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High intakes of *trans* monounsaturated fatty acids taken for 2 weeks do not influence procoagulant and fibrinolytic risk markers for CHD in young healthy men

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Dietary *trans* fatty acids are associated with increased risk of CHD. We hypothesized that the changes in plasma lipids associated with a high intake of *trans* fatty acids would cause adverse effects on procoagulant and fibrinolytic activities. A randomized crossover controlled feeding study was conducted in twenty-nine men. A *trans*-rich diet supplying 10 % energy as *trans*-18:1 was compared with diets in which the *trans* fatty acids were replaced either with carbohydrate or oleate; each diet was taken for 2 weeks in random order. Fasting fibrinogen and D-dimer concentrations and factor VII coagulant, plasminogen activator inhibitor type 1 and tissue plasminogen activator did not differ between diets. Postprandially, tissue plasminogen activator activity increased and plasminogen activator inhibitor type 1 activity decreased on all diets. Factor VIIc increased postprandially by 15 and 17 % on the *trans* and oleate diets respectively, compared with an 11 % increase on the carbohydrate diet; the mean difference between oleate and carbohydrate diets was 6 (95 % CI 0·2, 11·9) %. The LDL-cholesterol: HDL-cholesterol and apolipoprotein B : apolipoprotein A-I ratios increased by 13 (95 % CI 5·7, 21·8) and 10 (95 % CI 3·1, 17·2) % respectively on the *trans* diet compared with the oleate diet and by 6 (95 % CI 0·1,12·7) and 7 (95 % CI 0, 13·5) % respectively compared with the carbohydrate diet. Plasma HDL_2-cholesterol concentration was 18 (95 % CI 0·7, 35·9) % lower on the *trans* diet compared with the oleate diet. The results confirm adverse effects of *trans* fatty acids on HDL-cholesterol concentrations, but suggest that *trans* fatty acids do not have any specific effects on known haemostatic risk markers for cardiovascular disease in healthy young men in the short-term.

Lipids: Fibrinolysis: Coagulation: *Trans* fatty acids

The industrial hardening of edible oils employed in the manufacture of margarine and cooking fats results in the formation of monounsaturated fatty acids with a *trans* configuration. The major *trans* isomers in human diets are *trans*-18 : 1 isomers (British Nutrition Foundation, 1995). Dietary *trans* fatty acids have been associated with an increased risk of CHD in the USA and Europe (Ascherio *et al.* 1996; Hu *et al.* 1997; Pietinen *et al.* 1997; Oomen *et al.* 2001), but this finding has not been confirmed by studies in Europe, where the dietary intake of *trans* fatty acids has been assessed by measuring the proportion of *trans* fatty acids in adipose tissue (Aro *et al.* 1995; Roberts *et al.* 1995; van de Vijver *et al.* 2000). The mechanism by which *trans* fatty acids may increase risk is uncertain. *Trans* isomers of 18 : 1 fatty acids increase the plasma LDL-cholesterol:HDL-cholesterol ratio compared with oleate (Mensink & Katan, 1990; Nestel *et al.* 1992; Judd *et al.* 1994; Almendingen *et al.* 1995; Aro *et al.* 1997; Lichtenstein *et al.* 1999). Some (Nestel *et al.* 1992; Almendingen *et al.* 1995; Aro *et al.* 1997) but not all studies (Clevidence *et al.* 1997) report that diets enriched with *trans* fatty acids increase lipoprotein(a) (Lp(a))

Abbreviations: FVIIc, factor VII coagulant activity; Lp(a), lipoprotein(a); PAI-1, plasminogen activator inhibitor type 1; TAG, triacylglycerol; *t*-PA, tissue plasminogen activator.

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concentrations. Elevated Lp(a) concentrations have been postulated to decrease fibrinolytic activity (Stein & Rosenson, 1997), which in turn may increase risk of CHD (Meade *et al.* 1993). Fibrinolytic activity is determined largely by the balance between tissue plasminogen activator (*t*-PA) and plasminogen activator inhibitor type 1 (PAI-1) activities (MacCallum *et al.* 1998). Elevated PAI-1 activity is associated with increased risk of CHD in young survivors of CHD (Hamsten *et al.* 1985), and is also associated with elevated plasma triacylglycerol (TAG) concentrations and low HDL-cholesterol concentrations.

In vitro studies show that *trans*-18 : 1 leads to activation of factor VII in comparison with oleic acid (Mitropoulos *et al.* 1993). The Northwick Park Heart study (Meade *et al.* 1993) and the PROCAM study (Heinrich *et al.* 1994) found raised plasma fibrinogen and factor VII coagulant activity (FVIIc) to be associated with an increased risk of fatal CHD. Fasting FVIIc is positively associated with plasma cholesterol and TAG concentrations and increases following a meal high in fat. Fibrinogen is a more powerful risk factor for CHD, may act as marker for inflammation and is positively associated with obesity and smoking. Increased procoagulant activity is usually matched by increased fibrinolytic activity. The plasma concentration of D-dimer, which are products resulting from the degradation of fibrin by plasmin, is a sensitive indicator of the formation of fibrin and its digestion (Stoetzer *et al.* 1988) and has been used a global marker of fibrin turnover (Danesh *et al.* 2001).

Two previous studies (Almendingen *et al.* 1996; Mutanen & Aro, 1997) have reported the effects of longer-term intake of *trans* fatty acids on procoagulant and fibrinolytic CHD risk factors. However, both studies made comparison with diets rich in saturated fatty rather than carbohydrate or the corresponding *cis*-isomers. We hypothesized that an increased plasma concentration of *trans*-18 : 1 in lipoproteins would result in adverse changes in procoagulant and fibrinoytic activities in comparison with carbohydrate or oleate (*cis*-18 : 1*n*-9) in the diet. Because changes in plasma lipoprotein concentrations can be brought about in 2 weeks and the turnover of procoagulant and fibrinolytic factors is rapid (h rather than d), we decided to test this hypothesis by feeding male subjects for 2-week periods.

Subjects, materials and methods

Subjects and setting

Thirty-six healthy Caucasian men aged 18–35 years were recruited from among staff and students of King's College, London, UK. The screening procedure involved the completion of a detailed questionnaire to establish general health, details of prescribed medicines, pattern of alcohol consumption and food preferences and aversions that may interfere with acceptability of the diets. Exclusion criteria included current use of anticoagulant medication, current smoker, diabetes mellitus, CHD, fasting cholesterol $>6{\cdot}5$ mmol/l, fasting TAG concentration $>2{\cdot}3$ mmol/l, HDL-cholesterol $<0{\cdot}9$ mmol/l, BMI >28 kg/m^2, self-reported alcohol intake of more than twenty-one units per week, abnormal haematology or liver function tests, current use of vitamins or dietary supplements or following a vegetarian diet.

A small fasting venous blood sample (17 ml) was obtained for measurement of plasma total cholesterol, plasma HDL-cholesterol, plasma TAG, full blood count, FVIIc, fibrinogen, liver function tests and serum cotinine. Habitual dietary intake was assessed by a 3 d weighed dietary record; these were converted to nutrient intakes by computer from food tables based on data from *McCance and Widdowson's The Composition of Foods* (Holland *et al.* 1991). Height and weight were measured using a beam balance and stadiometer in order to establish BMI.

Smoking was an exclusion criterion because of its effect on fibrinogen concentration (Meade *et al.* 1987). However, as it is well known that a significant proportion of subjects who report themselves as non-smokers are deceivers. In order to verify non-smoking status, measurement of plasma cotinine (Feyerabend & Russell, 1990) was conducted prior to entry into the study and at intervals throughout the study. Body weight and whole blood counts were further recorded at the end of each dietary period. Of the forty-two subjects who expressed interest in the study, thirty-six subjects met the inclusion criteria; of these, four subjects withdrew before completing the first dietary period and three further subjects withdrew after the first treatment period for personal reasons. A total of twenty-nine subjects completed the study and their details are shown in Table 1; four of the subjects who completed the study were found to have plasma cotinine concentrations that exceeded the cut-off points for non-smokers during the intervention period, and their results were excluded from the statistical analyses for fibrinogen. Subjects were given a detailed outline of the study requirements and all provided written informed consent, as did their general practitioners. No financial incentive was given, but all food during the study period was provided free of charge. The study protocol was reviewed and approved by the Research Ethics Committee of King's College, London.

Table 1. Baseline characteristics of the twenty-nine male subjects who completed the study

(Mean values and standard deviations)

	Mean	SD
Age (years)	24·2	5·9
BMI (kg/m^2)	24·2	2·2
Energy intake (MJ/d)	12·6	2·9
Protein (% energy)	14·2	3·6
Fat (% energy)	35·3	8·1
Carbohydrate (% energy)	46·7	7·0
Alcohol (% energy)	3·6	5·0
Dietary fibre (g/d)	32	10
Plasma cholesterol (mmol/l)	4·63	0·69
LDL-cholesterol (mmol/l)	2·75	0·55
HDL-cholesterol (mmol/l)	1·32	0·33
Plasma triacylglycerol (mmol/l)	1·23	0·61
Fibrinogen (mg/l)	2·66	0·57
Factor VII coagulant activity (% standard)	103	34

Experimental design

A randomized crossover design of three diets was employed. Each experimental diet was taken for 14 d and the diets were taken consecutively without any washout period. The subjects were allocated in random order to receive each of the diets in one of six sequences (ABC, ACB, BAC, BCA, CAB and CBA) according to an orthogonal Latin square design in order to balance out any possible treatment order effects. A treatment period of 14 d was selected, as this is sufficient to bring about changes in plasma lipid concentration (Mensink & Katan, 1992). A washout period was not employed as previous studies with *trans* fatty acids have shown that *trans* fatty acids do not have a persistent effect on blood lipids (Mensink & Katan, 1990), unlike long-chain *n*-3 fatty acids (Sanders *et al.* 1997). As haemostatic variables were the main targets of the present study and these have a rapid turnover, measurable in h rather than d (Miller *et al.* 1991; de Maat, 2001), a study of 14 d was believed to be adequate to test whether a high intake of *trans* fatty acids had adverse effects on haemostatic function. In order to check for carryover effects, measurements of the concentration of *trans*-18 : 1 in total plasma lipids and platelets were made. The outcome variables were changes in lipid and haemostatic variables; these were derived from blood samples that were obtained after an overnight fast on the last 2 d of each treatment period and following a standardized meal that had a similar fat composition to the treatment diet. For plasma lipids, fibrinogen and FVIIc measurements were made on both days to obtain a more accurate estimate.

Experimental diets

The experimental diets supplied an energy intake of 13 MJ/d (consistent with energy balance in these subjects) and were designed so as to compare the effect of substituting 10 % energy either as *trans*-18 : 1 or as carbohydrate or as oleate. The intake of saturated, polyunsaturated fatty acids, cholesterol and fibre were held constant. The high carbohydrate, low-fat diet was similar to the American Heart Association Step 1 diet (Yu-Poth *et al*. 1999).

The diets were formulated using very-low-fat, ordinary foodstuffs, e.g. lean meat and low-fat dairy products. The majority of the fat was supplied by added oils and spreads used in the food preparation: these accounted for 80 % fat in the high-fat diets and 73 % fat in the low-fat diets, and were manufactured to specification by Unilever Research Laboratories (Vlaardingen, The Netherlands). A reduced-fat (600 g fat/kg) spread was supplied for use in the reduced fat, high-carbohydrate diet and two margarines (810 g fat/kg) were supplied for the high-fat diets. All the variation in the fatty acid composition between the high-oleic acid diet and the high-*trans* diet was accounted for in the composition of the spreads. Consequently these two diets were identical in every other respect. The *trans*- rich spread contained 336 g *trans* fatty acids/kg compared with 2 g *trans* fatty acids/kg for the high-oleate spread. The distribution of positional *trans* isomers of 18 : 1 in the *trans*- rich spread according to the manufacturer was as follows (%): *n*-6+*n*-7 + *n*-8 33·9, *n*-9 22·1, *n*-10 19·6, *n*-11 10·8, *n*-12 6·7, *n*-13 5·7, *n*-14 1·1.

Vitamins A (46·8 mg β-carotene/kg) and D (79·4 μg cholecalciferol/kg) were added to the oleate- and *trans*-rich margarines: this is a level consistent with that found in commercially available margarines in the UK. The reduced-fat margarine was supplemented with these vitamins at a higher concentration (57·72 mg β-carotene/kg and 97·9 μg cholecalciferol/kg) to account for the smaller amounts fed. Levels of vitamin E were standardized across the different oils and spreads by the addition of α-tocopherol to match the level found in the high-oleic acid oil. The low-fat diet comprised the same meals as the two high-fat diets except that the 10 % deficit in energy resulting from the use of the low-fat spread was supplied by additional carbohydrate in the form of bread and sucrose.

All food consumed by the subjects over the 6-week experimental period was specially prepared in a dietetic kitchen and provided in preweighed portions. The subjects were blind to the allocation of treatment with regard to *trans*- and oleate-rich diets. It was not possible to allocate the carbohydrate-rich diet blind. Coffee intake was restricted to not more than four cups per d. The subjects were not provided with alcoholic beverages, but were permitted to consume alcohol up to two standard units (20 ml ethanol) on each day during the study, except for the final 3 d of each dietary period when alcohol intake was forbidden. Black tea, white tea (with milk from the daily allowance) and low-energy soft drinks were permitted *ad libitum*.

Subjects were provided with a prepacked breakfast to consume at home and a prepacked lunchtime meal (sandwiches) each day. The subjects consumed their evening meal under supervision in a metabolic facility. At weekends subjects were provided with prepacked meals for consumption at home.

Test meals

On the last morning of each diet following the collection of a fasting blood sample, subjects consumed a test meal with a similar fatty acid composition to the background diet in the metabolic facility. The test meals, each supplying 6 MJ, consisted of food similar to that usually consumed in the main evening meal of the day; the fat content was calculated to be 90 g for the oleate- and *trans*-rich diets and 67 g for the carbohydrate-rich diet.

Replicate meal analysis

Replicates of the daily diet and test meals were analysed for total energy (bomb calorimetry), protein (Kjeldahl), total fat (Soxhlet) and fatty acids (GLC) using methods previously described (Sanders *et al.* 1997). The contribution of individual fatty acids to total energy intake was estimated assuming 37·7 kJ (9 kcal)/g fatty acid.

Blood sampling

Venous blood samples were obtained after an overnight fast on the last 2 d of each dietary period. Subjects were

asked to avoid strenuous activity on the days preceding blood samples. Additional samples were collected at 3 and 7 h after each test meal. Blood was collected using the vacutainer technique and particular care was taken to avoid excessive venous stasis and cold-activation of factor VII; the detailed protocol for handling of the blood samples for the haemostatic assays has been described previously (Sanders *et al.* 2001); samples for haemostatic assays were rapidly frozen in liquid N_2 and stored in sealed polypropylene tubes at −70°C until assayed. A 2 ml ETDA sample for blood counts was maintained at room temperature until analysed on the same day at the Department of Haematology, St Thomas's Hospital. Samples for lipid assays were collected into vacutainers (Becton Dickinson, Cowley, Oxford, UK) containing EDTA, chilled to 4°C and centrifuged within 3 h. Platelets were separated from the EDTA samples by low-speed centrifugation at 120 ***g*** for 20 min at 4°C to yield platelet-rich plasma. Platelet-poor plasma for lipid and lipoprotein analyses was prepared by centrifuging the platelet-rich plasma at 1500 ***g*** for 15 min at 4°C. The platelet pellet was washed three times with 5 ml ice-cold saline solution (8·9 g NaCl/l containing 40 mg EDTA/l); lipid extracts were prepared within 6 h of blood collection and stored in solvents containing 50 mg butylated hydroxytoluene/l at −20°C until analysed (Sanders & Roshanai, 1992). Plasma was kept at 4°C pending analysis of cholesterol, TAG and HDL subfractions within 5 d. Apolipoprotein concentrations were determined on samples of plasma stored at −70°C.

Laboratory measurements

Haemostatic assays were determined on frozen samples with all of the samples for one subject being assayed in the same batch in the coagulation laboratory of MRC Epidemiology and Medical Care Unit. The methods for determining plasma fibrinogen and FVIIc have previously been described (Sanders *et al.* 1997). Plasma D-dimer concentration was measured by ELISA (Chromogenix AB, Mölndal, Sweden); *t*-PA and PAI-1 activities were determined by chromogenic assay (Chromogenix AB). Full blood and platelet counts were determined on a Coulter counter (Coulter Instruments, Bedford, Beds., UK) in the Department of Haematology, St Thomas's Hospital. Liver function tests were undertaken in the Department of Clinical Pathology, St Thomas's Hospital and serum cotinine concentrations were determined by GC (Feyerabend & Russell, 1990) in the Medical Toxicology Unit (New Cross, London, UK).

Plasma cholesterol and TAG concentrations and apolipoprotein concentrations were determined by enzymatic and immunoturbidometric assays, as previously described, at the Wynne Institute of Metabolic Medicine, London, UK (Sanders *et al.* 1997). Platelet glycerophospholipid fatty acid composition was determined by GLC using a 25 m BP75 capillary column (SGE Europe, Milton Keynes, UK). Chylomicrons were isolated from plasma from the 3 h postprandial sample by overlaying plasma with 0·195-M NaCl containing 1 g EDTA/l and centrifuging for 15 min at 19 000 rpm in a SW41 swing bucket rotor (Beckman Ltd, High Wycombe, UK). Lipids were extracted with chloroform–methanol (1:1, v/v) from the chylomicron fraction, given a 'Folch wash' and separated by TLC on plates coated with Silica Gel G (Merck UK, Poole, Dorset, UK) and then developed in hexane–diethyl ether–acetic acid (80:20:1, by vol.). The TAG fraction was methylated with HCl (5 g/l methanol)–toluene (4:1, v/v). Fatty acid methyl esters were analysed by GLC on a 50 m capillary column coated with CpSil88 (Chrompak, London, UK).

Statistical analysis

Twenty-nine subjects completed all dietary treatments. Each individual's results for the two fasting samples were averaged in each period. Tests for differences between dietary periods were performed by ANOVA within subject. This approach assumes that the effects of treatment and the effects of period operate independently, and the model is invalid when significant interaction exists. Order effects and interaction were tested using the method of Pocock (1983). When significant differences were found between diets in the ANOVA, pairs of diets were compared using paired *t* tests. Adjustment for order effect was made when necessary using Bonferroni's correction for multiple comparisons. The study had >80 % power to detect a 5 % difference in FVIIc and fibrinogen between pairs of diets ($P<0·05$). For Lp(a), in which the distribution was highly skewed, period effects and interaction were sought on untransformed values using the Mann–Whitney test. In the comparison of three diets and non-paired comparisons, the non-parametric Friedman test and Sign test were employed on untransformed data respectively. Four subjects were found to be positive for plasma cotinine and their results were excluded from the statistical analysis for plasma fibrinogen.

Results

Table 1 shows the details of the twenty-nine subjects who completed the study; there were five subjects in each treatment sequence except for the sequence ACB where there were four. Body weights remained stable throughout the study. Four subjects had plasma cotinine values >14 μg/l and were, therefore, regarded as smokers and their results were excluded from the statistical analysis for plasma fibrinogen. Plasma cotinine levels were low in the other twenty-five subjects throughout the study: the median value was 0·7 (range 0·1–5·3) μg/l.

Dietary characteristics and fatty acid composition

Table 2 shows the analysed composition of the experimental diets. The intakes as a proportion of the dietary energy were close to those planned. The intake of *cis*-polyunsaturated fatty acids and saturated fatty acids was similar for all three diets. The proportion of energy derived from oleic acid (18 : 1*n*-9) was similar on the carbohydrate and *trans* diets but was twice as high in the oleate diet. *Trans* fatty acids accounted for 0·1 % energy intake on both the carbohydrate and oleate diets compared with 9·6 % on the *trans* diet. The oleate and *trans* test meals were designed to provide 6 MJ, 90 g fat, 41 g protein and 116 g carbohydrate.

Table 2. Composition of the experimental diets as determined by chemical analyses*

Diet...	*Trans*		Oleate		Carbohydrate	
	Mean	SD	Mean	SD	Mean	SD
Protein (% energy)	13·1	1·2	13	1	14	1·2
Carbohydrate (% energy)	46·8	2·2	47	2	53	1·8
Fat (% energy)	40·1	2·4	40	2	32	1·8
Fatty acids (% energy)	37·5	2·2	37·5	2·2	30·2	1·7
12 : 0	0·3	0·17	0·3	0·17	0·2	0·16
14 : 0	0·2	0·02	0·2	0·02	0·3	0·03
16 : 0	5·4	0·58	5·9	0·58	6·2	0·62
16 : 1	0·4	0·16	0·4	0·16	1	0·1
18 : 0	2·1	0·34	2·4	0·33	2·8	0·29
trans-18 : 1	9·6	0·74	0·1	0·01	0·1	0·01
cis-18 : 1	11·9	0·69	20·3	1·09	11·8	0·57
18 : 2*n*-6	5·5	0·53	5·9	0·55	5·8	0·61
18 : 3*n*-3	0·7	0·05	0·6	0·04	0·6	0·04
Saturated fatty acids	9·5	1·42	10·2	1·41	10·9	1·45
12 : 0 + 14 : 0 + 16 : 0	5·9	0·74	6·4	0·74	6·6	0·79
cis-Monounsaturated fatty acids	12·3	0·8	20·7	1·14	12·8	0·55
trans-Monounsaturated fatty acids	9·6	0·74	0·1	0·01	0·1	0
cis-Polyunsaturated fatty acids	6·1	0·56	6·5	0·57	6·4	0·62

* For details of analytical procedures, see p. 768.

On analysis, the oleate and *trans* meals supplied 87 g fat compared with the target value of 90 g. The carbohydrate meal was designed to provide 6 MJ, 67 g fat, 48 g protein and 167 g carbohydrate. On analysis the carbohydrate test meal supplied 65 g fat compared with the target value of 67 g. The fatty acid composition of the test meals was found to be similar to that of the parent diets (results not shown).

Biochemical indices of compliance to the diet

The *trans* diet led to a marked rise in *trans*-18 : 1 in plasma, but there was no evidence of any carryover effect between treatment periods (Fig. 1). The proportion of *trans*-18 : 1 in platelet lipids ($P<0·001$) increased to 4·2 (SEM 0·28) compared with 0·2 (SEM 0·06) and 0·3 (SEM 0·8) g/100 g total fatty acids on the carbohydrate and oleate diets respectively. The increase in the proportion of *trans*-18 : 1 was paralleled by a significant reduction ($P<0·01$) in the proportion of stearic acid (18:0) to 15·4 (SEM 0·26) compared with 18·3 (SEM 0·29) and 17·3 (SEM 0·35) g/100 g total fatty acids on the carbohydrate and oleate diets respectively. This change was short-lived and there was no evidence of any carryover effect into the next treatment period.

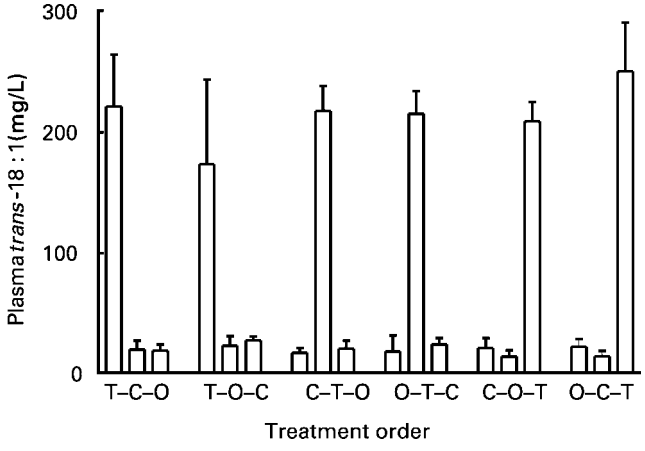

Fig. 1. Plasma concentrations of *trans*-18 : 1 in total plasma lipids according to treatment sequence. T, *trans*; C, carbohydrate; O, oleate. For details of subjects, diets and procedures, see Tables 1 and 2 and p. 768. Values are means with 95% CI for five subjects, except for sequence C–T–O when *n* 4.

Plasma lipids and lipoproteins

Table 3 shows the results for the fasting plasma lipids and lipoproteins concentrations. Statistically significant differences between diets in HDL- and HDL_2-cholesterol concentrations ($P=0·009$ and $P=0·042$) were noted between diets. Plasma HDL- and HDL_2-cholesterol concentrations were lower on the *trans* diet compared with the oleate diet ($P=0·018$ and $P=0·05$ respectively). Repeated-measures ANOVA revealed a significant effect of diet on the LDL-cholesterol:HDL-cholesterol and apolipoprotein B:apolipoprotein A-I ratios ($P=0·0004$ and $P=0·0027$). The LDL-cholesterol:HDL-cholesterol and apolipoprotein B:apolipoprotein A-I ratios were significantly higher on the *trans* diet compared with the oleate (both $P<0·01$) and carbohydrate diets (both $P<0·05$).

Table 4 shows the postprandial changes in plasma cholesterol and TAG concentrations. Plasma cholesterol fell compared with the fasting value on all diets ($P=0·0001$) following the test meals. There was a significant difference between diets in the postprandial changes in plasma cholesterol ($P=0·05$); the normalized area under the curve for the postprandial change in plasma cholesterol following the oleate diet was 18·5 (95% CI 6·9, 30·0) % lower ($P<0·01$) compared with the high-carbohydrate diet. As expected, the increase in plasma TAG concentration from fasting was greater on the oleate diet and the *trans* diet than on the carbohydrate

Table 3. Body weight and fasting plasma lipid and lipoprotein concentrations in subjects following diets containing 10 % energy from *trans*-18 : 1 compared with diets where the energy from *trans*-18 : 1 had been replaced with oleate or carbohydrate‡
(Mean values and standard deviations for twenty-nine subjects)

	Trans		Oleate		Carbohydrate		Statistical significance of effect (*F*): *P*
Diet. . .	Mean	SD	Mean	SD	Mean	SD	
Body weight (kg)	78·2	8·3	78	8·2	78·1	7·9	NS
Cholesterol (mmol/l)	4·28	0·44	4·18	0·64	4·30	0·62	NS
LDL-cholesterol (mmol/l)	2·60	0·44	2·45	0·57	2·54	0·51	0·07
HDL cholesterol (mmol/l)	1·18††	0·28	1·26	0·29	1·23	0·27	0·009
HDL_2-cholesterol (mmol/l)	0·16 ††	0·09	0·20	0·13	0·18	0·09	0·042
HDL_3-cholesterol (mmol/l)	1·01	0·19	1·04	0·2	1·04	0·16	NS
LDL-cholesterol:HDL-cholesterol	2·20*†††	0·67	1·94	0·62	2·07	0·62	0·0004
Triacylglycerol (mmol/l)§	1·05	0·47	0·97	0·45	1·12	0·69	NS
Lp(a) (mg/l)¶	64	24–275	50	29–236	58	27–288	NS
Apolipoprotein A-I (g/l)	1·12	0·20	1·18	0·17	1·16	0·17	NS
Apolipoprotein A-II (g/l)	0·38	0·06	0·39	0·05	0·4	0·05	NS
Apolipoprotein B (g/l)	0·63	0·13	0·60	0·12	0·61	0·14	NS
Apolipoprotein B:apolipoprotein A-I	0·56††*	0·15	0·51	0·13	0·52	0·13	0·0027

Lp(a), lipoprotein (a).
Mean values were significantly different from those of the carbohydrate diet: *$P<0·05$.
Mean values were significantly different from those of the oleate diet: ††$P<0·01$, †††$P<0·001$.
‡ For details of subjects, diets and procedures, see Tables 1 and 2 and p. 768.
§ Geometric mean values with approximate SD.
¶ Median values with interquartile range.

diet ($P<0·0001$ and $P=0·0003$ respectively) at 3 h (the differences between the oleate and *trans* diets were not significant). The differences in the normalized area under the curve for plasma TAG on the oleate and *trans* diets compared with the carbohydrate diet were both statistically significant ($P<0·01$); the mean difference was 430 (95 % CI 247, 613) on the oleate diet and 315 (95 % CI 147, 483) arbitrary units on the *trans* diet. Analysis of the fatty acid composition of the chylomicron-TAG collected 3 h after the test meal showed that the proportions of fatty acids were similar to those in the test meals (results not shown).

Haemostatic variables

Fasting FVIIc, fibrinogen concentration, PAI-1 and *t*-PA activities, and D-dimer concentration did not differ following all three diets (Table 3). However, plasma PAI-1 activity declined postprandially ($P=0·0001$) and t-PA activity increased postprandially ($P=0·0001$), but the differences between diets were not significant. Fibrinogen concentration changed postprandially ($P=0·002$). Analysis of the deviations from fasting revealed a significant time effect ($P<0·00001$) but no significant time or time×diet interactions. Values tended to fall at 3 h compared with fasting, then rise at 7 h. Plasma FVIIc increased postprandially on all diets ($P=0·0002$) and there was a significant difference between diets in the change. The increase in FVIIc at 3 h compared with the fasting value was greater ($P=0·02$) following the oleate compared with the carbohydrate meal: the mean difference was 6·1 (95 % CI −11·9, −0·20) %. Plasma FVIIc almost returned to the fasting value after the carbohydrate meal at 7 h, but was still significantly greater than the fasting value after the oleate and *trans* meals ($P=0·04$ and $P=0·004$ respectively). No other statistically significant differences were noted. Blood counts and platelet counts remained within normal ranges and no statistically significant differences were noted between treatments (results not shown).

Discussion

The intake of *trans* fatty acids in the UK diet is estimated to be approximately 2 % energy intake, equivalent to 5 g/d (British Nutrition Foundation, 1995). The present study used an intake of *trans* fatty acids some five-fold greater than this when expressed in terms of % energy. The absolute intake of *trans* fatty acids was even greater, about 38 g/d, as the energy intake of the subjects was relatively high. Margarine was chosen as the source of *trans* fatty acids as studies have reported an association between *trans* fatty acids provided by margarine, as opposed to those provided by ruminant animal fats, and risk of CHD (Willett *et al.* 1993). A high intake was employed in order to provoke potentially adverse effects on haemostatic function. The *trans* diet supplied a mixture of isomers of *trans*-18 : 1, typical of that found in industrially hydrogenated fat (Wolff *et al.* 1998). Platelet glycerophospholipid fatty acid composition was measured as a biomarker of *trans* fatty acid intake (Mensink & Hornstra, 1995). *Trans*-18 : 1 was incorporated into the platelets mainly at the expense of

Table 4. Fasting and postprandial changes in plasma cholesterol, triacylglycerol and haemostatic variables in male subjects following diets containing 10% energy from *trans*-18 : 1 compared with diets where the energy from *trans*-18 : 1 had been replaced with oleate or carbohydrate‡ (Mean values and standard deviations)

Diet...			Carbohydrate		Oleate		*Trans*	
	n	Time	Mean	SD	Mean	SD	Mean	SD
Cholesterol (mmol/l)	29	Fasting	4·27	0·62	4·20	0·66	4·32	0·46
		3 h	4·06††	1·01	4·00††	0·71	4·15††	0·63
		7 h	4·24	0·68	4·08	0·64	4·27	0·46
Triacylglycerol (mmol/l)	29	Fasting	1·04	0·44	0·88	0·39	0·97	0·40
		3 h	1·64††	0·82	2·23**††	1·50	2·26**††	1·54
		7 h	1·64	0·70	1·72††	0·70	1·86††	0·95
PAI-1 (units × 10^3/l)	27	Fasting	13·92	5·47	12·84	6·79	12·67	5·97
		3 h	9·03††	3·34	8·88††	4·39	8·49††	5·36
		7 h	6·93††	3·79	6·89††	3·40	7·14††	3·59
t-PA (units × 10^3/l)§	28	Fasting	1·68	1·14	1·86	1·10	1·86	1·08
		3 h	2·19††	0·89	2·65††	1·03	2·52††	1·21
		7 h	2·70††	0·91	2·77††	0·79	3·00††	1·11
D-dimer (μg/l)‖	29	Fasting	37	29–83	37	27–97	37	25–125
		3 h	42	26–92	39	20–93	42	22–110
		7 h	43	24–83	40	29–78	39	26–104
Fibrinogen (g/l)¶	25	Fasting	2·43	0·44	2·37	0·44	2·41	0·37
		3 h	2·42	0·51	2·35	0·51	2·27†	0·39
		7 h	2·53	0·45	2·37	0·45	2·40	0·37
FVIIC (%)	29	Fasting	101	27	101	23	101	22
		3 h	112††	31	118*††	30	116††	29
		7 h	103	31	106†	28	107††	26

PAI-1, plasminogen activator inhibitor type 1; *t*-PA, tissue plasminogen activator; FVIIC, Factor VII coagulant activity.
Mean values were significantly different from those of the carbohydrate diet: *$P<0·05$, **$P<0·01$.
Mean values were significantly different from fasting values: †$P<0·05$, ††$P<0·01$.
‡ For details of subjects, diets and procedures, see Tables 1 and 2 and p. 768.
§ Geometric mean values and standard deviations.
‖ Median values and interquartile ranges.
¶ Values for non-smoking subjects only.

stearic acid (18 : 0) and demonstrated good compliance to the dietary regimen. The absorption of *trans* fatty acids appeared to be high as the proportion of *trans* fatty acids in chylomicron TAG was similar to that in the dietary fat. The plasma concentrations of *trans*-18 : 1 in plasma total lipids increased markedly following the consumption of the *trans* diet but importantly returned to normal values following the high-carbohydrate and high-oleate diets. This demonstrates that the effects of *trans* fatty acids on plasma lipids are short-lived and the study was not confounded by carryover effects. Furthermore, statistical testing for carryover effects did not reveal any significant effect of treatment order on the outcome variables.

The main effect of the *trans* diet was to lower HDL- and HDL_2-cholesterol concentrations, which is in agreement with another report (Judd *et al.* 1994). In agreement with previous reports (Sanders *et al.* 1997), plasma cholesterol concentrations were found to fall significantly following a meal. Plasma cholesterol concentrations fell to a greater extent postprandially following the oleate diet compared with carbohydrate diet. The reason for this requires further study. Patsch *et al.* (1992) have argued that elevated TAG concentrations 6–8 h following a meal indicate impaired clearance of TAG-rich lipoproteins and are associated with risk of CHD. Both the 3 h plasma TAG concentrations and normalized areas under the curves were greater on the oleate and *trans* diets compared with the carbohydrate diet. This finding that the *trans* isomers of 18 : 1 does not differ from oleic acid is in agreement with the results of a test meal study (Sanders *et al.* 2000). As neither fasting nor postprandial TAG concentrations were greater on the *trans* diet, the lower HDL-cholesterol concentrations cannot be attributed to increased VLDL synthesis. The lower HDL_2-cholesterol could result from increased hepatic lipase activity or cholesteryl ester transfer protein activity (Abbey & Nestel, 1994), which would cause a higher rate of catabolism of HDL. In the present study the *trans*-18 : 1 diet did not differ with regard to its effects on Lp(a) from oleate, in agreement with Clevidence *et al.* (1997), or from carbohydrate. We have previously argued (Sanders *et al.* 1997) that Lp(a) concentrations are influenced by dietary fatty acid chain-length rather than *cis*–*trans* configuration.

The main purpose of the present study was to investigate the effects of *trans* fatty acids on haemostatic risk factors for CHD in both the fasted and fed state. In the present study, we were careful to ensure that venepunctures were atraumatic and we have previously demonstrated that under the conditions used plasma concentrations of fibrinopeptide A are low (Sanders *et al.* 2000, 2001). FVIIc and fibrinogen were also measured on two occasions at the end of each treatment period to decrease error. Fasting plasma FVIIc has been reported to be similar on diets containing *trans* fatty acids compared with diets rich in saturated fatty acids (Almendingen *et al.* 1996; Mutanen & Aro, 1997). In the present study, fasting plasma FVIIc did not differ between oleate or *trans*- 18 : 1 rich diets. However, plasma FVIIc activity increased postprandially

to a greater extent on the oleate diet than on the carbohydrate diet. Furthermore, FVIIc remained elevated 7 h following the test meal on both the oleate and *trans*-18 : 1 diets, but had returned to the fasting value on the carbohydrate diet. Our present observations do not support the contention by Larsen *et al.* (1999) that a diet rich in oleic acid results in a fall in FVIIc and are consistent with our previous study (Sanders *et al.* 1997).

Almendingen *et al.* (1996) compared the effects of partially hydrogenated fish oil, partially hydrogenated soyabean oil and butterfat on fibrinolytic and coagulation variables in thirty-one young men using a crossover design with each diet being taken for 3 weeks. A criticism of that study is that the intakes of saturated fatty acids were not controlled and consequently the observed effects cannot be attributed to the *trans* fatty acid content of the diet. Mutanen & Aro (1997) used a parallel design to study the effects of changing from a butter-rich diet either to one containing 8·7 % energy as *trans*-18 : 1 from partially hydrogenated vegetable oil or to a diet high (9·3 % energy) in stearic acid for 5 weeks in predominantly young female subjects. However, in that study the comparison was between diets high in saturated fatty acids, which may have adverse effects on coagulation and fibrinolysis, and menstrual cycle variations are known to lead to substantial variations in haemostatic variables. Furthermore, neither of the previous studies adequately controlled for smoking habit, which can affect plasma fibrinogen.

The PAI-1 and *t*-PA activities reported in the present study are similar to the values reported (Sanders *et al.* 1997) in male subjects consuming diets supplying 30 % energy from fat, which were either high (butter) or low (olive oil) in saturated fat. Almendingen *et al.* (1996) found that the consumption of partially hydrogenated soyabean oil resulted in higher PAI-1 antigen concentrations and PAI-1 activity compared with a butter-rich diet or a diet containing partially hydrogenated fish oil. However, Mutanen & Aro (1997) found no change on a diet high in *trans*-18 : 1 compared with a stearate-rich or a butter-rich diet. Muller *et al.* (2001) compared the effects of three diets with three different margarines, one based on palm oil, one based on partially hydrogenated soyabean oil and one with a high content of polyunsaturated fatty acids, on diurnal postprandial haemostatic variables in nine subjects. These authors found no effect on PAI-1 activity, but reported lower *t*-PA activity on the partially hydrogenated soyabean oil diet. The results of the present study, which suggest that *trans*-18 : 1 (where the *trans* fatty acids were derived from isomerized high-oleic acid sunflower) had no effect on PAI-1 or *t*-PA activity. It is possible that partially hydrogenated soyabean oil contains other isomeric fatty acids not found in the isomerized high-oleic acid sunflower oil used in the present study and that these exert differing effects, especially as isomers are formed from linolenic acid (18 : 3*n*-3), which is present in significant concentrations in soyabean oil. Increased PAI-1 activity is strongly associated with plasma TAG concentration and, therefore, might be expected to increase following the consumption of a high-fat meal. However, in the present study, PAI-1 activity was found to decline, in agreement with previous studies from our group (Oakley *et al.* 1998; Sanders *et al.* 2001). It is notable that PAI-1 activity was not increased on the carbohydrate diet compared with the oleate diet; this is in contrast with a study by Lopez-Segura *et al.* (1996), who reported a significant decline in PAI-1 activity in twenty-one young male subjects following a high-oleate diet compared with a reduced-fat diet (National Cholesterol Education Program Step 1 diet; Yu-Poth *et al*. 1999).

Plasma fibrinogen is an acute-phase protein and can reflect inflammation. Almendingen *et al.* (1996) reported that plasma fibrinogen decreased on a diet containing partially hydrogenated fish oil compared with a butter-rich diet. However, Mutanen & Aro (1997) reported a significant increase in plasma fibrinogen on a stearic acid-rich diet but not on the *trans*-18 : 1-rich diet compared with a butter-rich diet. In the present study care was taken to exclude any influence of cigarette smoking, which is known to elevate plasma fibrinogen, and non-smoking status was confirmed by measurement of plasma cotinine. Furthermore, replicate measurements of fibrinogen were made to decrease error. The variation in plasma fibrinogen was lower and the geometric mean values differed by <2 %. Our present results suggest that plasma fibrinogen is not influenced by the oleic or *trans*-18 : 1 content of the diet in healthy men. There was no evidence of any change in D-dimer concentrations: D-dimer is a global marker of fibrin degradation and this would be consistent with a lack of effect on procoagulant and fibrinolytic activity.

The *trans*-18 : 1 diet increased the LDL-cholesterol:HDL-cholesterol ratio by 13 % compared with a high-oleate diet and by 6 % compared with a carbohydrate-rich diet similar to the National Cholesterol Education Programme Step I diet (Yu-Poth *et al.* 1999). The changes were even less favourable when compared with the high-oleate diet: the *trans*-18 : 1 diet decreased the concentrations of HDL- and HDL_2-cholesterol by 6 and 18 % respectively. In conclusion, *trans*-18 : 1 has an unfavourable effect on some lipid indices of CHD risk, but does not appear to differ from oleate with regard to its effects on known procoagulant and fibrinolytic risk markers in healthy young non-obese men, at least in the short-term. Other mechanisms are required to explain the apparent adverse effects of *trans* fatty acids on risk of CHD.

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