https://doi.org/10.1017/S0007114598001299 Published online by Cambridge University Press

Effect on plasma lipids and lipoproteins of replacing partially hydrogenated fish oil with vegetable fat in margarine

Hanne Müller[1], Odd Jordal[2], Ingebjørg Seljeflot[3,4], Peter Kierulf[3], Bente Kirkhus[5], Oddlaug Ledsaak[1] and Jan I. Pedersen[1,2]*

[1]*Akershus College, 1340 Bekkestua, Norway*
[2]*Institute for Nutrition Research, University of Oslo, PO Box 1046 Blindern, 0316 Oslo, Norway*
[3]*Clinical Chemistry Department and* [4]*Research Forum, Ullevaal University Hospital, 0407 Oslo, Norway*
[5]*Mills DA, 0506 Oslo, Norway*

(Received 26 November 1997 – Revised 18 March 1998 – Accepted 27 March 1998)

We have compared the effects on lipoproteins and haemostatic variables of two hard margarines with similar functional properties, one traditional margarine containing partially hydrogenated fish oil (PHFO), and one experimental margarine based on vegetable oil (VO). Both were all-purpose cooking margarines with nearly identical functional properties. *Trans* fatty acids from PHFO in the traditional margarine were replaced mostly by saturated, monounsaturated and *trans* fatty acids of vegetable origin in the new formulation. Both test margarines contained approximately the same amount of *cis* polyunsaturated fatty acids. Sixteen female normolipidaemic students consumed each diet with the two test margarines for 14 d in random order (crossover design). The amount of fat was 31 % energy in the PHFO diet and 32 % energy in the VO diet. The test margarines provided approximately 26 % energy in both diets. In the PHFO diet 7·8 % of the energy was derived from *trans* fatty acids and 9·2 % from saturated fatty acids (12:0, 14:0 and 16:0) while in the VO diet, 1·1 % energy was derived from *trans* fatty acids and 13·3 % from saturated fatty acids (12:0, 14:0 and 16:0). The natural content of cholesterol in PHFO was deliberately not balanced by addition of cholesterol to the VO diet, thus the PHFO diet contained 215 mg and the VO diet 86 mg cholesterol per 8·5 MJ. LDL-cholesterol concentration was 19 % higher in subjects on the PHFO diet compared with the VO diet ($P < 0·01$). The ratio LDL-cholesterol:HDL-cholesterol was 12·6 % higher in subjects on the PHFO diet compared with the VO diet ($P < 0·01$). The level of apolipoprotein (apo)A-I was 6 % lower in subjects on the PHFO diet compared with the VO diet ($P < 0·01$). The ratio apoB:apoA-I was 10·4 % higher in subjects on the PHFO diet than on the VO diet ($P < 0·01$). There were no significant differences in total cholesterol, HDL-cholesterol, triacylglycerols, apoB, lipoprotein(a) and haemostatic variables between the diets. Our results demonstrate that PHFO, with its unfavourable effects on plasma lipids, can be replaced by vegetable oils in margarine without appreciable loss of functional properties but with significant improvement in the effects on plasma lipoproteins.

Plasma lipoproteins: Fish oil: Hydrogenated fat: *Trans* fatty acids

Partially hydrogenated oils, both vegetable oils and fish oils, have for a very long time been important ingredients in margarine production. Accordingly, the consumption of *trans* fatty acids has increased considerably in industrialized countries since the beginning of this century. Like saturated fatty acids, *trans* fatty acids from partially hydrogenated oils raise plasma total- and LDL-cholesterol levels (Mensink & Katan, 1990; Nestel *et al*. 1992; Zock & Katan, 1992; Judd *et al*. 1994; Almendingen *et al*. 1995) and in addition lower plasma HDL-cholesterol (Mensink & Katan, 1990; Zock & Katan, 1992) and increase lipoprotein(a) (Lp(a)) levels (Mensink *et al*. 1992; Nestel *et al*. 1992; Almendingen *et al*. 1995; Aro *et al*. 1997). Concerns have therefore been raised as to the continued use of partially hydrogenated oils in margarine production. We have previously shown that partially hydrogenated fish oil (PHFO) is at least as potent as butterfat and significantly more potent than partially hydrogenated soyabean oil in raising plasma total- and LDL-cholesterol levels (Almendingen *et al*. 1995). In addition it reduces HDL-cholesterol and increases Lp(a) levels

Abbreviations: apo, apolipoprotein; Lp(a), lipoprotein(a); PAI-1, plasminogen activator inhibitor-1; PHFO, partially hydrogenated fish oil; t-PA, tissue plasminogen activator; VO, vegetable oil.

***Corresponding author:** Dr Jan I. Pedersen, fax +47 22 85 15 30, email j.i.pedersen@basalmed.uio.no

(Almendingen *et al*. 1995). It would therefore seem prudent to reduce the use of PHFO in margarine products. PHFO has functional properties, however, that make it particularly suitable for the baking industry which requires margarines of certain hardness, as well as for household food preparation. It is, therefore, of interest to find alternative fat blends that maintain the functional properties of the margarine but with improved effects on plasma lipids. The aim of the present study was to compare the effects on plasma lipoproteins and haemostatic variables of two hard test margarines with similar functional properties, one traditional margarine based on PHFO and one based entirely on vegetable oils.

Methods

Participants and their baseline characteristics

Young female students of home economics were invited to participate in a strictly controlled dietary study. The sixteen volunteers who entered the study with a mean age of 22 (SD 2·7, range 19–30) years were all normolipidaemic, in good health and showed no intolerance with normal dietary habits. Except for oral contraceptives none was taking any medication known to affect serum lipids. Criteria for inclusion were that the participants should be reliable and have a regular meal pattern. We had no screening criteria with regard to smoking habits, age, physical activity or body weight.

All participants were requested to maintain their regular lifestyles and usual level of physical activities throughout the study. They were asked to abstain from alcohol consumption during the study period. In addition they were asked to report in a diary any deviation from their usual behaviour. Nine women were taking oral contraceptives and nine were smokers. Four subjects had relatives with a known history of cardiovascular disease, none of which was identified as familial hypercholesterolaemia. One person had a diagnosis of asthma while another had allergy. Both of them used medicines.

The average weight of the participants was 64·5 (SD 7·6) kg ranging from 54·6 to 83·3 kg and the BMI ranged from 20 to 29 kg/m^2 (mean 23 (SD 2·3) kg/m^2). All except one had a BMI below 25 kg/m^2. The mean diastolic blood pressure was 70 (SD 6·3) mmHg and the mean systolic blood pressure was 119 (SD 11·9) mmHg. For baseline levels of serum lipids, lipoproteins and fibrinolytic variables, see Table 3.

The protocol and the objective of the study were explained to the subjects in detail and they gave their written consent before entry into the study. They received free food but no payment during the study. The study protocol was approved by the Regional Committee for Ethics in Biomedical Research of Norway.

Habitual diet

The participants filled in a quantitive food-frequency questionnaire and each questionnaire was thoroughly checked. The quantitative food-frequency questionnaire, which is designed to cover the whole diet, has been validated and described elsewhere (Nes *et al*. 1992). The questionnaire is meant to give the usual intake during the past year. The calculated mean amount of total fat in the habitual diet was found to be 29 (SD 4·9) % of energy. The mean daily intakes of saturated, monounsaturated and polyunsaturated fatty acids were 24, 22 and 10·9 g respectively, and the intakes of linoleic and α-linolenic acids were 8·5 g and 1·1 g respectively. The calculated mean intake of dietary cholesterol was 220 mg/d.

Study design

The study ran from May to June 1995, during periods of 14 d. Mensink & Katan (1987) recently confirmed previous studies which showed that new stable levels of serum lipids and lipoproteins are attained within 14 d on a controlled diet (Keys *et al*. 1957; Brussaard *et al*. 1982; Mensink & Katan, 1987). It cannot be excluded, however, that the maximal response to *trans* fat may require more than 2 weeks (Judd *et al*. 1994). If this is the case any observed differences between the high and the low *trans* fat diets used here may tend to be underestimated. The study was designed as an intra-individidual crossover comparison of the effects on blood lipids and haemostatic variables of the two diets. The participants received the diets simultaneously and both diets were given in both periods. The participants were randomized into two groups and each group received the two diets in a different order. In this way, variation due to residual effects of the previous diet or to drift of variables over time could be minimized. After the end of the first test period, the participants crossed over to the second diet with a washout period of 4 d. During this period the subjects returned to their normal eating habits.

Body weight was monitored before lunch twice weekly. The weight was measured with light clothes on a digital balance, and read to the nearest 0·1 kg. Body height was measured without shoes and read to the nearest 1 mm. BMI was calculated as weight (kg)/height (m)^2. Dietary compliance was checked by interviewing and by evaluating the diaries.

Test margarines

One of the test margarines was a traditional hard margarine with a high content of PHFO in addition to some soyabean and coconut oils (PHFO margarine). The other was a hard margarine based on palm oil, soyabean oil, coconut oil, partially hydrogenated palm oil and fractionated palm oil (VO margarine). *Trans* fatty acids in the PHFO margarine were replaced mostly by saturated (8·8 g/100 g total fatty acids), monounsaturated (13·7 g/100 g) and *trans* fatty acids (3·4 g/100 g) from vegetable oils in the VO margarine. Both test margarines contained approximately the same content of *cis* polyunsaturated fatty acids. The fatty acid compositions of the two test margarines are given in Table 2. Both margarines (all-purpose cooking margarines) were subjected to sensory analyses, texture measurements (Stevens LFRA Texture analyser, Stevens Advanced Weighing Systems, Blackburn, Lancs., UK; 15°), solid fat measurements (Bruker minispec p20i; Bruker Analytical Instruments, Rheinstetten, Germany) as well as baking and frying tests.

https://doi.org/10.1017/S0007114598001299 Published online by Cambridge University Press

https://doi.org/10.1017/S0007114598001299 Published online by Cambridge University Press

The products were considered to be similar with regard to functional properties. However, the VO margarine was slightly harder and more brittle at lower temperature and became slightly softer at room temperature. The overall quality of both was considered equal to that of commercial margarines.

Since fish oil has a natural content of cholesterol the PHFO margarine contained 2310 mg cholesterol/kg. The VO margarine contained a negligible amount of cholesterol (210 mg/kg) derived from the added emulsifier. The margarines were produced by the addition of water, vitamin A, vitamin D, NaCl, flavour, emulsifier and β-carotene as colour. The PHFO margarine contained 165, and the VO margarine 155 g water/kg.

Experimental test diets

The two diets were based on a 7 d menu. They were calculated by using a computer-based, nutrient-calculation programme and were designed to have the same nutrient composition except for the fatty acid composition and cholesterol content. Both diets were calculated to contain (% energy): 15·4 protein, 34 fat and 50·6 carbohydrate. The fat from the background diet was calculated to supply a minimal amount of 6 % energy as fat while the test fat was planned to provide 28 % energy as fat. The menu for the two experimental diets contained the same basic food items and the fat from the background diet came from meat, fish, bread, dairy products and cereals. The two experimental diets differed only in the source of test margarine used for spreading, baking and cooking. The test fats were incorporated into the menus in several foods including bread, buns, porridge and sauces for dinner. All meals including weekend meals were prepared at the college. Lunch and dinner were served under supervision in a dining room from Monday to Friday. Supper and breakfast for the next day were sent home. Each Friday weekend meals were packaged for home consumption. All sauces for weekend dinner were provided frozen. During the controlled feeding periods no foods other than those in the menu were allowed. If the participants lost weight or were hungry, they were allowed to eat buns with the same nutrient composition as the rest of the diet. The participants were allowed to drink coffee, tea, and mineral water with artificial sweeteners. Each diet was assigned a colour code and an energy level. All foodstuffs were weighed for each individual subject. The subjects were supplied with food to meet 100 % of their mean daily energy requirements. The PHFO and VO diets were calculated to contain 89·5 g fat/10 MJ, of which the test margarines provided 74 g.

Compliance with the diets was judged by direct observation of consumption of weekday lunch and dinner, and by evaluation of food diaries. All participants complied well and only minor deviations from the diet were noted, e.g. light yoghurt was omitted from the menu twice by two participants. The fasting body weights were not significantly different at the end of each of the two periods (mean reduction of 0·4 kg).

Chemical analysis

Duplicate portions were taken of the two diets corresponding to a daily energy intake of 8·5 MJ. The duplicate portions were kept frozen at –20°. After homogenization the homogenates were freeze-dried and the homogenates from 7 d were pooled into one portion.

The N content in the duplicate portions was determined by the Kjeldahl technique. The factor used for conversion of N content to protein was 6·25. The total fat content was determined by chloroform–methanol extraction (Folch *et al.* 1957). The metabolizable energy content of the diets was determined as described by Andersson *et al.* (1975).

For analysis of fatty acid composition, the lipid fractions of the duplicate portions were isolated by Soxhlet extraction of about 5 g of freeze-dried homogenate from each series. The solvent used was diethyl ether, analytical grade, and the extraction time was 4 h. Quantitatively the lipid recovery was almost the same as extraction with the more polar solvent mixture chloroform–methanol (Folch *et al.* 1957). The fatty acids of the respective fat extracts were converted to methyl esters by the BF_3 method (American Oil Chemist's Society, 1989*a*) and analysed by GLC using the polar SP-2560 fused silica capillary column (100 m × 0·25 mm i.d.) as described in an earlier study (Almendingen *et al.* 1995).

The cholesterol content in the test fats and the homogenates was determined on the lipid extract as the trimethylsilyl ether derivative on GLC (American Oil Chemist's Society, 1989*b*). 5β, 3α-Cholestanol was used as internal standard, and the separation of cholesterol, tocopherols, and plant sterols was acceptable on the nonpolar DB-5 capillary column used (30 m × 0·25 mm).

Blood sampling and analyses

Blood samples were collected after an overnight fast before breakfast at the end (day 15) of each period. During the week before the start, and at the start of the first period baseline samples were taken. Serum was obtained by low-speed centrifugation within 1 h of venepuncture and stored at –70° until analysed.

Serum cholesterol and serum triacylglycerols were measured by enzymic methods (Nägele *et al.* 1985) using automated analyser equipment (Hitachi 737, Hitachi Limited, Tokyo, Japan). LDL-cholesterol was calculated using the Friedewald equation (Friedewald *et al.* 1972).

Serum HDL-cholesterol was measured essentially by a similar enzymic technique (Siedel *et al.* 1985) after precipitation of the LDL-fraction with dextran sulfate and Mg (Technicon Reagent T 0,1-2801-56, Tarrytown, NY, USA). Serum apolipoprotein (apo)A-I (Orion Diagnostika, Espoo, Finland) and serum apoB (Behringwerke Ag, Marburg, Germany) were both quantified immunoturbidometrically using a seven-point standard curve and an automated enzyme analyser (Cobas Fara, Hoffman–La Roche, Basel, Switzerland) essentially according to the manufacturer's instructions. Serum Lp(a) was quantified by a commercial kit (TintElize Lp(a), Biopool AB, Umeå, Sweden) according to the manufacturer's instructions. The interassay CV were as follows (%): total cholesterol 2, HDL-cholesterol 5, triacylglycerols 3, apoA-I 6·3, apoB 5·5 and Lp(a) 7·7 % at 100 mg/l, and 2·7 %, at 400 mg/l.

For haemostatic variables citrated plasma was obtained

Table 1. Content of energy and nutrients of duplicate portions of test diets containing partially hydrogenated fish oil (PHFO) or vegetable oil (VO) (portions at estimated 8·5 MJ/d)

	PHFO diet	VO diet
Energy (MJ)	8·75	8·77
Protein (% energy)	16·2	15·8
Carbohydrate (% energy)	53·1	51·8
Fat (% energy)	30·7	32·4
12:0, 14:0, 16:0 (% energy)	9·2	13·3
trans fatty acids (% energy)	7·7	1·1
cis monounsaturated fatty acids (% energy)	5·8	9·0
cis polyunsaturated fatty acids (% energy)	4·1	4·9
Cholesterol (mg)	215	86

from Vacutainer tubes containing 0·129 mM-trisodium citrate (dilution 1:10) by centrifugation within 15 min at 2500 ***g*** (30 min at 4°) for determination of plasminogen activator inhibitor type-1 (PAI-1) activity, PAI-1 antigen and fibrinogen. Acidified plasma for tissue plasminogen activator (t-PA) activity measurements was obtained using Stabilyte^R tubes as described by Rånby *et al.* (1989). All samples were stored at –70° until analysis.

PAI-1 activity and t-PA activity were measured amidolytically, according to Wiman *et al.* (1983) and Chmielewska *et al.* (1983). An ELISA method with a double-antibody technique was used for determinations of PAI-1 antigen (measuring free PAI-1 as well as that complexed with t-PA). Commercially available kits (Biopool AB) were used: Spectrolyse/pL, Spectrolyse/fibrin and TintElize PAI-1 respectively. The interassay CV were 4·5 % for PAI-1 activity, 9·8 % for PAI-1 antigen, and 8·0 % for t-PA activity. Fibrinogen was measured according to Clauss (1957) using an ACL-3000 Coagulation System Analyzer (Instrumentation laboratory, Milan, Italy), interassay CV 4·8 %.

All blood samples from the same individual were analysed within one run. The analyses were performed at the Clinical Chemistry Department and Clinical Research Unit, Ullevaal University Hospital, Oslo.

Statistical methods

Pairwise comparisons between the two diet groups were performed and mean differences among treatments were analysed by paired *t* tests with 95 % CI. *P* values < 0·05 were considered significant. All *P*-values are two-tailed. Serum Lp(a) and the haemostatic variables had a skewed distribution and were log-transformed before pairwise comparisons were performed by paired *t* test. Pairwise comparisons of the untransformed data were also performed by the Wilcoxon rank test. The *P* values were somewhat higher in *t* tests compared with the Wilcoxon rank tests.

Pearson correlation coefficients between baseline levels of serum lipids and apolipoproteins are presented when appropriate. Spearman correlation coefficients were also calculated for haemostatic variables. The statistical package SPSS Advanced Statistics, version 6.1 (SPSS Inc., Chicago, IL, USA) was used for the data analysis. A test for carry-over effect was performed as described by Jones & Kenwood (1989).

The power for detecting differences in serum lipids was calculated to be approximately 0·5 for total serum cholesterol, 0·9 for LDL-cholesterol, 0·6 for HDL-cholesterol, 0·8 for LDL-cholesterol:HDL-cholesterol, 0·9 for apoA, 0·8 for apoB, 0·6 for Lp(a) and <0·5 for triacylglycerols.

Results

Test diets

The duplicate portions of the two diets corresponding to an estimated intake of 8·5 MJ were analysed and the energy level was found to be identical in both diets (Table 1). The intake of energy from protein was identical in both diets, and that from fat was slightly lower in the PHFO diet than in the VO diet (Table 1). The PHFO diet contained the highest amount of energy derived from *trans* fatty acids while the VO diet had the highest content of energy derived from saturated fatty acids. The content of cholesterol was higher in the PHFO diet than in the VO diet; the levels were 246 mg and 98 mg/10 MJ respectively.

The fatty acid compositions of the margarines and duplicate portions of the diets as determined by GLC are shown in Table 2. The PHFO margarine contained a total of 30·1 g *trans* fatty acids/100 g total fatty acids (16·2 g *trans* monoenes and 13·9 g *trans* dienes). The VO margarine contained 3·4 g *trans* fatty acids/100 g total fatty acids, almost all as *trans* monoenes.

The PHFO diet had the highest content of very-long-chain saturated fatty acids (20:0–24:0). The sum of saturated fatty acids (12:0-16:0) and *trans* fatty acids was highest in the PHFO diet, 55 *v.* 45 g/100 g total fatty acids in the VO diet (corresponding to 16·9 and 14·6 % energy respectively).

Serum lipids and apolipoproteins

Table 3 shows concentrations of total-, LDL-, and HDL-cholesterol, apoB, apoA-I and triacylglycerols at baseline and after the two test diets. Also shown are the mean differences between the two diets and their statistical significance probabilities. No carry-over effects were observed from the first to the second period.

Total serum cholesterol was highest on the PHFO diet, but the difference between the two diets was not significant. The levels of LDL-cholesterol were significantly higher in subjects on the PHFO diet compared with the VO diet ($P \leq 0·01$). The levels of HDL-cholesterol were not significantly different between the two diets.

Serum apoB level was slightly higher on the PHFO diet but the difference did not reach statistical significance ($P = 0·08$). Compared with the level on the PHFO diet, the level of apoA-I was significantly increased on the VO diet ($P < 0·001$).

Significant differences of the ratios LDL-cholesterol: HDL-cholesterol and apoB:apoA-I were observed between the two diets, both being highest on the PHFO diet. Thus, the ratio LDL-cholesterol:HDL-cholesterol was 12·6 % higher, and the ratio apoB:apoA-I 10·4 % higher on the PHFO diet than on the VO diet.

There were no significant differences in the levels of Lp(a) between the two diets.

https://doi.org/10.1017/S0007114598001299 Published online by Cambridge University Press

https://doi.org/10.1017/S0007114598001299 Published online by Cambridge University Press

Table 2. Fatty acid composition (g/100 g total fatty acids) of the fat extracts of margarines containing partially hydrogenated fish oil (PHFO) or vegetable oil (VO) and their corresponding diets

Fatty acid	PHFO margarine	PHFO diet	VO margarine	VO diet
6:0	-	0·1	0·8	0·2
8:0	1·0	0·8	1·4	1·2
10:0	0·8	0·8	0·9	1·2
12:0	5·6	4·8	7·3	6·5
14:0	6·7	6·8	3·5	4·2
15:0	0·4	0·5	0·1	0·2
15:1	0·1	0·1	-	-
16:0	16·0	18·2	31·9	30·4
16:1*t*	4·5	4·0	0·2	0·2
16:1*c*	2·2	2·5	0·2	0·4
17:0	0·6	0·5	0·1	0·2
17:1	-	0·2	-	-
18:0	5·8	6·8	4·8	5·9
18:1*t*	6·1	6·6	3·0	2·9
18:1*c*	9·1	12·2	28·3	27·5
18:2*t* *	1·5	1·0	0·2	0·3
18:2*c*	10·0	10·2	14·1	13·5
18:3*c*	1·2	1·2	1·4	1·7
20:0	2·0	1·5	0·4	0·4
20:1*t*	3·6	3·0	-	-
20:1*c*	2·1	2·2	0·2	0·2
20:2*t* *	7·2	5·2	-	-
20:2*c*	1·4	1·2	-	-
22:0	1·0	0·8	0·2	0·2
22:1*t*	1·7	1·6	-	-
22:1*c*	1·5	1·6	-	-
22:2*t* *	5·2	3·6	-	-
22:2*c*	1·2	0·9	-	-
24:0	0·4	0·4	0·2	0·3
24:1*t*	0·3	0·1	-	-
24:1*c*	0·1	0·2	-	-

c, *cis* isomer; *t*, *trans* isomer.
* Includes *trans,trans, cis,trans* and *trans,cis.*

Table 3. Serum lipid and lipoprotein levels of female subjects at baseline and after 14 d of consuming diets containing partially hydrogenated fish oil (PHFO) or vegetable oil (VO) margarines, and differences between the two test periods*

(Mean values and standard deviations for sixteen subjects)

	Baseline		PHFO diet		VO diet		Mean differences (PHFO−VO)	*P* value	95 % CI
	Mean	SD	Mean	SD	Mean	SD			
Total cholesterol (mmol/l)	4·44	0·54	4·64	0·68	4·46	0·53	0·18	0·11	(−0·04, 0·39)
LDL-cholesterol (mmol/l)	2·41	0·53	2·87	0·52	2·63	0·47	0·24	0·01	(0·06, 0·42)
HDL-cholesterol (mmol/l)	1·46	0·28	1·28	0·30	1·32	0·27	−0·05	0·21	(−0·12, 0·03)
LDL-cholesterol:HDL-cholesterol	1·74	0·44	2·32	0·58	2·06	0·54	0·26	0·01	(0·06, 0·46)
Triacylglycerols (mmol/l)	1·04	0·27	1·10	0·41	1·13	0·47	−0·025	0·74	(−0·18, 0·13)
ApoB (g/l)	0·96	0·14	1·05	0·17	1·00	0·14	0·05	0·08	(−0·01, 0·97)
ApoA-I (g/l)	1·75	0·25	1·53	0·23	1·62	0·28	−0·10	0·01	(−0·17, −0·03)
ApoB:ApoA-I	0·55	0·10	0·69	0·12	0·63	0·11	0·07	0·01	(0·02, 0·11)
Lp(a) (mg/l)	175	191	225	222	212	227	0·04†	0·22†	(−0·03, 0·12)† (−47·0, 21·4)‡

Apo, apolipoprotein; Lp(a), lipoprotein(a).
* For details of diets and procedures, see Tables 1 and 2 and pp. 244–246.
† Log-transformed data.
‡ Untransformed data.

Table 4. Levels of haemostatic variables in female subjects after 14 d of consuming diets containing partially hydrogenated fish oil (PHFO) or vegetable oil (VO) margarines, and differences between the two test periods*

(Mean values and standard deviations for sixteen subjects, with lower quartile, median and upper quartile values in parentheses)

	PHFO diet		VO diet		Mean differences† (PHFO–VO)	*P* value	95 % CI†
	Mean	SD	Mean	SD			
Fibrinogen (g/l)	2.4 (2·1, 2·4, 2·7)	0·4	2·4 (2·1, 2·4, 2·5)	0·3	0·01	0·24	(−0·009, 0·032)
PAI-1 activity (U/ml)	5·3 (1·1, 4·6, 8·2)	4·3	3·9 (1·4, 2·9, 6·9)	3·3	0·09	0·51	(−0·36, 0·189)
PAI-1 antigen (ng/ml)	10·0 (4·3, 9·8, 13·2)	6·0	8·1 (5·0, 7·5, 10·6)	3·8	0·05	0·52	(−0·11, 0·22)
t-PA activity (IU/ml)	0·9 (0·7, 0·8, 1·2)	0·3	1·05 (0·7, 1·0, 1·4)	0·6	-0·04	0·30	(−0·041, 0·125)

PAI-1, plasminogen activator inhibitor-1; t-PA, tissue plasminogen activator.
* For details of diets and procedures, see Tables 1 and 2 and pp. 244–246.
† Log-transformed data.

The Pearson coefficient of correlation between the baseline levels of apoB and LDL was 0·50 ($P = 0{\cdot}05$) and between apoA-I and HDL was 0·81 ($P < 0{\cdot}01$).

Haemostatic variables

In Table 4 are given the levels of haemostatic variables and the mean differences between the two diets and their statistical significance probabilities.

There were no significant differences in the levels of fibrinogen, PAI-1 activity, PAI-1 antigen and t-PA activity between the two diets. No significant correlations between Lp(a) and haemostatic variables were found. There was a significant correlation between PAI-1 antigen and PAI-1 activity at baseline (Spearman coefficient, r 0·88, $P < 0{\cdot}01$). No significant correlations between triacylglycerols and PAI-I activity were found.

Discussion

In this strictly controlled crossover dietary study with sixteen well-motivated young women we have compared two different hard margarines, one traditional margarine based on PHFO and one based on VO, both all-purpose margarines with similar functional properties. The two test margarines contained approximately the same content of polyunsaturated fatty acids (Table 2). In general, the results presented here confirm our previous findings (Almendingen *et al*. 1995) that PHFO has unfavourable effects on serum lipids and lipoproteins even when compared with a vegetable fat blend of comparable functional properties and melting temperatures.

Most often male individuals are used as participants in dietary studies on the effects of fatty acids on serum lipids. Several studies have reported similar effects of fatty acids on serum lipids in both men and women (Mensink & Katan, 1989; Judd *et al*. 1994; Howard *et al*. 1995). A possible exception may be HDL-cholesterol, which may increase in men but not in women in response to polyunsaturated fatty acids (Yu *et al*. 1995). The effects of *trans* fatty acids on both HDL-cholesterol and LDL-cholesterol concentrations were found to be similar, however, in both men and women (Mensink & Katan, 1990). Thus, there is no reason to believe that the results obtained in the present study are only valid for females. Possible sex differences in the effects on Lp(a) are discussed later.

The PHFO margarine contained cholesterol and part of the increasing effect that was observed both in total- and LDL-cholesterol on the PHFO diet may be due to dietary cholesterol. The aim of the study was to compare two all-purpose hard margarines with similar functional properties, made from raw materials taken directly from the production line. Thus, the results should be of direct relevance to consumers. We, therefore, deliberately did not balance the difference in dietary cholesterol between the diets. The difference in cholesterol content between the PHFO diet and the VO diet on an average energy intake of 9·24 MJ was 137 mg/d (Table 1) and both diets were low in cholesterol. According to the mathematical model of Hopkins (1992) the predicted difference in plasma total cholesterol due to dietary cholesterol (PHFO diet − VO diet) was calculated to be 0·084 mmol/l which is also in accordance with Ginsberg *et al*. (1995) who found that total fasting cholesterol concentration increased by 0·073 mmol/l per 100 mg dietary cholesterol in young women. The observed difference was 0·18 mmol/l (Table 3) which indicates that a large fraction of the cholesterol-raising effect of the PHFO diet may have been due to its content of cholesterol.

The difference in serum total cholesterol concentration between the two diets was somewhat smaller and not statistically significant, compared with the difference in LDL-cholesterol which was highly significant (Table 3). This more potent increasing effect on LDL-cholesterol than on total cholesterol can be explained by the lowering effect on HDL-cholesterol by *trans* fatty acids in PHFO (Mensink & Katan, 1990; Zock & Katan, 1992; Almendingen *et al*. 1995). Our previous finding of an HDL-cholesterol-lowering effect of PHFO (Almendingen *et al*. 1995) was indicated also in the present study. The difference between

https://doi.org/10.1017/S0007114598001299 Published online by Cambridge University Press

https://doi.org/10.1017/S0007114598001299 Published online by Cambridge University Press

the two diets was not statistically significant, however, possibly because of the lack of power. In addition the HDL-cholesterol-lowering effect of PHFO may have been partly obscured by the HDL-cholesterol-raising effect of dietary cholesterol. Based on the findings of Ginsberg *et al.* (1995) this would amount to approximately 0·02 mmol/l. At the apoA-I level the HDL-lowering effect was also highly significant in the present study.

The principal differences in fatty acids between our two test margarines were the content of *trans* fatty acids, which was highest in the PHFO diet, and the content of saturated and monosaturated 18:1*cis* fatty acids, which were highest in the VO diet. Our results indicate that the replacement of *trans* fatty acids in the PHFO margarine with saturated (8·8 g/100 g total fatty acids), monounsaturated (13·7 g/100 g) and *trans* fatty acids (3·4 g/100 g) of vegetable origin contributes to a reduction in LDL-cholesterol, LDL-cholesterol:HDL-cholesterol ratio, apoB:apoA-I ratio and an increase in apoA-I (Table 3). Mensink & Katan (1990) and Troisi *et al.* (1992) reported a particularly strong influence of *trans* fatty acid intake on the ratio LDL-cholesterol:HDL-cholesterol, which is in accordance with our findings.

The PHFO diet resulted in a small but non-significant increase in Lp(a) concentration. Lp(a) is considered to be an independent risk factor for CHD (Schreiner *et al.* 1993) although a significant association has not always been found in women (Wild *et al.* 1997). A number of studies have recently demonstrated that *trans* fatty acids increase Lp(a) when given at a level of 7 % energy or more (Mensink *et al.* 1992; Nestel *et al.* 1992; Almendingen *et al.* 1995; Aro *et al.* 1997). In a previous study we also demonstrated that PHFO increases Lp(a) compared with partially hydrogenated soyabean oil or butterfat (Almendingen *et al.* 1995). The reason why this effect did not reach statistical significance in the present study may be related to lack of power. Furthermore, only women participated in the present study. In two previous studies on the effect of *trans* fatty acids on Lp(a) only men participated (Nestel *et al.* 1992; Almendingen *et al.* 1995). In the study of Mensink *et al.* (1992) both men and women participated and in one of the experiments of that study a significant increase in Lp(a) was observed in men but not in women consuming a *trans* fatty acid diet compared with either a stearic acid or a linoleic acid diet (Mensink *et al.* 1992). Thus, it cannot be excluded that the effect of *trans* fatty acids on Lp(a) is more pronounced in men than in young women.

Vegetable and hydrogenated vegetable oils contain only small quantities of fatty acids with C chain lengths greater than 18 while hydrogenated fish oils contain a large proportion of very-long-chain fatty acids (C20–C24) both saturated and *trans* unsaturated. Very little is known about the effects of these fatty acids on serum lipoproteins. In the present study the PHFO diet contained a total of 22·3 g C20–C24 fatty acids/100 g total fatty acids. One study by Wardlaw *et al.* (1995) showed that a caprenin diet which contained (100 g total fatty acids): 45 g 22:0 and 50 g 8:0 + 10:0 and a negligible content of 12:0–16:0 did not decrease mean total cholesterol, LDL-cholesterol or apoB levels compared with a palm-oil–palm-kernel-oil diet and a butter diet. This means that one or more of 8:0, 10:0 or 22:0 may be serum cholesterol-raising. PHFO also contains appreciable amounts of myristic acid (14:0), known to be strongly cholesterol-raising (Hegsted *et al.* 1993; Yu *et al.* 1995), as well as *cis* and *trans* 16:1 (Table 2). Smith *et al.* (1996) tested the effect of myristoleic (14:1*cis*) and palmitoleic acids (16:1*cis*) in pigs. They observed that 36 g 14:1+16:1/100 g total fatty acids resulted in the greatest increase in total- and LDL-cholesterol levels from pretreatment values compared with a diet containing (g/100 g total fatty acids) 52 palmitic acid, 51 stearic acid or 38 linoleic acid. Nestel *et al.* (1994) also found that palmitoleic acid increased total- and LDL-cholesterol levels in human subjects to a similar extent to palmitic acid and significantly more than oleic acid. This indicates that in addition to *trans* fatty acids and the known cholesterol-raising saturated fatty acids, several other saturated and unsaturated fatty acids found in PHFO but not present, or only present in small amounts, in other types of fats may be cholesterol-raising.

The content of linoleic and linolenic acids was 3·5 % of energy in the PHFO diet and 4·9 % energy in the VO diet. The effects of the test margarines on blood lipids can therefore hardly be explained by differences in polyunsaturated fatty acids.

In a previous study we found that a PHFO diet resulted in significantly lower levels of PAI-1 antigen and PAI-1 activity than a partially hydrogenated soyabean oil diet and a significantly lower fibrinogen level than a butterfat diet (Almendingen *et al.* 1996). In the present study we did not find any significant differences in haemostatic variables when the PHFO diet was compared with the VO diet. The intra- and interindividual variations in fasting values of fibrinolytic variables were very large, however, and sixteen participants may be too small a number to detect any differences in a study of only 2 weeks duration. Also, postprandial changes in haemostatic variables may be more relevant than fasting values (Marckmann *et al.* 1993).

Recently, focus has been on the reduction of the use of partly hydrogenated oils in margarines because of the cholesterol-raising effect of *trans* fatty acids. In the present study the contents of *trans* fatty acids in the PHFO diet and the VO diet were 7·8 % energy and 1·1 % energy respectively. The intake of *trans* fatty acids has been estimated to represent 1–5 % energy in several countries (Becker, 1996). The amount of *trans* fatty acids in our PHFO diet was, thus, higher than the average intake in most countries. Some (Siguel & Lerman, 1993; Willett *et al.* 1993; Ascherio *et al.* 1994; Hu *et al.* 1997), but not all (Aro *et al.* 1995; Roberts *et al.* 1995) epidemiological studies show a connection between intake of *trans* fatty acids and CHD. Few epidemiological studies have looked more specifically into the association between consumption of PHFO and risk of cardiovascular disease. In one study a positive association between intake of PHFO and mortality from ischaemic heart disease was found (Thomas *et al.* 1975) but this was not confirmed in a later study by the same authors (Thomas *et al.* 1981). Because of a covariation between intake of saturated and *trans* fatty acids it may be difficult by epidemiological studies to establish a true association between CHD and consumption of *trans* fatty acids (Kromhout *et al.* 1995). It is reasonable to assume, however, that *trans* fatty acids contribute to the risk of CHD by their

effects on serum lipid risk factors. In a previous study we found that PHFO in particular has unfavourable effects on blood lipids, and this has been confirmed in the present study. The consumption of PHFO was recently reported to be approximately 11 g in Norway representing about 10 % of fat intake (Almendingen *et al*. 1995). By its content of cholesterol, *trans* fatty acids, saturated fatty acids and possibly other cholesterol-raising fatty acids this amount of PHFO may have a considerable influence on the population mean plasma cholesterol level. We therefore recommend that the use of this fat in margarines intended for the food and baking industry, or for household use, should be minimized.

Acknowledgements

We gratefully acknowledge the cooperation of the participants in the study. We thank Knud Detlefsen and our kitchen staff for their assistance, Kari Solvoll for help with data handling of the food-frequency questionnaires, and Anita B. Mathisrud for drawing blood samples. The Norwegian food company Mills DA is acknowledged for supplying the test margarines. We are also indebted to A/S Sigernes and Regal Mølle for making free breads and buns, and providing free flour respectively. The study was part of the Nordic R&D Programme for the Food Industry with financial support from The Nordic Industrial Fund and Mills DA.

References

Almendingen K, Jordal O, Kierulf P, Sandstad B & Pedersen JI (1995) Effects of partially hydrogenated fish oil, partially hydrogenated soybean oil, and butter on serum lipoproteins and Lp(a) in men. *Journal of Lipid Research* **36**, 1370–1384.

Almendingen K, Seljeflot I, Sandstad B & Pedersen JI (1996) Effects of partially hydrogenated fish oil, partially hydrogenated soybean oil, and butter on haemostatic variables in men. *Arteriosclerosis Thrombosis Vascular Biology* **16**, 375–380.

American Oil Chemist's Society (1989*a*) Method CE2-66. In *Official Methods and Recommended Practices of the American Oil Chemist's Society* [D Firestone, editor]. Champaign, IL: AOCS.

American Oil Chemist's Society (1989*b*) Method CE3-74. In *Official Methods and Recommended Practices of the American Oil Chemist's Society* [D Firestone, editor]. Champaign, IL: AOCS.

Andersson I, Borgström B, Dahlquist A & Åkesson B (1975) A study of the food consumption by the duplicate portion technique in a small sample of the Dalby population. *Scandinavian Journal of Social Medicine* **10**, Suppl., 36–41.

Aro A, Jauhiainen M, Partanen R, Salminen I & Mutanen M (1997) Stearic acid, *trans* fatty acids, and dairy fat: effects on serum and lipoprotein lipids, apolipoproteins, lipoprotein (a), and lipid transfer proteins in healthy subjects. *American Journal of Clinical Nutrition* **65**, 1419–1426.

Aro A, Kardinaal AFM, Salminen I, Kark JD, Riemersma RA, Delgado-Rodriquez M, Gomez-Aracena J, Huttunen JK, Kohlmeier L, Martin BC, Martin-Moreno JM, Mazaev VP, Ringstad J, Thamm M, van't Veer P & Kok FJ (1995) Adipose tissue isometric *trans* fatty acids and risk of myocardial infarction in nine countries: the EURAMIC Study. *Lancet* **345**, 273–278.

Ascherio A, Hennekens CH, Buring JE, Master C, Stampfer MJ & Willett WC (1994) *Trans*-fatty acids intake and risk of myocardial infarction. *Circulation* **89**, 94–101.

Becker W (1996) Intake of *trans* fatty acids in the Nordic countries. *Scandinavian Journal of Nutrition* **40**, 16–18.

Brussaard JH, Katan MB, Groot PHE, Havekes LM & Hautvast JGAJ (1982) Serum lipoproteins of healthy persons fed a low-fat diet or a polyunsaturated fat diet for three months. A comparison of two cholesterol-lowering diets. *Atherosclerosis* **42**, 205–219.

Chmielewska J, Rånby M & Wiman B (1983) Evidence for a rapid inhibitor to tissue plasminogen activator in plasma. *Thrombosis Research* **31**, 427–436.

Clauss A (1957) Gerinnungsphysiologische Schnellmetode zur Bestimmung des Fibrinogens (A rapid coagulation test for the determination of fibrinogen). *Acta Haematologica* **17**, 237–246.

Folch J, Lees M & Stanley GHS (1957) A simple method for the isolation and purification of total lipides from animal tissues. *Journal of Biological Chemistry* **226**, 497–509.

Friedewald WT, Levy RI & Fredrickson DS (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical Chemistry* **18**, 499–502.

Ginsberg HN, Karmally W, Siddiqui M, Holleran S, Tall AR, Blaner WS & Ramakrishnan R (1995) Increases in dietary cholesterol are associated with modest increases in both LDL and HDL cholesterol in healthy young women. *Arteriosclerosis Thrombosis Vascular Biology* **15**, 169–178.

Hegsted M, Ausman LM, Johnson JA & Dallal GE (1993) Dietary fat and serum lipids: an evaluation of the experimental data. *American Journal of Clinical Nutrition* **57**, 875–883.

Hopkins PH (1992) Effects of dietary cholesterol on serum cholesterol: a meta-analysis and review. *American Journal of Clinical Nutrition* **55**, 1060–1070.

Howard BV, Hannah JS, Heiser CC, Jablonski KA, Paidi MC, Alarif L, Robbins DC & Howard WJ (1995) Polyunsaturated fatty acids result in greater cholesterol lowering and less triacylglycerol elevation than do monounsaturated fatty acids in a dose-response comparison in a multiracial study group. *American Journal of Clinical Nutrition* **62**, 392–402.

Hu FB, Stampfer MJ, Manson JE, Rimm E, Colditz GA, Rosner BA, Hennekens CH & Willett WC (1997) Dietary fat intake and the risk of coronary heart disease in women. *New England Journal of Medicine* **337**, 1491–1499.

Jones B & Kenwood MG (1989) *Design and Analysis of Cross-over Trials*, pp. 67–75. London: Chapman and Hall.

Judd JT, Clevidence BA, Muesing RA, Wittes J, Sunkin ME & Podczasy JJ (1994) Dietary *trans* fatty acids: effects on plasma lipids and lipoproteins of healthy men and women. *American Journal of Clinical Nutrition* **59**, 861–868.

Keys A, Anderson JT & Grande F (1957) Prediction of serum-cholesterol responses of man to changes in fats in the diet. *Lancet* **2**, 959–966.

Kromhout D, Menotti A, Bloemberg B, Aravanis C, Blackburn H, Buzina R, Dontas AS, Fidanza F, Giampaoli S, Jansen A, Karvonen M, Katan M, Nissinen A, Nedeljkovic S, Pekkanen J, Pekkarinen M, Punsar S, Räsänen L, Simic B & Toshima H (1995) Dietary saturated and *trans* fatty acids and cholesterol and 25 year mortality from coronary heart disease: the Seven Countries Study. *Preventive Medicine* **24**, 308–315.

Marckmann P, Sandström B & Jespersen J (1993) Dietary effects on circadian fluctuation in human blood coagulation factor VII and fibrinolysis. *Atherosclerosis* **101**, 225–234.

Mensink RP & Katan MB (1987) Effect of monounsaturated fatty acids versus complex carbohydrates on high density lipoproteins in healthy men and women. *Lancet* **1**, 122–125.

Mensink RP & Katan MB (1989) Effect of a diet enriched with monounsaturated or polyunsaturated fatty acids on levels of low-density and high-density lipoprotein cholesterol in healthy

https://doi.org/10.1017/S0007114598001299 Published online by Cambridge University Press

women and men. *New England Journal of Medicine* **321**, 436–441.

Mensink RP & Katan MB (1990) Effect of dietary *trans* fatty acids on high-density and low-density lipoprotein cholesterol levels in healthy subjects. *New England Journal of Medicine* **323**, 439–445.

Mensink RP, Zock PL, Katan MB & Hornstra G (1992) Effect of dietary *cis* and *trans* fatty acids on serum lipoprotein (a) levels in humans. *Journal of Lipid Research* **33**, 1493–1501.

Nâgele U, Wahlefeld AW & Ziegenhorn J (1985) Triglycerides: colorimetric method. In *Methods of Enzymatic Analysis*, 3rd ed., vol. 8, pp. 12–18 [HU Bergmeyer, editor]. Weinheim: Verlag Chemie GmbH.

Nes M, Frost Andersen L, Solvoll K, Sandstad B, Hustvedt BE, Løvø A & Drevon CA (1992) Accuracy of a quantitative food frequency questionnaire applied in elderly Norwegian women. *European Journal of Clinical Nutrition* **46**, 809–821.

Nestel P, Clifton P & Noakes M (1994) Effects of increasing dietary palmitoleic acid compared with palmitic and oleic acids on plasma lipids of hypercholesterolemic men. *Journal of Lipid Research* **35**, 656–662.

Nestel PM, Noakes M, Belling B, McArthur R, Clifton P, Janus E & Abbey M (1992) Plasma lipoprotein lipid and Lp(a) changes with substitution of elaidic acid for oleic acid in the diet. *Journal of Lipid Research* **33**, 1029–1036.

Roberts TL, Wood DA, Riemersma RA, Gallagher PJ & Lampe FC (1995) *Trans* isomers of oleic and linoleic acids in adipose tissue and sudden cardiac death. *Lancet* **345**, 278–281.

Rånby M, Sundell B & Nilsson T (1989) Blood collection in strong acidic citrate anticoagulant used in a study of dietary influence on basal tPA activity. *Thrombosis and Haemostasis* **62**, 917–922.

Schreiner PJ, Morrisett JD, Sharret AR, Patsch W, Tyroler HA, Wu K & Heiss G (1993) Lipoprotein (a) as a risk factor for preclinical atherosclerosis. *Arteriosclerosis and Thrombosis* **13**, 826–833.

Siedel J, Rollinger W, Rôschlau P & Ziegenhorn J (1985) Total cholesterol, end-point and kinetic method. In *Methods of Enzymatic Analysis,* 3rd ed., vol. 8, pp. 139–148 [HU Bergmeyer, editor]. Weinheim: Verlag Chemie GmbH.

Siguel EN & Lerman RH (1993) *Trans*-fatty acid patterns in patients with angiographically documented coronary artery disease. *American Journal of Cardiology* **71**, 916–920.

Smith DR, Knabe DA, Cross HR & Smith SB (1996) A diet containing myristoleic plus palmitoleic acids elevates plasma cholesterol in young growing swine. *Lipids* **31**, 849–858.

Thomas LH (1975) Mortality from arteriosclerotic disease and consumption of hydrogenated oils and fats. *British Journal of Preventive and Social Medicine* **29**, 82–90.

Thomas LH, Jones PR, Winter JA & Smith H (1981) Hydrogenated oils and fats: the presence of chemically modified fatty acids in human adipose tissue. *American Journal of Clinical Nutrition* **34**, 877–886.

Troisi R, Willett WC & Weiss ST (1992) *Trans*-fatty acid intake in relation to serum lipid concentrations in adult men. *American Journal of Clinical Nutrition* **56**, 1019–1024.

Wardlaw GM, Snook JT, Park S, Patel PK, Pendley FC, Lee M-S & Jandacek RJ (1995) Relative effects on serum lipids and apolipoproteins of a caprenin-rich diet compared with diets rich in palm oil/palm-kernel oil or butter. *American Journal of Clinical Nutrition* **61**, 535–542.

Wild SH, Fortmann SP & Marcovina SM (1997) A prospective case control study of lipoprotein (a) levels and apo (a) size and risk of coronary heart disease in Stanford Five-City Project participants. *Arteriosclerosis Thrombosis Vascular Biology* **17**, 239–245.

Willett WC, Stampfer MJ, Manson JE, Colditz GA, Speizer FE, Rosner BA, Sampson LA & Hennekens CH (1993) Intake of *trans* fatty acids and risk of coronary heart disease among women. *Lancet* **341**, 581–585.

Wiman B, Mellbring G & Rånby M (1983) Plasminogen activator release during venous stasis and exercise as determined by a new specific assay. *Clinica Chimica Acta* **127**, 279–288.

Yu S, Derr J, Etherton TD & Kris-Etherton PM (1995) Plasma cholesterol-predictive equations demonstrate that stearic acid is neutral and monounsaturated fatty acids are hypocholesterolemic. *American Journal of Clinical Nutrition* **61**, 1129–1139.

Zock PL & Katan MB (1992) Hydrogenation alternatives, effects of *trans* fatty acids and stearic acid versus linoleic acid on serum lipids and lipoproteins in humans. *Journal of Lipid Research* **33**, 399–410.

© Nutrition Society 1998

https://doi.org/10.1017/S0007114598001299 Published online by Cambridge University Press