ELSEVIER

A residential study comparing the effects of diets rich in stearic acid, oleic acid, and linoleic acid on fasting blood lipids, hemostatic variables and platelets in young healthy men

Kirsty A. Hunter,* Lynn C. Crosbie,* Alison Weir,* George J. Miller,† and Asim K. Dutta-Roy*

**Rowett Research Institute, Aberdeen, Scotland, UK and †MRC Epidemiology and Medical Care Unit, St. Bartholomew's and Royal London College of Medicine, London, UK*

Dietary fat is known to influence the variables of blood coagulation and fibrinolysis associated with vascular disease. However, the role of fat content and/or fat composition of the diet in this regard is still not well understood. In the present study, we investigated the effects of three isoenergic diets of differing fat composition in nine healthy young men in a strictly controlled residential study. Subjects consumed the three experimental diets for periods of 2 weeks each, separated by a washout period of at least 5 weeks in a randomized crossover design. The diets provided 38% of total energy intake as fat, 45% as carbohydrate, and 17% as protein, and differed only with respect to the fatty acid composition (stearic acid-rich diet: 34.1% stearic acid, 36.6% oleic acid; oleic acid-rich diet: 65.8% oleic acid; linoleic acid-rich diet: 36.5% linoleic acid, 38% oleic acid). Blood samples were collected at the beginning and at the end of each dietary period from fasted subjects for determination of factor VII coagulant activity (FVIIc), activated factor VII (FVIIa), factor VII antigen (FVIIag), tissue plasminogen activator (tPA) activity, plasminogen activator inhibitor type 1 (PAI-1) activity, fibrinogen, prothrombin fragment 1+2 (F_{1+2}), and plasma lipids. There were no significant differences between diets in fasting plasma concentrations of FVIIc, FVIIa, FVIIag, fibrinogen, F_{1+2}, PAI-1 activity, and tPA activity. Plasma concentrations of lipids (high density lipoproteins, low density lipoproteins, triacylglycerols, and total cholesterol) were also unaffected. Although there were no changes in platelet aggregation response and membrane fluidity observed in any of the diets, increased anti-aggregatory prostaglandin E_1 binding to platelet membranes was observed only in the case of linoleic acid-rich diet. In conclusion, diets with very different fatty acid compositions, at 38% of energy as fat intake, did not significantly influence blood coagulation, fibrinolysis, or blood lipids in the fasting state in young healthy men. (J. Nutr. Biochem. 11:408–416, 2000)

Keywords: stearic acid; oleic acid; linoleic acid; plasma lipids; FVIIc; t-PA activity; PAI-1 activity; F_{1+2}; fibrinogen; platelets; prostaglandin E_1 receptor; membrane fluidity

Introduction

Epidemiologic and clinical studies have demonstrated that specific components of all three limbs of the hemostatic system (i.e., the coagulation cascade, platelets, and fibrinolysis) are independent risk markers of cardiovascular disease.[1–5] In the hemostatic system plasma factor VII coagulant activity (FVIIc), concentration of fibrinogen, and plasminogen activator inhibitor type 1 (PAI-1) are risk factors for an acute coronary event.[1,6,7] Furthermore, elevated plasma concentrations of tissue plasminogen activator (tPA) and fibrinogen were associated with enhanced risk of further myocardial infarction and stroke among men.[8–10]

Address correspondence to Professor Asim K. Dutta-Roy, Rowett Research Institute, Aberdeen AB21 9SB, Scotland, United Kingdom.
Received January 14, 2000; accepted June 28, 2000.

655 Avenue of the Americas, New York, NY 10010
0955-2863/00/$–see front matter
PII S0955-2863(00)00097-8

Dietary lipids are thought to influence the development of cardiovascular disease via a number of processes such as the hemostatic system and platelet function.[11–15] Plasma FVIIc is positively associated with plasma cholesterol and triglyceride concentrations in healthy adults, raising the possibility that dietary fat intake may influence hemostatic status in addition to its well-recognized influence on plasma lipids.[16] Attempts to identify the specific lipid characteristics affecting the hemostatic system suggest that not only total fat content but also the types of fat in the diet may be influential.[11,16]

Of the various fatty acids that have been suggested to influence hemostatic function, several are becoming increasingly important in the U.K. diet and warrant further investigation. The so-called Mediterranean diet, characterized by a moderately low intake of polyunsaturated fatty acids and high intake of monounsaturated fatty acids from olive oil, has gained popularity.[17] The principal fatty acid of olive oil, oleic acid (18:1 n-9), is an effective hypocholesterolemic agent,[18,19] and, as one of the key components of the Mediterranean diet, has been considered to be potentially useful in the prevention of cardiovascular disease. Claims have been made of both beneficial and detrimental alterations in hemostasis when diets are supplemented with or contain large amounts of oleic acid.[19–22] The cholesterol-lowering properties of linoleic acid (18:2 n-6) have been known for many years,[22] but its effects on hemostasis are less well documented. As with stearic acid (18:0) and oleic acid, no firm conclusions have been drawn as to the net hemostatic response to large amounts of dietary linoleic acid. Stearic acid has a minimal or neutral effect on plasma cholesterol concentrations but its influence on hemostasis also remains uncertain.[23] A high intake of stearic acid taken as shea butter has been reported to affect blood lipids and FVIIc favorably in young men when compared with fats high in saturated fatty acids such as lauric acid (12:0), myristic acid (14:0), or palmitic acid (16:0).[24]

The present dietary study examined the effects of isoenergic diets differing in content of stearic acid, oleic acid, or linoleic acid on fasting plasma concentrations of hemostatic variables and lipids and on platelet function in healthy, young individuals in highly metabolically controlled conditions. In this study, volunteers were supplied with these diets, which contained physiologic amounts of the major dietary components (38% of energy as fat, 45% as carbohydrate, and 17% as protein) with different fatty acid compositions (stearic acid-rich diet, 34.1% stearic acid and 36.6% oleic acid; oleic acid-rich diet, 65.8% oleic acid; and linoleic acid-rich diet, 36.5% linoleic acid and 38% oleic acid).

In this article, we compare the effects of the stearic acid-, oleic acid-, and linoleic acid-rich diets on plasma lipids [triacylglycerols, high density lipoprotein (HDL) cholesterol, and low density lipoprotein (LDL) cholesterol] and hemostatic variables [FVIIc, activated factor VII (FVIIa), factor VII antigen (FVIIag), tPA activity, prothrombin fragment (F_{1+2}), and PAI-1 activity] and platelet aggregation response, membrane fluidity, and prostaglandin E_1 (PGE_1) receptor activity of platelet membranes in a random crossover study in young, healthy men in highly controlled conditions.

Materials and methods

Chemicals

1,6 Diphenylhexatriene (DPH) was obtained from Molecular Probes (Eugene, OR USA). [5,6(n)^{3}H] PGE_1 (specific activity 56 Ci/mmol) was from Amersham International (Bucks, UK). Unlabeled PGE_1 and lipid standards were obtained from Sigma (Poole, UK). All other chemicals used were of high-grade analytical quality.

Subjects

The study protocol was approved by the Joint Ethical Committee of the Grampian Health Board and the University of Aberdeen (Aberdeen, Scotland, UK). Healthy male subjects (aged 20–35 years) were recruited after assessment of their medical and dietary histories. Exclusion criteria were the presence of overt vascular, hematologic, or respiratory disease, hypertension, infection, hyperlipidemia, body mass index (BMI) less than 20 or greater than 28 kg/m^2, frequent consumption of drugs that affect lipid metabolism or hemostatic function (e.g., aspirin, paracetamol, ibuprofen, steroids), habitual consumption of fatty acid supplements, smoking, frequent blood donations, and more than 8 hr of vigorous exercise per week.

General protocol

Subjects consumed three test diets that were rich in one of three blended oils, each for a period of 2 weeks. During these periods of dietary manipulation, subjects were residing at the Rowett Research Institute's Human Nutrition Unit. Each period was separated by a washout interval of greater than 5 weeks when subjects returned home and consumed their habitual diet.

Test oils

Three blended oils were chosen that were similar in composition to those used in the postprandial study described previously[25] [i.e., rich in stearic and oleic acids (stearic acid-rich diet), oleic acid (oleic acid rich-diet), and linoleic and oleic acids (linoleic acid-rich diet)]. Oils were donated by Unilever Research (Vlaardingen, The Netherlands). Fatty acid composition and partial positional distribution of these oils are shown in *Table 1*. Cholesterol intake per day was approximately 350 mg/day. To standardize the vitamin E content of each diet that has been recognized as a determinant of cardiovascular disease,[26] the blended diets were analyzed by extraction of lipids, followed by high performance liquid chromatography as described previously.[27] The stearic acid-rich diet contained most vitamin E and therefore the oleic acid- and linoleic acid-rich diets were altered by the addition of tocopheryl acid succinate (donated by Henkel Corporation, Cincinnati, OH USA) to give a final standardized α-tocopherol intake of 75 mg/day.

Dietary design

All food was prepared in the metabolic kitchen of the Human Nutrition Unit at the Rowett Research Institute. The diet consumed over each 2-week period consisted of a 4-day rotating menu such that, for the three test periods, the diets were identical apart from the fatty acid composition. Daily energy intake was modified for each subject to provide the product of their basal metabolic rate (measured using a Deltatrac ventilated hood system) (Datex-Ohmeda, Stirling, Scotland) and a physical activity factor of 1.6 KJ/day, an amount estimated to maintain body weight.

The 4-day rotating menu was designed using the Food Base Computer Package (developed by the Institute of Brain Chemistry and Human Nutrition, Hackney Hospital, London, UK) and

Table 1 Fatty acid composition and partial positional distribution of blended oils supplied by Unilever

Fatty acid	Stearic acid-rich diet		Oleic acid-rich diet		Linoleic acid-rich diet	
	% Total	% Sn-2	% Total	% Sn-2	% Total	% Sn-2
C8-C10	0	0	0	0	0	0
C12-C14	0	0	0	0	0	0
C16:0	4.0	0.6	4.2	0.3	6.2	0.5
C18:0	41.5	3.4	4.5	0.3	4.5	0.3
C18:1n-9	40.1	79.3	79.1	89.2	40.6	43.5
C18:2n-6	10.3	15.8	9.9	10.1	45.4	54.3
C18:3n-3	1.0	0.9	0.1	0.0	1.3	1.3
C20:0	1.4	0.1	0.4	0.0	0.4	0
Total	98.3	100.1	98.2	99.9	98.4	99.4

confirmed by biochemical compositional analyses. The final proportions of the three primary nutritional components were similar to a typical U.K. diet: 38% of energy as fat, 45% as carbohydrate, and 17% as protein.[28] Eighty percent of the dietary lipid was provided by a test oil that was added to cooked dishes prepared from selected low-fat ingredients such as potatoes, soya protein, skimmed milk, and pasta. *Table 2* shows the average fatty acid composition of each diet.

Body weight was monitored every other day and, if weight fluctuated, daily energy intake was adjusted accordingly. In general, subjects' weights tended to fall, and to compensate their experimental diet was supplemented with a turkey salad roll that contained the same proportions of fat, carbohydrate, and protein as the diet itself. Because certain dietary components, such as garlic, onion, some spices, and alcohol, are known to modify platelet metabolism, they were excluded from the diet. No alcohol was allowed on the day before and during the dietary intervention periods.

Procedure for blood sampling

Venous blood samples were taken using a 20-gauge butterfly needle and syringe system and minimal stasis after subjects had lain quietly for 30 min. Time of sampling was standardized across the three dietary periods to minimize any effects of circadian variation. Subjects were asked to refrain from vigorous exercise on the day before samples were taken to remove its influence on certain hemostatic factors. Blood samples were taken after an overnight fast on days 0 and 14.

Analysis of blood samples

Blood for the measurement of FVIIc, FVIIa, FVIIag, and F_{1+2} were taken into 10% (v/v) 3.8% trisodium citrate and centrifuged immediately at 2,000 × g for 10 min at room temperature. Plasma was frozen at −80°C until analysis. Plasma FVIIc concentrations were measured by a one-stage clotting assay using rabbit brain thromboplastin (Diagnostic Reagents, Thames, Oxford, UK) and a factor VII-deficient plasma as described by Miller et al.[29] Plasma FVIIa concentrations were determined by a one-stage clotting assay as described by Morrissey et al.[30] FVIIag was measured by an enzyme-linked immunosorbent assay (Asserachrom VII:Ag, Diagnostica Stago, Asnieres-sur-seine, France). Chromogenic methods were used for both tPA (Coatest tPA, Chromogenix AB, Molndal, Sweden) and PAI-1 (Coatest PAI-1, Chromogenix AB) activity measurements. Plasma F_{1+2} was measured by enzyme immunoassay (Behring Diagnostics, Marburg, Germany). Blood for the measurement of plasma fibrinopeptide A (FPA) concentration was taken into anticoagulant and concentrations were determined by radioimmunoassay (Byk-Sangtec, Dietzenbach, Germany). The F_{1+2} data were excluded when plasma concentrations of FPA were over 6 ng/mL, indicative of a substandard venepuncture. Plasma fibrinogen level was measured according to Clauss.[31]

Table 2 Fatty acid composition (% total) of the blended experimental diets

Fatty acid	Stearic acid-rich diet	Oleic acid-rich diet	Linoleic acid-rich diet
C12:0	0.6	0.6	0.5
C14:0	1.6	1.5	1.5
C16:0	10.0	9.9	11.2
C18:0	34.1	6.0	6.0
C18:1n-9	36.6	65.8	38.0
C18:2n-6	10.8	11.0	36.5
C18:3n-3	1.1	0.7	1.3
Total	94.8	95.5	95.0

Blood for the measurement of plasma triglyceride and C-reactive protein concentrations was taken in 0.054 mL of 15% ethylenediamine-tetraacetic acid (EDTA). After centrifuging at 2,000 × g for 10 min, plasma was removed and stored at 4°C for the measurement of triglyceride concentration and at −20°C for the determination of C-reactive protein concentration. Plasma triacylglycerols were measured within 4 days by an automated enzymatic method (Kone Dynamic Selective Chemistry Analyser, Ruukintie, Finland) and C-reactive protein concentration was determined using a latex agglutination kit (Behring Diagnostics). If plasma C-reactive protein concentrations were elevated (indicating the presence of an acute phase response), the blood sample was excluded from all analyses.

Plasma HDL and total cholesterol concentrations were determined using kits from Sigma. LDL cholesterol was calculated from the difference. Fatty acid composition of red blood cells (measured to assess compliance with the diet) and diets was determined according to the method of Bligh and Dyer.[32] Fatty acid methyl esters (FAME) were analyzed using (Hewlett Packard, Palo Alto, CA USA) gas chromatograph (HP 6890 series), fitted with a 30 m cyanopropyl methyl polysiloxane capillary column with 0.25 mm internal diameter, using a split ratio of 1:50. The injection and detection temperatures were 250°C and helium was used as carrier gas. The starting temperature of the column was 80°C, which increased to 180°C at a rate of 25°C/min. After 4 min at 180°C, the temperature was increased to 220°C at a rate of 1°C/min. A standard mixture of FAME was used to identify the FAME in the samples by means of retention time. Using computer-assisted analysis (Hewlett Packard, HP 9680 series), the fatty acid profiles were corrected for blank runs.

Table 3 Subjects' habitual diet estimated by food frequency questionnaire (FFQ)

Energy (MJ/day	Protein		Fat		Carbohydrate		Saturated fatty acids	
	% of energy	g	% of energy	g	% of energy	g	% energy	g/day
13.9 ± 2.2	17.3 ± 1.7	143.6 ± 29.6	33.0 ± 7.8 37.7[a]	124.8 ± 47.1 93.4[1]	44.6 ± 8.2	388.9 ± 63.4	12.4 ± 3.7 15.5[2]	47.5 ± 20.8

Results shown are the mean ± SD for eight subjects.
[1]Data from Gregory et al.[28] for average fat intakes of Scottish men are also shown for comparison.
[2]Remaining 5% of energy from alcohol.

Platelet aggregation study

Venous blood was collected through siliconized needles into plastic syringes. Coagulation was prevented by mixing 9 volumes of blood with 1 volume sodium citrate (final concentration, 13 mM).[33] Platelet-rich plasma (PRP) was obtained by centrifugation of blood for 10 min at 180 × g. Platelet counts were performed in a Coulter cell counter (Serono Baker Diagnostics, Allentown, PA USA) and the platelet numbers in PRP were adjusted to 2 to 3 × 10^8 cell/mL. Platelet aggregation was monitored on a Packs-4 aggregometer (Helena Lab, Beaumont, TX USA) at a constant stirring speed of 1,000 rpm at 37°C. Aggregation of platelets in PRP was induced by adding adenosine diphosphate (ADP). Platelet aggregation was performed within a certain time period (120 min) after the blood was taken and results from delayed samples were rejected. The aggregating agent ADP was used at 5, 7.5, and 10 μM concentrations.

Determination of fluorescence polarization

Platelet membrane fluorescence polarization was determined according to Dutta-Roy et al.[34] Platelet membranes were prepared as described previously.[35] Typically, 50 μg of platelet membrane in 50 mM Tris-HCl, pH 7.4, containing 2 mM $MgCl_2$ were labeled with a fluorescence probe, by incubating an equal volume of 2 μM DPH dispersion in the same buffer for 1 hr at 23°C. The steady-state fluorescence polarization was measured in a Perkin-Elmer (Foster City, CA USA) luminescence spectrophotometer (LS-5B) fitted with a polarizer accessory. Excitation and emission wavelengths were 358 and 430 nm, respectively. Excitation and emission slits were 5 and 10 nm, respectively. The steady-state fluorescence polarization (P) was calculated from equation 1:

$$P = \frac{I_{VV} - G.I_{VH}}{I_{VV} + G.I_{VH}}, \qquad G = \frac{I_{HV}}{I_{HH}} \tag{1}$$

where I_{VV} and I_{VH} and are the fluorescence intensities recorded with the analyzing polarizer oriented, parallel and normal, respectively, to the vertically oriented of the polarized excitation beam. G is the grating correction factor. I_{HV} and I_{HH} are the fluorescence intensities determined with the emission polarizer vertically and horizontally, respectively, when the excitation polarizer was set in the horizontal position. Light scattering errors were minimized by assuring that measured anisotropies were concentration-independent. The steady-state fluorescence anisotropy (r_s) was obtained from equation 2:

$$r_s = \frac{I_{VV} - G.I_{VH}}{I_{VV} + 2G.I_{VH}} = \frac{2P}{3 - P} \tag{2}$$

The fluorescence background of a blank sample (unlabeled membranes) never exceeded 15% of the fluorescence signal of membranes labeled with DPH.

Platelet membrane PGE_1 receptor assay

Platelet membrane PGE_1 receptor activity was analyzed as described by Dutta-Roy and Sinha.[35] Typically, 50 μg of platelet membranes were incubated with 9 nM [^{3}H]PGE_1 for 30 min at 37°C. After the incubation of the membrane, the mixtures was vacuum-filtered through Whatman (Kent, UK) glass micro fiber filter paper (GF/C) that had been pre-soaked with the assay buffer. Each assay tube and buffer was washed with 10 mL of the buffer at 4°C. The filters were then dried and suspended in scintillation fluid for measurement of radioactivity. Parallel experiments were run using more than 1,000-fold excess unlabeled PGE_1 (9 μM) in the above incubation mixture to determine the nonspecific binding. This value was subtracted from the total binding to calculate the specific binding.

Statistical analysis

Blood measurements at day 14 were analyzed by randomized block analysis of variance using Genstat (Lawes Agricultural Trust, Edinburgh, UK). Blocking was by subject, and diet was assessed in the within-subject stratum. Day 0 values were fitted as a covariate. Data on red blood fatty acids were expressed as mean ± SD and a paired t-test was used to compare day 0 and day 14 values for the individual diets. A P-value of less than 0.05 was taken as indicating statistical significance.

Results

Dietary study and subjects

Of the nine subjects who entered the study, six successfully completed the three dietary periods and three completed two periods. The mean age, weight, and BMI of these subjects were 28.0 ± 5.6 years, 78.1 ± 10.74 kg, and 24.7 ± 2.2 kg/m^2, respectively. The diets were well tolerated without any adverse effect. During the 2-week period, no significant change in BMI was observed. A food frequency questionnaire (FFQ) developed in Aberdeen specifically for the North-Eastern Scottish population was used to assess habitual diet. The Rowett's Own Nutritional Analysis (RONA) system developed for use in conjunction with the questionnaire was used to give a detailed estimation of components of the habitual diet. The results of this analysis are shown in *Table 3*. Data from Gregory et al.[28] giving average fat intakes of Scottish men are also shown for comparison. The results generated by the FFQ suggested that subjects were habitually consuming a diet low in fat (*Table 3*), the mean ± SD intake being 33 ± 8% of energy (compared with 38% in the test diet). To check the reliability of this finding, three volunteers completed a 5-day weighed dietary intake and the results of this are compared with those from the

Monounsaturated fatty acids		Polyunsaturated fatty acids		Sugars	Starch	Fiber	Alcohol
% of energy	g/day	% of energy	g/day	(mg/day)	(g/day)	(g/day)	(g/day)
12.7 ± 3.0 11.8[2]	47.4 ± 16.3	5.6 ± 1.5 5.6[2]	20.7 ± 7.0	181.1 ± 53.0	204.8 ± 52.3	32.3 ± 8.4	25.0 ± 23.9

questionnaire in *Table 4*. Agreement between the two methods was reasonable, suggesting that the finding of a moderate fat intake was reliable.

As an indication of compliance with the dietary regimen, fatty acid profiles in red blood cells were determined at the beginning and at the end of each dietary period. The test diets differed markedly in their effects on the proportions of fatty acids in red blood cells, as shown in *Table 5*. These oils clearly increased the proportion of one of the main fatty acids present in the diet in red blood cells by decreasing other major fatty acids. The stearic acid-rich diet, which contained 34% stearic acid, increased the proportion of red blood cell stearic acid (from 13.3 ± 0.57% to 14.6 ± 0.84%, $P < 0.01$) with concomitant decreased proportions of linoleic (from 10.12 ± 0.94% to 9.52 ± 0.78, $P < 0.01$) and palmitic acids (19.7 ± 0.68% to 18.1 ± 0.41%, $P < 0.01$) but without effect on the level of oleic acid. The oleic acid rich-diet, which contained 66% oleic acid, increased the proportion of oleic acid (from 12.6 ± 1.07% to 13.6 ± 1.22%, $P < 0.01$) with decreased concentrations of linoleic (9.8 ± 0.95% to 9.0 ± 0.75%, $P < 0.01$) and palmitic acids (19.7 ± 1.09% to 18.7 ± 0.87, $P < 0.01$) but had no effect on stearic acid. The linoleic acid-rich diet, which contained 37% linoleic acid, increased linoleic acid (9.8 ± 1.3% to 10.8 ± 1.1%, $P < 0.001$), but significantly decreased palmitic (from 19.58 ± 1.02% to 18.94 ± 1.32%, $P < 0.05$) and oleic acids (from 12.55 ± 1.29% to 12.02 ± 0.79%, $P < 0.05$) without affecting the proportion of stearic acid. The level of arachidonic acid did not change in the case of any of these test diets. These results are indicative of subject compliance with each dietary regimen. No carryover effect was observed on habitual samples collected before the second or third experimental dietary period.

Effect of diets on plasma lipids and blood coagulation/fibrinolytic factors

Table 6 shows the median values for plasma concentrations of total cholesterol, HDL, LDL, and triacylglycerols at day 0 (habitual diet) and day 14. No significant differences were observed in fasting concentrations of plasma lipids when day 14 values were compared for the three diets. *Table 7* shows the effects of the three test diets on various hemostatic variables of blood coagulation and fibrinolysis. Again, no significant differences were observed in fasting plasma concentrations of FVIIc, FVIIag, FVIIa, fibrinogen, and F_{1+2} when day 14 values were compared for the three experimental diets. There were also no differences in PAI-1 activity and tPA activity when day 14 values were compared for the three experimental diets. In addition, no significant differences were observed between hemostatic variables and plasma lipids in samples taken immediately before the three interventions.

Effects of diets on platelets

Table 8 shows the platelet aggregation response to 10 μM ADP at day 0 (habitual diet) and day 14. No significant differences were observed in aggregation response when day 14 values were compared for the three diets. Platelet counts also did not change during the dietary study (data not shown).

To determine the effects of diets on platelet membranes, membrane fluidity was determined by a fluorescence polarization method. *Table 9* shows the fluorescence anisotropy (r_s) of platelet membranes at days 0 and 14. No significant

Table 4 Comparison of separate estimates of habitual diet obtained for three subjects by food frequency questionnaire (FFQ) and 5-day weighed intake (5 day)

	Energy (MJ/day)	Protein (% energy)	Fat (% energy)	Carbohydrate (% energy)	Protein (g)	Fat (g)	Carbohydrate (g)	Staurated fatty acids (g)	Mono-unsaturated fatty acids	Poly-unsaturated fatty acids (g)
Subject A										
FFQ	15.5	16.2	33.9	49.7	149.9	139.6	490.5	65	48	16
5 day	16.0	10.0	31.3	56.5	106.2	152.6	637.9	57.9	47.8	20
Subject B										
FFQ	10.6	18.5	30.0	49.7	116.7	84.2	335.0	22.0	40.1	18.9
5 day	10.6	18.3	34.9	46.1	114.7	100.6	307.5	27.7	39.2	18.9
Subject C										
FFQ	11.8	17.2	24.3	53.7	121.0	76.3	404.2	33.1	28.2	10.8
5 day	7.4	16.2	26.9	55.6	76.3	57.9	276.9	20.4	16.2	10.8

Table 5 Effect of diets rich in stearic, oleic, and linoleic acids on red blood cells fatty acid composition (mean ± SD, $n = 6$)

Fatty acids (% of total fatty acids)	Stearic acid-rich diet		Oleic acid-rich diet		Linoleic acid-rich diet	
	Day 0	Day 14	Day 0	Day 14	Day 0	Day 14
Palmitic acid, 16:0	19.66 ± 0.68	18.12 ± 0.41[a]	19.7 ± 1.09	18.71 ± 0.87[a]	19.58 ± 1.02	18.94 ± 1.32[b]
Stearic acid, 18:0	13.32 ± 0.57	14.63 ± 0.84[a]	13.5 ± 0.97	13.64 ± 1.02	13.75 ± 0.70	13.54 ± 0.73
Oleic acid, 18:1n-9	12.27 ± 0.44	12.33 ± 0.33	12.61 ± 1.07	13.64 ± 1.22[a]	12.55 ± 1.29	12.02 ± 0.79[b]
Linoleic acid, 18:2n-6	10.12 ± 0.94	9.52 ± 0.78[a]	9.8 ± 0.95	9.07 ± 0.75[a]	9.84 ± 1.29	10.78 ± 1.15[c]
Arachidonic acid, 20:4n-6	13 ± 1.03	13.23 ± 1.29	12.97 ± 0.84	13.18 ± 0.81	12.78 ± 1.86	14.3 ± 1.53

[a] $P < 0.01$, [b] $P < 0.05$, [c] $P < 0.001$ (significantly different compared with day 0).

differences in membrane fluidity were observed at days 0 and 14, and no diet effect was observed.

PGE_1 receptor activity of platelets was determined by direct radiolabeled PGE_1 binding to platelet membranes. Linoleic acid-rich diet increased PGE_1 binding to platelet membranes (3.09 ± 0.34 fmol PGE_1 bound/50 μg protein at day 0 vs. 3.98 ± 0.61 fmol PGE_1 bound/50 μg protein at day 14, $P < 0.05$). Stearic acid-rich and oleic acid-rich diets had no effect of on this anti-aggregatory PGE_1 receptor (*Table 10*).

Discussion

This study was designed to assess the short-term effect of changes in dietary fatty acid composition at a fixed fat content on the cardiovascular risk profiles. Young healthy men consumed diets of physiologic composition (38% of energy as fat, 45% as carbohydrate, and 17% as protein) containing oils enriched with stearic and oleic acids (stearic acid-rich diet), oleic acid (oleic acid-rich diet), and oleic and linoleic acids (linoleic acid-rich diet), each for a 2-week period in a random crossover study. The experimental diets were completely controlled and were identical with respect to practically all food components except the fatty acid composition.

High-fat diets increase the plasma FVIIag concentration, and an acute effect whereby a small proportion of FVII is converted from its proenzyme to active serine protease for several hours postprandially.[11] In adults whose usual diet is rich in long-chain saturated fatty acids, postprandial activation of FVII occurs irrespective of the fatty acid composition.[11] The underlying mechanism appears to require lipolysis and the presence of another coagulant protein, factor IX. However, the effect of dietary fatty acid composition on FVIIc still remains unsolved. To address this question, we designed three experimental diets using natural fat blends to create differences in their fatty acid composition. We observed no significant differences between the three test diets with regard to coagulation and fibrinolytic variables associated with cardiovascular disease (factor VII, t-PA activity, fibrinogen, and PAI-1 activity). The factors known to affect hemostatic parameters (i.e., the amount of total fat, cholesterol, fiber, and physical activity[1,16,36]) were kept constant during the study, so comparisons could be made solely between differences in the fatty acid compositions of the diet. The present investigation supports previous human studies that reported no influence on FVIIc activities of changes in the fatty acid composition of diet.[11,37–39]

The experimental diets with different fatty acid compositions did not seem to affect fibrinolytic system, because activity of both tPA and PAI-1 activities remained unaltered in the present study. Several prospective studies have shown that plasma fibrinogen is a powerful, independent predictor for myocardial infarction and stroke.[6] However, the present study agrees with previous findings that diet, with the possible exception of fish oils, does not seem to regulate the plasma level of fibrinogen.[40,41] The effects of dietary fatty acids on plasma lipids and the cardiovascular system are variable depending on their chain length and number of double bonds.[11] In the present study, we demonstrated that a change in the fatty acid composition (stearic, oleic, or linoleic acids) of dietary fat has virtually no effect on plasma lipids (LDL, HDL, total cholesterol, and triacylglycerols). All three experimental diets, however, had plasma cholesterol-lowering effects when compared with habitual diets. This was probably due to the facts that these diets had very low myristic acid and contained at least 36% oleic acid. The cholesterol-increasing effect of myristic acid and the cholesterol-lowering effect of linoleic and oleic acids are known[18,22]; however, the effect of stearic acid is yet to be

Table 6 Effects of diets rich in stearic, oleic, and linoleic acids on plasma lipids and linoproteins[1]

Plasma lipids (mmol/L)	Habitual diets[2]	Stearic acid-rich diet	Oleic acid-rich diet	Linoleic acid-rich diet
Triglyceride	0.79 (0.39–2.14)	0.54 (0.39–1.70)	0.52 (0.41–1.57)	0.51 (0.38–1.28)
LDL	3.87 (1.24–5.17)	2.79 (1.97–4.09)	3.12 (1.74–4.79)	2.58 (1.36–4.19)
HDL	1.125 (0.76–1.57)	0.96 (0.63–1.37)	1.06 (0.69–1.44)	1.04 (0.77–1.24)
Total cholesterol	5.06 (2.55–6.16)	3.76 (2.92–4.72)	3.91 (2.80–5.48)	3.71 (2.40–5.12)

[1] Values are median and range.
[2] $n = 18$ (3 observations of each individual, $n = 6$) measured at day 0. For details please see the Materials and methods section.
LDL—low density lipoprotein. HDL–high density lipoprotein.

Table 7 Effect of diets rich in stearic, oleic, and linoleic acids on hemostatic variables for blood coagulation and fibrinolysis[1]

Hemostatic factors	Habitual diets[2]	Stearic acid-rich diet	Oleic acid-rich diet	Linoleic acid-rich diet
FVIIc (% standard)	107.5 (78–142)	84.5 (61–135)	89.0 (67–117)	91 (68–115)
FVIIa (μg/L)	3.02 (0.96–6.42)	1.7 (0.94–7.4)	2.42 (1.09–4.06)	2.12 (1.0–4.69)
FVIIag (% standard)	76.5 (63–89)	70.5 (67–80)	67 (64–87)	75 (66–75)
*t*PA activity (IU/L)	875 (220–6,770)	1,250 (910–2,990)	1,060 (160–1,870)	1,230 (640–4,610)
PAI-1 activity (IU/L)	10,850 (3,100–38,250)	6,260 (1,180–37,110)	10,110 (4,520–27,720)	10,880 (2,062–35,650)
F_{1+2} (nmol/L)	0.66 (0.28–1.08)	0.51 (0.39–0.71)	0.74 (0.39–0.88)	0.55 (0.34–1.11)
Fibrinogen (g/L)	2.65 (1.92–3.5)	2.88 (2.23–3.79)	2.78 (2.44–3.36)	2.77 (2.23–3.50)

[1] Values are median and range.
[2] $n = 27$ (3 observations of each individual, $n = 6$) measured at day 0. For details please see the Materials amd methods section.
FVIIc—factor VII coagulant. FVIIa–activated factor VII. FVIIag—factor VII antigen.
*t*PA—tissue plasmonogen activator. PAI-1—plasminogen activator inhibitor type 1.
F_{1+2}—prothrombin fragment 1+2.

resolved. The effect of stearic acid on serum total and LDL cholesterol concentrations is generally considered to be neutral or even hypocholesterolemic.[24,42,43] Stearic acid is considered less harmful for cardiovascular risk than are saturated fatty acids with 12 to 16 carbon atoms. Moreover, when compared with 12 to 16 chain lengths, stearic acid has been shown to lower FVIIc.[24,44] In the present study, stearic acid had no effect on plasma lipids and hemostatic variables in young healthy men when compared with those of either oleic or linoleic acids at fixed fat content of the diet. A possible mechanism for the neutral or decreasing effect of stearic acid on plasma total cholesterol concentration is thought to be due to its rapid conversion to oleic acid, which does not increase plasma total cholesterol concentration. However, the relationship of dietary fat intake to blood cholesterol concentrations is very complex. It is possible that the ratio of the various fatty acids in the diets determines the changes in blood cholesterol levels upon changes in fat intake, not the percentage of calories from fat, either saturated or unsaturated fatty acids.

PGE_1 and prostacyclin (PGI_2) share the same receptor in platelet membranes and inhibit platelet aggregation by increasing cyclic AMP (cAMP) levels through activation of receptor-linked adenylate cyclase.[35] PGE_1/PGI_2 receptors in platelets have been shown to be important in the prevention of platelet activation/aggregation.[12,45] In cardiovascular diseases, PGE_1 receptor numbers have been shown to be decreased/impaired and thus their platelets become hyperactive because of lack of production of cAMP.[12,35,45] Therefore, decreased PGE_1 receptor number of platelets is considered a risk factor associated with cardiovascular disease.[12,46] The underlying mechanism of up-regulation of PGE_1 receptor activity by linoleic acid-rich diet is not known at present, but it is possible that this diet up-regulates PGE_1 receptor activity by increasing insulin sensitivity.[12,34,45] Hyperactivity of platelets, and their adhesion and aggregation at the site of the injury in atherosclerotic vessel walls, is critically important in the pathogenesis of cardiovascular disease.[1–4] Given the high incidence of cardiovascular disease in developed countries, there is still a great need for research into the prevention of platelet hyperactivity by dietary intervention. A linoleic acid-rich diet may be beneficial compared with the stearic acid- and oleic acid-rich diets with respect to platelet function.

In conclusion, short-term intake of diets with similar fat content (38% as energy) but with distinctly different fatty acid compositions have no influence on plasma concentration of FVII, tPA activity, and PAI-1 activity in young, healthy men. Further research is needed to evaluate the effects of these diets on blood lipids and hemostatic variables in different population groups at risk for coronary heart disease and in subjects with disorders in lipid metabolism.

Acknowledgments

This work was supported by the Ministry of Agriculture, Food and Fisheries (MAFF), United Kingdom, and in part by the Scottish Executive Rural Affairs Department (SERAD). We thank Unilever (Vlaardingen, The Netherlands) and the Henkel Corporation for the gifts of test oils and vitamin E, respectively. We thank Ms. Anna M. Clohessy for excellent work in the metabolic kitchen. We are also grateful to Dr. Gert W. Meijer (Unilever) for his valuable comments on the diet formulation and Dr. Graham Horgan (BIOSIS) for statistical analysis of the data.

Table 8 Effect of diets on platelet aggregation[1]

Diet	Day 0	Day 14
Stearic acid-rich diet	80.6 ± 11	83.3 ± 13
Oleic acid-rich diet	83.8 ± 13	79.9 ± 13
Linoleic acid-rich diet	86.6 ± 8	86.3 ± 9

[1] Platelet aggregation is expressed as % of maximun platelet aggregation in response to 10 μM adenosine diphosphate.

Table 9 Effect of diets on platelet membrane anisotropy (r_s) parameter

Diet	Day 0	Day 14
Stearic acid-rich diet	0.236 ± 0.014	0.241 ± 0.017
Oleic acid-rich diet	0.237 ± 0.013	0.234 ± 0.013
Linoleic acid-rich diet	0.236 ± 0.013	0.238 ± 0.012

Table 10 Effect on platelet prostaglandin E_1 receptor activity

Diet	Day 0	Day 14
	(fmol prostaglandin E_1 bound/50 μg protein)	
Stearic acid-rich diet	3.40 ± 0.70	3.95 ± 1.1
Oleic acid-rich diet	3.64 ± 0.7	3.40 ± 0.99
Linoleic acid-rich diet	3.09 ± 0.34	3.98 ± 0.61[a]

[a] $P < 0.05$ vs. day 0.

References

1 Meade, T.W., Brozovic, M., Chakrabarti, R.R., Haines, A.P., Imeson, J.D., Mellow, S., Miller, G.J., North, W.R.S., Stirling, Y., and Thompson, S.G. (1986). Haemostatic function and ischaemic heart disease principal results of the Northwick Park Heart study. *Lancet* **2,** 533–537

2 Hamsten, A., De Fair, Y., Walldius, G., Dahlen, G., Szamosi, A., Landou, C., Blomback, M., and Wiman, B. (1987). Plasminogen activator inhibitor in plasma: Risk factor for recurrent myocardial infarction. *Lancet* **2,** 3–9

3 Nordoy, A., Hatcher, L., Goodnight, S., Fitzgerald, G.A., and Connor, W.E. (1994). Effects of dietary fat content, saturated fatty acids, and fish oil on eicosanoid production and hemostatic parameters in normal men. *J. Lab. Clin. Med* **123,** 914–920

4 Dutta-Roy, A.K., Ray, T.K., and Sinha, A.K. (1986). Prostacyclin stimulation of the activation of blood coagulation factor X by platelets. *Science* **231,** 385–389.

5 Gram, J., Jespersen, J., Kuft, C., and Rijken, D.C. (1987). On the usefulness of fibrinolysis variables in the characterisation of a risk group for myocardial infarction. *Acta Med. Scand.* **221,** 149–153

6 Hamsten, A., Wirman, B., de Faire, U., and Blomback, M. (1985). Increased plasma levels of a rapid inhibitor of tissue plasminogen activator in young survivors of myocardial infarction. *N. Engl. J Med* **313,** 1557–1563

7 Ernst, E. (1993). The role of fibrinogen as a cardiovascular risk factor. *Atherosclerosis* **100,** 310–318

8 Ridker, P.M., Vaughan, D.E., Stampfer, M.J., Manson, J.E., and Hennekens, C.H. (1993). Endogenous tissue type plasminogen activator and risk of myocardial infarction. *Lancet* **341,** 1165–1168

9 Ridker, P.M., Hennekens, C.H., Stampfer, M.J., Manson, J.E., and Vaughan, D.E. (1994). Prospective study of endogenous tissue plasminogen activator and risk of stroke. *Lancet* **343,** 940–943

10 Thompson, S.G., Kienast, J., Pyke, D.M., Haverkate. F., and van deloo, J.C.W. (1994). Haemostatic factors and the risk of myocardial infarction or sudden death in patients with angina pectoris. *N. Engl. J. Med.* **332,** 635–641

11 Miller, G.J. (1997). Dietary fatty acids and blood coagulation. *Prostaglandins Leukot. Essent. Fatty Acids* **57,** 389–394

12 Dutta-Roy, A.K. (1994). Insulin mediated processes in platelets, erythrocytes and monocytes/macrophages: Effects of essential fatty acid metabolism. *Prostaglandins Leukot. Essent. Fatty Acids* **51,** 385–399

13 Orth, M., Luley, C., Mayer, H., and Wieland, H. (1995). Responsiveness of ATIII and coagulation factors V and VII to a standardised oral fat load. *Thromb. Res.* **80,**265–270

14 Jackson, R.L., Taunton, O.D., Morrissett, J.D., and Gotto A.M. Jr. (1978). The role of dietary polyunsaturated fats in lowering basal cholesterol in man. *Circ. Res.* **42,** 447–453

15 Bladbjerg, E.M., Marckmann, P., Sandstrom, B., and Jespersen, J. (1994). Non-fasting factor VII coagulant activity (FVII:C) increased by high-fat diet. *Thromb. Haemost.* **71,** 755–758

16 Miller, G.J. (1997). Some interactions between the haemostatic system and lipids. *Fibrinolys. Proteolys.* **11(suppl 1),** 67–69

17 Salvadori, R. (1984). *La Dieta Mideterranea*. Idee Libro Publishers, Rome, Italy

18 Mattson, M.A. and Grundy, S.M. (1985). Comparison of the effects of dietary saturated, monounsaturated and polyunsaturated fatty acids on plasma lipids and lipoproteins in man. *J. Lipid Res.* **26,** 194–202

19 Barradas, M.A., Christofides, J.A., Jeremy, J., Mikhailidis, D.P., Fry, D.E., and Dandona P. (1990). The effect of olive oil supplementation on human platelet function, serum cholesterol-related variables and plasma fibrinogen concentrations: A pilot study. *Nutr. Res.* **10,** 403–411

20 Lopez-Segura, F., Velasco, F., Lopez-Miranda, J., Castro, P., Lopez-Pedrera, J., Torres, A., Trujillo, J., Ordovas, J.M., and Perez-Jimenez, F. (1996). Monounsaturated fatty acid-enriched diet decreases plasma plasminogen activator inhibitor type 1. *Arterioscler. Thromb. Vasc. Biol.* **16,** 82–88

21 Sanders, T.A.B. , Miller, G.J., de Grassi, T., and Yahia, N. (1996). Postprandial activation of coagulant factor VII by long-chain dietary fatty acids. *Thromb. Haemost.* **76,** 369–371

22 Bronte-Stewart, B., Keys, A., Brock, J.F., Moodie, A.D., Keys, M.H., and Anronis, A. (1995). Serum cholesterol, diet and coronary heart disease: An inter-racial survey in the Cape Peninsula. *Lancet* **ii,** 1103–1108

23 Hoak, J.C. (1994). Stearic acid, clotting, and thrombosis. *Am. J. Clin. Nutr.* **60(S),** 1050S–1053S

24 Tholstrup, T., Marckmann, P., Jespersen, J., and Sandstrom, B. (1994). Fat high in stearic acid favourably affects blood lipids and factor VII coagulant activity in comparison with fats high in palmitic acid or high in myristic and lauric acids. *Am. J. Clin. Nutr.* **59,** 371–377

25 Hunter, K.A., Crosbie, L.C., Weir, A., Miller, G.J., and Dutta-Roy, A.K. (1999). The effects of structurally defined triacylglycerols of differing fatty acid composition on postprandial haemostasis in young, healthy men. *Atherosclerosis* **142,** 151–159

26 Dutta-Roy, A.K., Gordon, M.J., Campbell, F.M., Duthie, G.G., and James, W.P.T. (1994). Vitamin E requirements, transport, and metabolism: Role of α-tocopherol-binding proteins. *J. Nutr. Biochem.* **5,** 562–570

27 Bellizzi, M.C., Dutta-Roy, A.K., Duthie, G.G., and James, W.P.T. (1996). α-Tocopherol binding activity of red blood cells. *Free Radic. Res.* **27,** 105–116

28 Gregory, J., Foster, K., and Wiseman, M. (1990). *The Dietary and Nutritional Survey of British Adults: A Survey of the Dietary Behaviour, Nutritional Status and Blood Pressure of Adults Aged 16 to 64 Living in Great Britain*. Office of Population Census and Surveys, Social Survey Division, HMSO, London, UK

29 Miller, G.J., Stirling, Y., Esnouf, M.P., Heinrich, J., van de Loo, J., Kienast, J., Wu, K.K., Morrissey, J.H., Meade, T.W., Martin, J.C., Imeson, J.D., Cooper, J.A., and Finch, A. (1994). Factor VII-deficient plasmas depleted of protein C raise the sensitivity of the factor VII bioassay to activated factor VII: An international study. *Thromb. Haemost.* **71,** 38–41

30 Morrissey, J.H., Macik, B.G., Neuenschwander, P.F., and Comp, P.C. (1993). Quantitation of activated factor VII levels in plasma using a tissue factor mutant selectively deficient in promoting factor VII activation. *Blood* **81,** 734–744

31 Clauss, A. (1957). Gerinnugsphysiologische Schnell-methode zur Bestimmug des Fibrinogens. *Acta Haematol.* **17,** 237–246

32 Bligh, B.E. and Dyer, W.J. (1959). A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37,** 911–917

33 Dutta-Roy, A.K., Gordon, M.J., Campbell, F.M., and Crosbie, L.C. (1996). Arachidonic acid uptake by human platelets is mediated by CD36. *Platelets* **7,** 291–295

34 Dutta-Roy, A.K., Hoque, L., and Paterson, B.J. (1993). Prostaglandin E_1 binding sites in erythrocyte membranes. *Eur. J. Biochem.* **213,** 1167–1173

35 Dutta-Roy, A.K. and Sinha, A.K. (1987). Purification and properties of prostaglandin E_1/prostacyclin receptor of human blood platelets. *J. Biol. Chem.* **262,** 12685–12691

36 Stevenson, E.T., Davy, K.P., and Seals, D.R. (1995). Hemostatic, metabolic and androgenic risk factors for coronary heart disease in physically active and less active post-menopausal women. *Arterioscler. Thromb. Vasc. Biol.* **15,** 669–677

37 Marckmann, P., Sandstrom, B., and Jespersen, J. (1990). Effects of total fat content and fatty acid composition in diet on factor VII coagulant activity and blood lipids. *Atherosclerosis* **80,** 227–233

38 Miller, G.J., Martin, J.C., Webster, J., Wilkes, H., Miller, N.E., Wilkinson, W.H., and Meade, T.W. (1986). Association between dietary fat intake and plasma factor VII coagulant activity—a predictor of cardiovascular mortality. *Atherosclerosis* **60,** 269–277

39 Mitropoulos, K.A., Miller, G.J., Martin, J.C., Reeves, B.E.A., and Cooper, J. (1994). Dietary fat induces changes in factor VII coagulant activity through effects of plasma free stearic acid concentration. *Arterioscler. Thromb.* **14,** 214–222
40 Marckmann, P., Jespersen, J., Leth, T., and Sandstrom, B. (1991). Effects of fish diet versus meat diet on blood lipids, coagulation and fibrinolysis in healthy young men. *J. Intern. Med.* **229,** 317–323
41 Slabber, M., Barnard, H.C., Kuyl, J.M., and Badenhorst, C.J. (1992). Effect of a short-term very low calorie diet on plasma lipids, fibrinogen, and factor VII in obese subjects. *Clin. Biochem.* **25,** 334–335
42 Bonanome, A. and Grundy, S.M. (1988). Effect of dietary stearic acid on plasma cholesterol and lipoprotein levels. *N. Engl. J. Med.* **318,** 1244–1248
43 Zock, P.L. and Katan, M.B. (1994). Hydrogenation alternatives: Effects of trans fatty acids and stearic acid versus linoleic acid on serum lipids and lipoproteins in human. *J. Lipid Res.* **33,** 399–410
44 Bladbjerg, E.M., Tholstrup, T., Marckmann, P., Sandstrom, B., and Jesepersen, J. (1995). Dietary changes in fasting levels of factor VII coagulant activity (VII:C) are accompanied by changes in factor VII protein and other vitamin K-dependent proteins. *Thromb. Haemost.* **73,** 239–242
45 Dutta-Roy, A.K., Kahn, N.N., and Sinha, A.K. (1991). Interaction of receptors for prostaglandin E_1/prostacyclin and insulin in human erythrocytes and platelets. *Life Sci.* **49,** 1129–1139
46 Chirkov, Y.V., Chirkova, L.P., Sage, R.E., and Horowitz J.D. (1995). Impaired responsiveness of platelets from patients with stable angina-pectoris to antiaggregating and cyclic AMP elevating effects of protsaglandin E_1. *J. Cardiovasc. Pharmacol.* **25,** 961–966