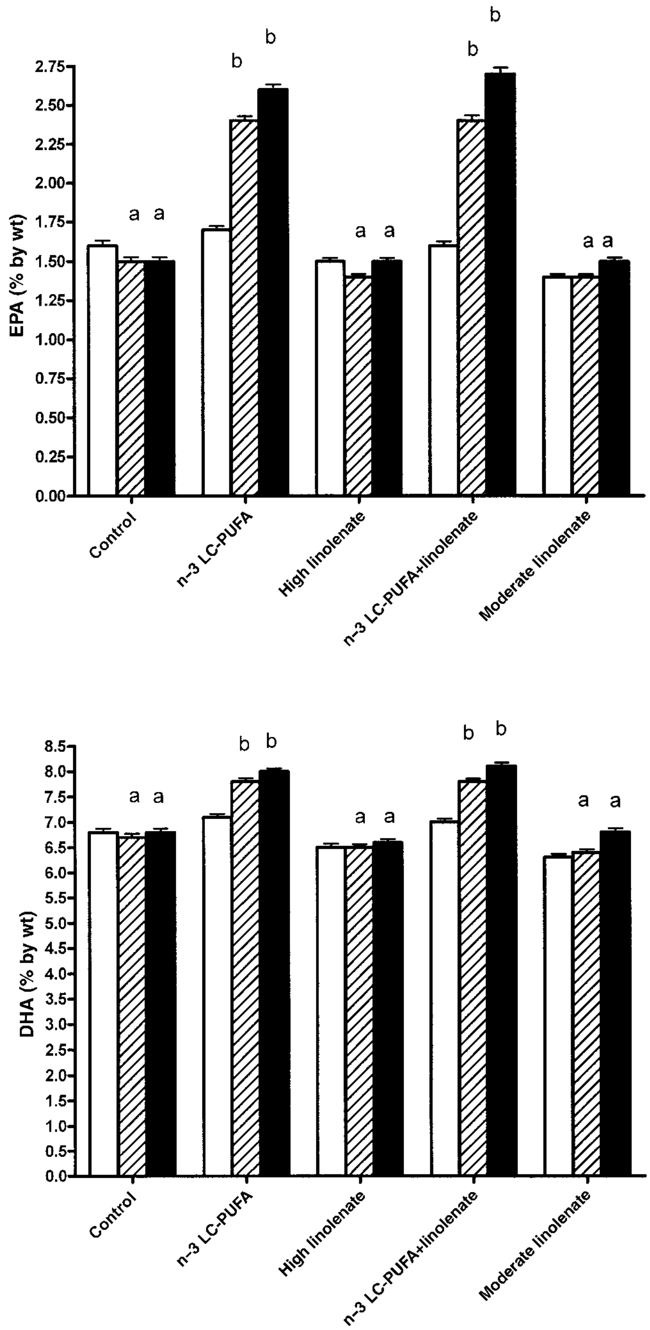

FIGURE 1. Mean (95% CI) proportions of eicosapentaenoic (EPA) and docosahexaenoic (DHA) acid in erythrocyte lipids during the dietary treatments. □, Baseline; ▨, 3 mo of treatment; ■, 6 mo of treatment. The groups did not differ significantly at baseline. The diet × time (baseline, 3 mo, or 6 mo) interaction was significant for EPA and DHA ($P < 0.0001$ for both, repeated-measures ANOVA); bars with different letters are significantly different, $P < 0.01$ (Bonferroni multiple-comparison test).

changes in body weight did not differ significantly between treatments. The fasting and postprandial plasma triacylglycerol concentrations are shown in **Table 3**. The n−3 LC-PUFA intake × follow-up interaction was significant ($P = 0.003$). The comparison of the results of the combined n−3 LC-PUFA treatments and of the diets containing no additional n−3 LC-PUFA are shown in **Table 4**. Overall plasma triacylglycerol concentrations were lower in the groups that received additional n−3 LC-PUFA

TABLE 3
Postprandial changes in plasma triacylglycerol concentration at baseline and follow-up with the different dietary treatments[1]

Treatment	Fasting	2 h	3 h	6 h
	Follow-up			
	mmol/L			
Control (n = 48)				
Baseline	1.28 ± 0.45	1.61 ± 0.64	1.89 ± 0.73	1.82 ± 1.08
Follow-up	1.20 ± 0.40	1.70 ± 0.64	1.98 ± 0.73	1.99 ± 1.13
n−3 LC-PUFA (n = 54)				
Baseline	1.38 ± 0.62	1.90 ± 0.82	2.21 ± 0.94	2.07 ± 1.15
Follow-up	1.19 ± 0.52	1.72 ± 0.68	1.99 ± 0.85	2.02 ± 1.07
High linolenate (n = 44)				
Baseline	1.40 ± 0.68	2.06 ± 0.81	2.36 ± 0.98	2.19 ± 1.22
Follow-up	1.33 ± 0.61	2.01 ± 0.78	2.29 ± 1.00	2.21 ± 1.42
n−3 LC-PUFA + linolenate (n = 44)				
Baseline	1.21 ± 0.44	1.66 ± 1.45	1.91 ± 0.78	1.63 ± 0.82
Follow-up	1.06 ± 0.37	1.66 ± 0.65	1.78 ± 0.71	1.55 ± 0.70
Moderate linolenate (n = 44)				
Baseline	1.35 ± 0.58	1.97 ± 0.85	2.14 ± 0.89	2.16 ± 1.15
Follow-up	1.36 ± 0.60	1.97 ± 0.83	2.30 ± 0.93	2.22 ± 1.19

[1] All values are geometric $\bar{x}$ ± SD (adjusted for age, sex, and BMI). LC-PUFA, long-chain polyunsaturated fatty acid. Data were log transformed and analyzed by 4-factor (follow-up, time, n−3 LC-PUFA, and 3 concentrations of linolenate) repeated-measures ANOVA. There was a significant effect of time ($P < 0.0001$) and a significant n−3 LC-PUFA × follow-up interaction ($P = 0.003$) but no significant linolenate × follow-up interaction ($P = 0.67$).

treatments than in those that did not. Plasma concentrations of NEFA decreased from the fasting value at 2 h and then rose after the test meal (**Figure 2**), but neither fasting values nor the postprandial response differed significantly between dietary treatments.

The results for fibrinogen, FVIIag, and FXIIa, none of which were affected by the dietary intervention, are shown in **Table 5**. However, at baseline, fibrinogen concentration was influenced by age, alcohol intake, and serum cholesterol concentration; after adjustment for age, sex, and BMI, plasma fibrinogen concentrations were 5.5% lower (95% CI: −9.6%, −1.3%; $P = 0.01$) in subjects consuming >1 unit alcohol/d (10 mL ethanol) than in those consuming < 1 unit/d. At baseline, fasting FVIIag concentrations (Table 5) were positively correlated with those of log plasma triacylglycerol ($r = 0.189$, $P = 0.003$) and cholesterol ($r = 0.259$, $P < 0.001$). Fasting FVIIc was positively correlated with plasma cholesterol ($r = 0.2205$, $P < 0.001$), log plasma triacylglycerol ($r = 0.206$, $P = 0.001$), and BMI ($r = 0.214$, $P = 0.001$). FXIIa was positively correlated with FVIIag ($\rho = 0.283$, $P < 0.001$). FXIIa concentrations were significantly higher in smokers than in nonsmokers [median: 1.35 ng/mL; interquartile range: 0.55, 2.54 in smokers (n = 40); median: 0.42 ng/mL; interquartile range: 0.70, 1.37 in nonsmokers (n = 214)], but no relation between alcohol intake and FXIIa concentration was found.

The results for FVII in the fasting state, which did not differ between treatments, are shown in **Table 6**. FVIIag concentrations did not differ significantly. Fasting values for FVIIc and FVIIa were 8% (95% CI: −9.4%, −6.4%; $P < 0.00001$) and 19.2% (95% CI: −21.9%, 16.4%; $P < 0.0001$) lower, respectively, before the test meal than before the other corresponding fasting measurements at baseline and follow-up. Subjects had been advised to restrict their fat intake on the day before the test meal, whereas no restriction was imposed before the other fasting measurement. The postprandial changes in FVIIa, which increased from the fasting value by a mean of 47% (95% CI: 42%, 52%) at 6 h but which were not influenced by the dietary treatments, are shown in **Table 7**. The results for fibrinogen, FXIIa,

TABLE 4
Postprandial changes in plasma triacylglycerol concentrations at baseline and follow-up in subjects according to whether they received additional dietary n−3 long-chain polyunsaturated fatty acids (LC-PUFA)[1]

	Fasting	2 h	3 h	6 h
	mmol/L			
n−3 LC-PUFA (n = 98)				
Baseline	1.38 ± 0.62	1.90 ± 0.82	2.21 ± 0.94	2.07 ± 1.15
Follow-up	1.19 ± 0.52	1.72 ± 0.68	1.99 ± 1.07	2.02 ± 1.07
No n−3 LC-PUFA (n = 126)				
Baseline	1.28 ± 0.45	1.61 ± 0.64	1.89 ± 0.73	1.82 ± 1.08
Follow-up	1.20 ± 0.40	1.70 ± 0.64	1.98 ± 1.13	1.99 ± 1.13

[1] All values are geometric $\bar{x}$ ± SD (adjusted for age, sex, and BMI). Data were log transformed and analyzed by 4-factor (follow-up, time, n−3 LC-PUFA, and 3 concentrations of linolenate) repeated-measures ANOV A. There was a significant effect of time ($P < 0.0001$) and a significant follow-up × n−3 LC-PUFA interaction ($P = 0.003$). Changes in fasting values between groups compared with analysis of covariance adjusted for age, sex, and BMI differed significantly between diets ($P = 0.009$).

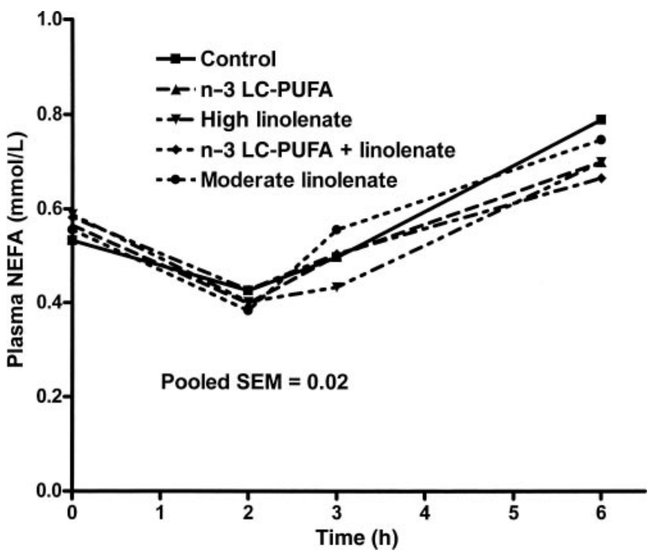

FIGURE 2. Geometric mean (95% CI) plasma nonesterified fatty acid (NEFA) concentrations after the test meal that followed the 6-mo dietary intervention (after adjustment for baseline value, age, sex, and body mass index). Data were analyzed by repeated-measures ANOVA of the log data. NEFA concentrations changed significantly over time ($P < 0.001$); however, the diet × time × follow-up interaction was not significant ($P = 0.21$), and the differences between diets at baseline were not significant.

and measures of FVII according to whether the subjects received additional n−3 LC-PUFA, after adjustment for concentration of dietary linoleate, are shown in **Table 8**; no significant differences were observed.

DISCUSSION

The aim of the current study was to ascertain the effects on hemostatic factors of manipulating the dietary n−6:n−3 in the context of the UK diet. The study was undertaken in men and women aged 45–70 y who by virtue of their age are at substantial risk of CVD. The subjects had an estimated 18% risk of CVD within 10 y. In the designing of this study, it was recognized that a low intake of LA is associated with a higher risk of IHD (21) and that rates of CVD have fallen in the United Kingdom as the ratio of PUFAs to saturated fatty acids has increased. Consequently, the evaluation of diets providing < 4% of energy as LA was excluded. Estimates of household food consumption have indicated that the consumption of PUFAs (mainly LA) increased from ≈4% in the 1970s to 7% of the dietary energy in the 2000 National Food Survey (22); the n−6:n−3 in the diet is estimated to have risen from ≈3:1 to ≈10:1 (23). On the basis of epidemiologic evidence, we hypothesized that the optimal n−6:n−3 would be ≈3:1. The current study was designed to test whether this ratio could be achieved in persons who increased their intake of ALA or n−3 LC-PUFAs or both while maintaining relatively constant intakes of saturated and monounsaturated fatty acids. Estimates of dietary intakes at 6 mo showed that they were close to those planned, even though the baseline estimates of total fat intake were slightly lower than had been estimated when planning the study. Measurements of the proportions of EPA and DHA in erythrocyte lipids indicated that compliance with the dietary advice was good.

Although numerous studies have shown that a greater intake of n−3 LC-PUFA decreases both fasting and postprandial triacylglycerol concentrations (24), most studies have used intakes of >3 g/d, as dietary supplements, and have been of relatively short

TABLE 5
Comparison of fasting plasma fibrinogen, factor VII antigen (FVIIag), and activated factor XII (FXIIa) concentrations at baseline and after a 6-mo dietary intervention[1]

	Diet					
	Control	n−3 LC-PUFA	High linolenate	n−3 LC-PUFA + linolenate	Moderate linolenate	P^2
Fibrinogen (g/L)						
n	44	61	52	51	49	
Baseline	3.22 ± 0.54[3]	3.13 ± 0.54	3.16 ± 0.57	3.25 ± 0.52	3.27 ± 0.60	
Follow-up	3.18 ± 0.63	3.17 ± 0.55	3.25 ± 0.60	3.19 ± 0.57	3.28 ± 0.50	
Percentage change (%)	−0.4 (−5.7, 5.3)[4]	0.7 (−3.6, 5.2)	2.8 (−2.0, 7.8)	−1.5 (−6.1, 3.2)	−0.1 (−5.0, 5.1)	0.87
FVIIag (%)						
n	43	57	46	49	47	
Baseline	135.4 ± 38.6	126 ± 38.2	127.5 ± 38.1	127.2 ± 40.8	136.5 ± 39.3	
Follow-up	136.6 ± 44.3	124.5 ± 43.1	123.7 ± 38.0	131.1 ± 41.6	136.7 ± 39.5	
Percentage change (%)	0.90 (−5.7, 7.9)	−1.20 (−6.6, 4.6)	−2.90 (−8.0, 2.4)	3.00 (−2.0, 8.2)	0.10 (−5.1, 5.7)	0.65
FXIIa (μg/L)[5]						
n	43	60	48	51	47	
Baseline	0.68 (0.36, 1.63)	1.63 (0.88, 0.43)	0.43 (1.49, 0.85)	0.85 (0.57, 1.36)	1.36 (0.67, 0.46)	
Follow-up	0.70 (0.35, 1.31)	1.31 (0.71, 0.37)	0.37 (1.36, 0.76)	0.76 (0.50, 1.30)	1.30 (0.70, 0.32)	
Change	0 (−0.32, 0.14)	−0.06 (−0.23, 0.08)	−0.11 (−0.30, 0.11)	−0.02 (−0.18, 0.08)	0.04 (−0.11, 0.22)	0.11

[1] LC-PUFA, long-chain polyunsaturated fatty acid.
[2] Analysis of covariance with adjustments for age, BMI, sex, and baseline value with the use of log-transformed data.
[3] Geometric $\bar{x}$ ± SD (all such values).
[4] $\bar{x}$; 95% CI in parentheses (all such values).
[5] Data were not normally distributed, and statistical analysis was by Kruskal-Wallis test: median values with interquartile range. The diets did not differ significantly at baseline.

TABLE 6
Fasting factor VII coagulant (FVIIc) activity and activated FVII (FVIIa) concentrations at baseline and at follow-up after a 6-mo dietary intervention[1]

	Diet				
	Control	n−3 LC-PUFA	High linolenate	n−3 LC-PUFA + linolenate	Moderate linolenate
FVIIc (%)[2]					
Background diet					
n	40	57	48	47	45
Baseline	121.6 ± 32.4[3]	115.4 ± 29.9	122.1 ± 36	117.5 ± 31.4	120.4 ± 33.1
Follow-up	125.9 ± 35.0	119.7 ± 30.1	122.4 ± 34.4	121.7 ± 31.0	125.2 ± 31.9
Percentage change (%)	3.50 (−2.6, 10.1)[4]	2.27 (−0.9, 8.5)	0.25 (−3.2, 3.9)	3.56 (−1.4, 8.8)	3.98 (−1.6, 9.9)
Pretest meal[5]					
n	40	57	48	47	45
Baseline	109.2 ± 25.7	108.5 ± 29.1	110.7 ± 27.9	110.2 ± 29.0	109.3 ± 29.2
Follow-up	115.8 ± 29.0	111.1 ± 28.1	112.5 ± 29.9	112.5 ± 28.5	111.3 ± 27.8
Percentage change (%)	6.10 (1.1, 11.3)	2.4 (−1.7, 6.8)	1.6 (−2.5, 5.9)	2.02 (−2.9, 7.1)	1.80 (−3.3, 7.1)
FVIIa (μg/L)[6]					
Background diet					
n	40	57	46	49	43
Baseline	51.4 ± 30.7	45.5 ± 26.2	49 ± 25.2	51.2 ± 29.7	55.4 ± 26.9
Follow-up	54.6 ± 27.7	52.1 ± 28.7	51.6 ± 26.1	53.4 ± 32.9	55.3 ± 28.5
Percentage change (%)	6.30 (−7.5, 22.1)	14.30 (1.6, 28.8)	5.40 (−4.7, 16.6)	4.29 (−8.1, 18.3)	−0.03 (−12.7, 4.0)
Pretest meal[5]					
n	40	57	46	49	43
Baseline	40.8 ± 19.8	40.7 ± 21.2	36.9 ± 17.5	41.1 ± 21.6	41.2 ± 19.7
Follow-up	45.7 ± 23.6	42.3 ± 22.2	39.1 ± 19.1	43.2 ± 29	44.3 ± 22.7
Percentage change (%)	12.1 (−1.4, 27.4)	4.1 (−5.5, 14.7)	5.9 (−4.8, 17.8)	5.1 (−6.1, 17.6)	7.4 (−2.4, 18.1)

[1] LC-PUFA, long-chain polyunsaturated fatty acids. Data were analyzed by repeated-measures ANOVA with the use of log-transformed data. The differences between diets in measures of FVIIc and FVIIa at baseline were not significant.
[2] Background vs pretest meal effect, $P < 0.0001$; background vs pretest meal × follow-up baseline interaction, $P = 0.83$; follow-up × follow-up vs baseline × diet interaction, $P = 0.89$; follow-up vs baseline × background vs pretest meal × diet interaction, $P = 0.83$.
[3] Geometric $\bar{x}$ ± SD (all such values).
[4] $\bar{x}$; 95% CI in parentheses (all such values).
[5] Subjects were advised to avoid foods high in fat on the day before the test meal, whereas no restrictions in diet were imposed on subjects after the background diet.
[6] Background vs pretest meal effect, $P < 0.0001$; background vs pretest meal × follow-up baseline interaction, $P = 0.95$; follow-up vs baseline × diet interaction, $P = 0.87$; follow-up vs baseline × background vs pretest meal × diet interaction, $P = 0.63$.

duration, typically < 3 mo. Finnegan et al (25) compared intakes of 0.8 or 1.7 g EPA+DHA/d, 4.5 or 9.5 g ALA/d, or control (LA) for 6 mo by using a parallel design in 150 subjects ranging in age from 25 to 72 y. In that study, fatty acids were incorporated into 25 g fat spread, which replaced the subject's normal spread, and EPA+DHA was provided as capsules rather than as food. Finnegan et al (25) reported a 7.7% reduction in fasting plasma triacylglycerol concentrations with an additional 1.7 g/d of a mixture of EPA and DHA and no effect of 4.5–9 g ALA/d, but they did not observe any effect on plasma triacylglycerol of an additional 0.9 g EPA+DHA. The current study showed that a daily intake of 1.3 g n−3 LC-PUFA (0.7% of total dietary energy) lowered fasting and 3-h plasma triacylglycerol concentrations by 11.1% and 7.2%, respectively, compared with the other dietary treatments. It is unlikely that this effect was mediated by a decreased rate of lipolysis of adipose tissue, because neither fasting nor postprandial NEFA concentrations changed with the treatments. The most likely mechanism for the triacylglycerol-lowering effect is the inhibition of hepatic triacylglycerol synthesis mediated via the activation of the peroxisome proliferator–activated receptor α by n−3 LC-PUFA (26).

Plasma triacylglycerol concentrations were positively correlated with FVIIc and FVIIag at baseline, and postprandial lipemia resulted in the activation of FVII. Consequently, it may be predicted that lower fasting plasma triacylglycerol concentrations and low postprandial lipemia after the diets containing the greater intake of n−3 LC-PUFA would lower values for FVIIc, FVIIag, and FVIIa. However, that was not the case. The lack of effect of a higher intake of n−3 fatty acids on the various measures of FVII in the fasting state is in agreement with Finnegan et al (27). Marckmann et al (28), however, reported a 9% decrease in FVIIag after the consumption of 0.9 g n−3 LC-PUFA, whereas Sanders et al (18) found no change in FVIIag but reported an increase in FVIIc after consumption of 5 g n−3 LC-PUFA in a low-saturated-fat diet that was 7% greater than that seen after consumption of a control diet that was high in saturated fatty acids. No relation was found between fasting or postprandial concentrations of NEFA and FVIIa. After a meal containing 50 g fat, FVIIa increased by a mean of 47%, but it was not affected by the background diet. The observation that a low intake of fat on the day before the measurement had a major influence on fasting FVIIc and FVIIa supports the view that meals high in fat acutely increase FVIIa for ≥12 h. It seems highly likely that the amount of fat in a single meal is a more important determinant of FVIIa than is the proportion of the total dietary energy derived from fat. Because meals containing <30 g fat do not result in significant postprandial lipemia, a case has been made for limiting the fat content of any one meal to <30 g

TABLE 7
Postprandial increase in activated factor VII (FVIIa) concentrations after a test meal containing 50 g fat at baseline and after the 6-mo diet intervention[1]

	Diet				
	Control	n−3 LC-PUFA	High linolenate	n−3 LC-PUFA + linolenate	Moderate linolenate
Baseline					
n	40	57	46	49	43
Fasting	40.8 ± 19.8[2]	40.7 ± 21.2	36.9 ± 17.5	41.1 ± 21.6	41.2 ± 19.7
6 h after a test meal	55.7 ± 27.8	56.5 ± 31.5	55.2 ± 29.7	61.7 ± 36.6	56.6 ± 31.7
Percentage change (%)	36.5 (21.5, 53.1)[3]	39.0 (29.4, 49.0)	49.8 (37.3, 63.3)	50.4 (40.0, 61.4)	37.2 (20.1, 56.7)
Follow-up					
n	40	57	46	49	43
Fasting	45.7 ± 23.6	42.3 ± 22.2	39.1 ± 19.1	43.2 ± 29.0	44.3 ± 22.7
6 h after a test meal	66.5 ± 28.4	64.2 ± 36.0	61.7 ± 32.7	65.5 ± 42.9	67.0 ± 36.6
Percentage change (%)	45.4 (32.2, 59.8)	51.7 (41.8, 62.6)	58.1 (42.2, 75.8)	51.7 (34.7, 70.9)	51.3 (38.7, 65.0)
Postprandial increment[4]					
n	40	57	46	49	43
Percentage change (%)	6.5 (−5.8, 20.6)	9.3 (−1.4, 21.0)	5.5 (−6.9, 19.8)	0.9 (−9.5, 12.6)	10.3 (−6.4, 30.0)

[1] LC-PUFA, long-chain polyunsaturated fatty acid. Data were analyzed by repeated-measures ANOVA using log-transformed data. Fasting vs postprandial effect, $P < 0.0001$; follow-up vs baseline × diet interaction, $P = 0.32$; follow-up vs baseline × diet × fasting vs postprandial interaction, $P = 0.94$. The differences between treatments at baseline and at follow-up were not significant.
[2] $\bar{x}$ ± SD (all such values).
[3] $\bar{x}$; 95% CI in parentheses (all such values).
[4] Measured on a log scale.

(29), particularly in older persons, a group in whom the postprandial increase in FVIIa is greater.

In a secondary prevention-of-IHD trial, De Lorgeril et al (30) reported lower plasma fibrinogen in subjects allocated to a diet with a low n−6:n−3, provided mainly by rapeseed oil (canola), than in subjects following usual dietary advice. In a crossover trial of nonsmoking subjects, plasma fibrinogen concentrations in subjects provided an intake of 5 g n−3 LC-PUFAs were lower than concentrations in subjects following an identical diet in which the n−3 LC-PUFAs were replaced with 5 g LA (18). Finnegan et al (27) found no effect of 4.5-9 g ALA or 0.9–1.7 g EPA+DHA on plasma fibrinogen in nonsmokers. Smoking is well known to influence plasma fibrinogen concentrations (31), and it can be a major source of confounding. In the current study, smoking status was assessed by measurement of urinary cotinine; smoking habits did not change during the study. The findings of the current study suggest that moderate increases in n−3 fatty acids in the diet, achieved by increasing fish consumption and by using rapeseed (canola) oil and spreads with a lower n−6:n−3, have no measurable effect on plasma fibrinogen. The observation that moderate alcohol intake was associated with lower plasma fibrinogen is in accord with other reports (32, 33). Increasing the intake of n−3 fatty acids did not affect FXIIa. There were large variations in FXIIa between subjects but little variation between measurements at baseline and follow-up. Zito et al (34) showed that much of the between-subject variation is determined by 46C→T polymorphism in the FXII gene. However, a strong effect of smoking on FXIIa was observed, which is

TABLE 8
Percentage changes in fasting and postprandial plasma factor VII (FVII) between baseline and follow-up in subjects given or not given additional dietary n−3 long-chain polyunsaturated fatty acids (LC-PUFA)[1]

	n−3 LC-PUFA		No n−3 LC-PUFA		
	n	Value	*n*	Value	*P*[2]
		%		%	
Fibrinogen	112	−0.3 (−3.4, 3.0)[3]	144	0.7 (−2.1, 3.7)	0.30
FXIIa	111	−0.03 (−0.23, 0.08)	138	−0.02 (0.19, 0.16)	0.58
FVIIag	106	0.7 (−3.0, 4.6)	136	−0.7 (−3.8, 2.7)	0.41
FVIIc background diet	110	3.8 (0.5, 7.2)	135	2.5 (−0.4, 5.4)	0.42
FVIIc pretest meal	106	2.3 (−0.8, 5.4)	141	3.6 (0.8, 6.4)	0.90
FVIIa background diet	107	9.70 (0.8, 19.4)	130	3.60 (−3.1, 11.3)	0.32
FVIIa pretest meal	108	5.3 (−2.0, 13.2)	136	9.5 (3.1, 16.3)	0.90
FVIIa postprandial increment[4]	106	0.90 (−5.5, 7.3)	133	2.4 (−3.9, 8.8)	0.996

[1] FXIIa, activated FXII; FVIIag, FVII antigen; FVIIc, FVII coagulant activity.
[2] *P* values from 2-factor ANOVA without interaction adjusted for concentration of linoleate.
[3] $\bar{x}$; 95% CI in parentheses (all such values).
[4] Measured on a log scale.

consistent with an earlier report (14), but we could find no relation between recent alcohol intake and FXIIa in the current study.

In conclusion, these findings indicate that lowering n−6:n−3 by increasing the intake of n−3 LC-PUFA decreases both fasting and postprandial plasma triacylglycerol concentrations in older men and women but does not influence fibrinogen, FXIIa, or indexes of FVII. Lowering the n−6:n−3 by increasing the intake of ALA and decreasing that of LA affected neither plasma triacylglycerol nor hemostatic variables. The findings of this study suggest that other lifestyle factors, such as high BMI, alcohol intake, cigarette smoking, and the fat content of a meal, are significant determinants of these hemostatic factors.

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REFERENCES

1. Rosenberg RD, Aird WC. Vascular-bed–specific hemostasis and hypercoagulable states. N Engl J Med 1999;340:1555–64.
2. Meade TW, Ruddock V, Stirling Y, Chakrabarti R, Miller GJ. Fibrinolytic activity, clotting factors, and long-term incidence of ischaemic heart disease in the Northwick Park Heart Study. Lancet 1993;342:1076–9.
3. Heinrich J, Balleisen L, Schulte H, Assmann G, van de LJ. Fibrinogen and factor VII in the prediction of coronary risk. Results from the PROCAM Study in healthy men. Arterioscler Thromb 1994;14:54–9.
4. Junker R, Heinrich J, Schulte H, van de LJ, Assmann G. Coagulation factor VII and the risk of coronary heart disease in healthy men. Arterioscler Thromb Vasc Biol 1997;17:1539–44.
5. Haines AP, Imeson JD, Meade TW. Skinfold thickness and cardiovascular risk factors. Am J Epidemiol 1987;126:86–94.
6. Bowles LK, Cooper JA, Howarth DJ, Miller GJ, MacCallum PK. Associations of haemostatic variables with body mass index: a community-based study. Blood Coagul Fibrinolysis 2003;14:569–73.
7. Miller GJ, Martin JC, Webster J, et al. Association between dietary fat intake and plasma factor VII coagulant activity–a predictor of cardiovascular mortality. Atherosclerosis 1986;60:269–77.
8. Miller GJ, Cruickshank JK, Ellis LJ, et al. Fat consumption and factor VII coagulant activity in middle-aged men. An association between a dietary and thrombogenic coronary risk factor. Atherosclerosis 1989;78:19–24.
9. Sanders TA, de Grassi T, Miller GJ, Humphries SE. Dietary oleic and palmitic acids and postprandial factor VII in middle-aged men heterozygous and homozygous for factor VII R353Q polymorphism. Am J Clin Nutr 1999;69:220–5.
10. Sanders TA, Oakley FR, Cooper JA, Miller GJ. Influence of a stearic acid-rich structured triacylglycerol on postprandial lipemia, factor VII concentrations, and fibrinolytic activity in healthy subjects. Am J Clin Nutr 2001;73:715–21.
11. Kjalke M, Silveira A, Hamsten A, Hedner U, Ezban M. Plasma lipoproteins enhance tissue factor-independent factor VII activation. Arterioscler Thromb Vasc Biol 2000;20:1835–41.
12. Miller GJ, Cooke CJ, Nanjee MN, et al. Factor VII activation, apolipoprotein A-I and reverse cholesterol transport: possible relevance for postprandial lipaemia. Thromb Haemost 2002;87:477–82.
13. Mitropoulos KA, Miller GJ, Watts GF, Durrington PN. Lipolysis of triglyceride-rich lipoproteins activates coagulant factor XII: a study in familial lipoprotein-lipase deficiency. Atherosclerosis 1992;95:119–25.
14. Miller GJ, Esnouf MP, Burgess AI, Cooper JA, Mitchell JP. Risk of coronary heart disease and activation of factor XII in middle-aged men. Arterioscler Thromb Vasc Biol 1997;17:2103–6.
15. Bucher HC, Hengstler P, Schindler C, Meier G. n−3 Polyunsaturated fatty acids in coronary heart disease: a meta-analysis of randomized controlled trials. Am J Med 2002;112:298–304.
16. Hu FB, Willett WC. Optimal diets for prevention of coronary heart disease. JAMA 2002;288:2569–78.
17. Kaaks R, Riboli E. Validation and calibration of dietary intake measurements in the EPIC project: methodological considerations. European Prospective Investigation into Cancer and Nutrition. Int J Epidemiol 1997;26 (suppl):S15–25.
18. Sanders TA, Oakley FR, Miller GJ, Mitropoulos KA, Crook D, Oliver MF. Influence of n−6 versus n−3 polyunsaturated fatty acids in diets low in saturated fatty acids on plasma lipoproteins and hemostatic factors. Arterioscler Thromb Vasc Biol 1997;17:3449–60.
19. Executive Summary of the third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA 2001;285:2486–97.
20. Zimmet P, Alberti KG, Shaw J. Global and societal implications of the diabetes epidemic. Nature 2001;414:782–7.
21. Roberts TL, Wood DA, Riemersma RA, Gallagher PJ, Lampe FC. Linoleic acid and risk of sudden cardiac death. Br Heart J 1993;70:524–9.
22. National Food Survey. Household food consumption, expenditure and nutrient intake. Internet: http://statistics.defra.gov.uk/esg/publications/nfs/default.asp (accessed 10 May 2005).
23. Sanders TA. Polyunsaturated fatty acids in the food chain in Europe. Am J Clin Nutr 2000;71(suppl):176S–8S.
24. Harris WS. n−3 Fatty acids and serum lipoproteins: human studies. Am J Clin Nutr 1997;65(suppl):1645S–54S.
25. Finnegan YE, Minihane AM, Leigh-Firbank EC, et al. Plant- and marine-derived n−3 polyunsaturated fatty acids have differential effects on fasting and postprandial blood lipid concentrations and on the susceptibility of LDL to oxidative modification in moderately hyperlipidemic subjects. Am J Clin Nutr 2003;77:783–95.
26. Evans RM, Barish GD, Wang YX. PPARs and the complex journey to obesity. Nat Med 2004;10:355–61.
27. Finnegan YE, Howarth D, Minihane AM, et al. Plant and marine derived (n−3) polyunsaturated fatty acids do not affect blood coagulation and fibrinolytic factors in moderately hyperlipidemic humans. J Nutr 2003;133:2210–3.
28. Marckmann P, Bladbjerg EM, Jespersen J. Dietary fish oil (4 g daily) and cardiovascular risk markers in healthy men. Arterioscler Thromb Vasc Biol 1997;17:3384–91.
29. Sanders TA. Dietary fat and postprandial lipids. Curr Atheroscler Rep 2003;5:445–51.
30. de Lorgeril M, Renaud S, Mamelle N, et al. Mediterranean alpha-linolenic acid-rich diet in secondary prevention of coronary heart disease. Lancet 1994;343:1454–9.
31. Meade TW, Imeson J, Stirling Y. Effects of changes in smoking and other characteristics on clotting factors and the risk of ischaemic heart disease. Lancet 1987;2:986–8.
32. Rimm EB, Williams P, Fosher K, Criqui M, Stampfer MJ. Moderate alcohol intake and lower risk of coronary heart disease: meta-analysis of effects on lipids and haemostatic factors. BMJ 1999;319:1523–8.
33. Wannamethee SG, Lowe GD, Shaper G, et al. The effects of different alcoholic drinks on lipids, insulin and haemostatic and inflammatory markers in older men. Thromb Haemost 2003;90:1080–7.
34. Zito F, Drummond F, Bujac SR, et al. Epidemiological and genetic associations of activated factor XII concentration with factor VII activity, fibrinopeptide A concentration, and risk of coronary heart disease in men. Circulation 2000;102:2058–62.