European Journal of Clinical Nutrition (1997) 51, 527–534

Interesterification of fats in margarine: effect on blood lipids, blood enzymes, and hemostasis parameters

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Objectives: In 60 healthy humans, a blend of commonly used edible vegetable fats was compared with the same fat blend after random chemical interesterification, for their effects in terms of nutritional safety on blood lipids, blood enzymes and hemostasis parameters.
Design: Both fat blends were supplied double-blind at two energy levels (4 and 8% of energy) in margarine according to a parallel design. At either energy level, the two fat blends were consumed according to a cross-over design for two periods of three weeks, without an intermediate wash-out period.
Results: The 30 parameters studied revealed no statistically significant differences between the two fat blends, except for slightly ($\sim$10%) lower D-dimers concentrations after consumption of the interesterified fat blend.
Conclusions: Increased levels of the fibrin-degradation products D-dimers are positively associated with risk for coronary heart disease. Thus, it was concluded that the inclusion of a chemically interesterified vegetable fat blend in the diet of healthy people does not influence fasting blood lipids, blood enzymes and/or hemostasis parameters in an adverse way, when compared with a non-interesterified fat blend with the same fatty acid composition.
Descriptors: random interesterification; hydrogenation; lipoproteins; fatty acids; blood enzymes; thrombosis

Introduction

Interesterification of triglycerides has been used for many years to modify edible fats and oils. The process is functional for the food industries because it leads to different melting and crystallisation behaviour of the fat. Hardening of liquid oil through interesterification with solid fat offers an alternative to the use of partial hydrogenation in margarine and spread manufacture. During interesterification the arrangement of the fatty acids on the glycerol backbone of a triglyceride is changed, without producing or introducing *trans* fatty acids or positional isomers of fatty acids. The product of the most widely practised chemical catalysed interesterification process is a triglyceride where the fatty acids are randomly distributed over the glycerol backbone.

Possible direct nutritional consequences of interesterification could be expected for the process of triglyceride absorption. This process starts with the splitting off of the fatty acids at the *sn*-1,3 positions of the triglyceride under the influence of intestinal lipases (Small, 1991). The remaining 2-monoglyceride is efficiently absorbed. The degree of absorption of the free fatty acids is dependent on their type (Matton *et al*, 1979). Following absorption, in the enterocyte the 2-monoglyceride is resynthesised into a triglyceride, incorporated in chylomicrons, and secreted into lymph (Small, 1991). The chylomicron triglycerides largely retain (approximately 75%) of the original fatty acid at the *sn*-2 position. However, the fatty acids at the *sn*-1,3 positions tend towards a random selection of active fatty acids in the intestinal mucosal cell.

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Received 17 December 1996; revised 28 March 1997; accepted 14 April 1997

Fatty acids attached to the *sn*-2 position of triglycerides in chylomicrons might be preferentially transported to the liver instead of to the extrahepatic tissues because lipoprotein lipase primarily hydrolyses the *sn*-1,3 positions of triglycerides. The hepatocyte is the major site of action of fatty acids on LDL-metabolism (Spady *et al*, 1993). In line with the hypothesis by Dietschy's group that in the hepatocyte saturated fatty acids inhibit cholesterol esterification and elevate the ratio of cholesterol over cholesteryl esters (Spady *et al*, 1993), saturated fatty acids at the *sn*-2 position of dietary triglycerides might elevate LDL concentrations more than the same fatty acids at the *sn*-1,3 positions. In vegetable fats and oils (long chain) saturated fatty acids are largely located at the *sn*-1,3 positions. Thus, especially random interesterification of these fats could alter the influence of the fat on lipid metabolism.

With respect to the effect of interesterification of dietary fats on blood lipids, three recent studies indicated that large differences in dietary fatty acid configuration has negligible effects on fasting or postprandial blood lipoprotein concentrations in humans (Zock *et al*, 1995; Nestel *et al*, 1995; Zampelas *et al*, 1994). Two other reports of studies in humans concluded that random interesterification of butter fat lowered the (postprandial) hypercholesterolemic effect of this fat (Christophe *et al*, 1978; Mutanen *et al*, 1996). No other human trials have been reported, designed to directly compare commonly used dietary fat blends after chemical interesterification, in terms of nutritional safety, with similar non-interesterified fat blends on blood lipids, clinical chemical, and/or hemostasis parameters. The present study investigated the effect of chemical interesterification of a blend of vegetable oils on fasting blood lipids, blood enzymes, and hemostatis parameters in healthy human subjects.

Methods

We had this strictly controlled dietary study conducted, according to Good Clinical Practice, in a double-blind fashion, at the TNO Nutrition and Food Research Institute, Zeist, the Netherlands. The study protocol was approved by the TNO Medical Ethical Committee.

Subjects

Subjects were recruited from the pool of volunteers of the TNO Nutrition and Food Research Institute and by advertisements in local newspapers. No TNO-employees were included. Respondents received a verbal briefing and a copy of written information. They filled in two copies of the informed consent form, one of which they retained, and a questionnaire on personal data, life style, disease history and dietary habits. Each of the respondents was medically examined after an overnight fast for urine and blood collection. After examination, instructions were given by a dietician on a three-day diary to be filled in. Routine blood chemistry and hematology and urinalysis was performed. None of the volunteers included in the study did suffer from anemia, glucosuria, or proteinuria, and they were apparently healthy, did not use vitamin or any other nutritional supplements, and were not on a diet for medical reasons. Some characteristics of the subjects on entry in the study are given in Table 1. Post study screening (physical examination, and routine blood chemistry, hematology and urinalysis) was performed within a week after the last blood sampling of the dietary phase.

Experimental design

A fat blend was composed of 36% (w/w) coconut fat, 33% palm oil, 22% dry-fractionated palm oil-stearin fraction, and 9% low-trans partially hydrogenated rapeseed oil. Part of this fat blend was used as such. The remainder was chemically interesterified using sodium-methylate (0.05%) as a catalyst, according to standard food industries protocols. The non-interesterified and the interesterified fat blends were refined, and each of the two so called hardstocks was mixed with soybean oil in a ratio of 58:42. Preparation of two margarines containing 80% of the mixed fats was performed at Unilever Research Laboratory Vlaardingen, according to Good Manufacturing Practice. Characterization and determination of the homogeneity and stability of the margarines was performed according to Good Laboratory Practice. The fatty acid profile and the positional distribution of the triglycerides in the margarines are given in Table 2. Essentially, interesterification resulted in relatively more myristic (C14:0), palmitic (C16:0), and stearic (C10:0) acids of the sn-z position, at the expense of lauric (C12:0) and oleic (C18:1) acids. During interesterification, esterification of tocopherols and tocotrienols appeared to be negligible (data not shown).

Table 2 Fatty acid profile and positional distribution of the test margarines (namely mixes of interesterified or non-interesterified fat blend with soybean oil in a ratio of 58:42)[a]

	Control fat blend			*Interesterified fat blend*		
Fatty acid	*Total*	*sn-2*	*sn-1,3*	*Total*	*sn-2*	*sn-1,3*
	% by wt of total fatty acids					
C8:0	1.5	0.1	2.2	1.5	0.9	1.8
C10:0	1.2	0.5	1.6	1.2	1.1	1.3
C12:0	9.8	14.2	7.6	10.1	8.7	10.8
C14:0	4.2	2.1	5.3	4.3	3.8	4.6
C16:0	22.3	7.1	29.9	22.4	18.0	24.6
C16:1	0.1	0.1	0.1	0.1	0.1	0.1
C18:0	4.8	1.8	6.3	4.7	3.6	5.3
C18:1,c[b]	23.2	31.9	18.9	23.2	23.9	22.9
C18:1,t[b]	2.0	2.5	1.8	2.0	2.2	1.9
C18:2	26.2	36.6	21.0	26.0	34.4	21.8
C18:3	2.6	3.0	2.4	2.7	3.1	2.5
C20:0	0.3	0.0	0.5	0.3	0.2	0.4
C20:1	0.2	0.0	0.3	0.2	0.1	0.3
C22:0	0.2	0.0	0.3	0.2	0.1	0.3

[a] Average value in three samples taken at the beginning, middle, and end of either margarine production.
[b] c = *cis*-isomers; t = *trans*-isomers: these values were determined in the fat blends prior to margarine production.

Either fat blend was supplied at two levels of energy, 4 en% and 8 en%, according to a parallel design to 32 subjects. The 4 en% fat blend level corresponded with a margarine intake of 20–40 g per day, namely 7 en%, in this group of volunteers. This range would comprise the average daily margarine intake of most Western countries. The higher level of fat blend (8 en% = 14 en% of margarine) was included in the study to enable drawing conclusions on the effect of interesterification of fats at substantially higher than average margarine intake levels. Typically, at the 4 en% fat blend level, interesterification resulted in an increased dietary intake of palmitic acid at the *sn*-2 position of about 0.25 en% and a decreased intake of oleic acid at that position of about 0.15 en%. In either energy level group, the two fat blends were supplied to all subjects according to a cross-over design, with each fat blend being included in the diet for a period of three weeks. Each experimental group included equal numbers of males and females. The selected males and females were clustered in groups of four subjects of the same sex, based on their age, BMI and serum total cholesterol concentration. Individuals from each cluster were randomly allocated to one of the four experimental conditions at entrance. All subjects participated simultaneously from March 22 to May 4, 1995. During the study, subjects maintained their daily routines. The objective of the study was to investigate the effect of interesterification of a blend of vegetable oils on blood lipids, blood enzymes, and hemostasis parameters. The statistical power of the trial as executed, for the parameters measured, is given in Table 3.

Table 1 Characteristics of subjects on entry in the study

	Control fat blend				*Interesterified fat blend*			
	4 en%		*8 en%*		*4 en%*		*8 en%*	
Characteristic[a]	*Male (n = 8)*	*Female (n = 8)*	*Male (n = 7)*	*Female (n = 7)*	*Male (n = 8)*	*Female (n = 8)*	*Male (n = 7)*	*Female (n = 7)*
Age (y)	31 ± 13	39 ± 14	33 ± 13	43 ± 12	32 ± 11	31 ± 10	41 ± 15	34 ± 9
Serum total cholesterol (mM)	5.1 ± 1.2	5.3 ± 0.8	4.8 ± 1.1	5.6 ± 0.8	4.5 ± 1.2	5.4 ± 1.1	4.9 ± 1.2	4.5 ± 0.4
BMI (kg/m^2)	24.6 ± 4.2	23.6 ± 2.3	24.6 ± 2.3	23.9 ± 1.6	24.4 ± 3.3	23.8 ± 2.8	23.0 ± 3.2	22.0 ± 1.8

[a]Characteristics are given for those subjects that completed the study, and were included in the statistical analyses.

Table 3 The statistical power of the study as executed ($n = 60$) for the detection of an overall significant difference between fat blends at the probability level of $P = 0.05$, expressed as percent of the grand mean

Parameter [unit]	*Grand mean*	*Difference (%)*
Alkaline phosphatase (AP) [U/L]	65	2
Alanine transaminase (ALT) [U/L]	13	6
Aspartate transaminase (AST) [U/L]	19	4
γ-Glutamate transaminase (γ-GT) [U/L]	16	4
Lactate dehydrogenase (LDH) [U/L]	315	3
α-Hydroxybutyrate dehydrogenase (α-HBDH) [U/L]	126	3
Bilirubin [μM]	12	9
Protein [g/L]	73	1
Albumin [g/L]	51	1
C-reative protein (CRP) [mg/L]	2.3	19
Creatinine [μM]	69	1
Urea [mM]	4.6	3
Glucose [mM]	5.0	1
Total cholesterol [mM]	5.1	2
LDL cholesterol [mM]	3.3	3
HDL cholesterol [mM]	1.4	2
Triglyceride [mM]	1.1	7
Lp[a] [mg/L]	213	6
Free fatty acids (FFA) [mM]	0.51	13
Vitamin E [μM]	26	2
LDL-oxidation		
lag time [min]	98	1
propagation rate [nmol/mg/min]	9.7	2
Plasminogen activator-inhibitor-1 antigen (PAI-1 Ag) [ng/mL]	28	9
Tissue-plasminogen activator antigen (t-PA Ag) [ng/mL]	4.8	10
Tissue-plasminogen activator (t-PA) activity [U/mL]	0.52	9
Von Willebrand Factor (vWF) [%]	102	3
Activated coagulation factor VII (Factor VIIa) [mU/mL]	95	6
Fibrinogen [g/L]	2.1	4
D-dimers [ng/mL]	17	15

Individual energy intake was calculated using a three-day (two working and one weekend day) dietary record method (Cameron & van Staveren, 1988) and the NEVO Food Composition Table (1993). A 10% surplus was added to the calculated energy level to correct for underreporting.

The margarines containing the fat blends were provided to the volunteers as an ingredient in cake and as margarine for spreading on bread. Whole diets were provided at ten different levels of energy to meet the habitual energy intake of all subjects. The energy content of the daily diets was between 2000 and 4250 kcal (8.4 and 17.8 MJ), with incremental steps of 250 kcal (1 MJ). The diets were composed to be nutritionally adequate and to meet the dietary guidelines of the Netherlands Food and Nutrition Board. All food was provided and the volunteers were instructed not to consume any other food than that supplied. Compliance with the diets was improved by recording of uncomsumed products, asking for additional food consumption, and rating of the feeling of hunger on adverse events forms. Dinner was provided daily at the metabolic unit of the TNO Institute between 5 and 7 pm. After dinner, the foods for snacking, breakfast and lunch until dinner on the next day were provided. Dietary adjustments were made when a weight gain or loss had occurred on two consecutive weighing days that was greater than 1.5 kg. Diets were also adjusted when minor weight changes were accompanied by complaints about the amount of food.

Measurements

The volunteers daily filled in a questionnaire on adverse events and on compliance with the foodstuffs. Body weights were determined twice a week. At the end of each three-week dietary period, blood samples were collected from each subject on two separate days with a two-day interval. Blood was sampled from the cubital vein after an overnight fast. Blood was mixed with clot activator for determination of serum chemistry: Alkaline phosphatase, AP; alanine transaminase, ALT; aspartate transaminase, AST; γ-glutamate transaminase, γ-GT; lactate dehydrogenase, LDH, α-hydroxybutyrate dehydrogenase, α-HBDH; total bilirubin; total protein; albumin; C-reactive protein, CRP, creatinine; urea; glucose; triglycerides; total cholesterol; HDL-cholesterol, after precipitation with polyethylene glycol according to Siedel *et al* (1983); and free fatty acids (FFA) were determined using test combinations purchased from Boehringer, Mannheim, Germany. Serum LDL-cholesterol concentration was calculated using the formula by Friedewald *et al* (1972). Serum lipoprotein[a] (Lp[a]) concentration was analyzed using the test combination from DAKO products, Glostrup, Denmark. Whole serum fatty acids was analyzed using a standard method (AOCS Ce 1-62). Stabylite blood was used for determination of tissue-plasminogen activator (tPA) activity (Meijer *et al*, 1992). Buffered and non-buffered citrate blood was used for determination of plasma fibrinogen, using the reagent from DiaMed (DiaMed AG, Cressier sur Monat, Switzerland) on a Biomatic 2000 analyzer (Sarstedt BV, Einhoven, Netherlands), and the activated form of coagulation factor VII (Factor VIIa) (Morrissey *et al*, 1993), respectively. Citrate-Theophylline-Adenosine-Dipyridamole (CTAD) blood was used for analysis of tPA-antigen (Ranby *et al*, 1986), plasminogen activator-inhibitor-1 (PAI-1)-antigen (Meijer *et al*, 1994), D-dimers (using the Enzygnost® micro method from Behringwerke, Marburg, Germany), and von Willebrand Factor (vWF) (Silveira *et al*, 1986). EDTA-blood was used for the determination of LDL oxidation parameters (Princen *et al*, 1992), only in the samples taken on the first day of each of two-days sampling period.

Characterization (mean value) and homogeneity (CV; coefficient of variance) of the margarines was determined by measuring water (AOCSce 920.116) and fat content, by

extraction with dichloromethane after lyophilization, overall fatty acid profile (AOCS Ce 1-62), and fatty acid profile at the *sn*-2 position (Off J Eur Commun, 1991; IUPAC, 1987) in three samples taken at the beginning, middle and end of the manufacture. Stability of the margarines was determined by performing the same analyses and by determination of the peroxide value (AOCS Cd 8b-90) in margarine samples that were returned by the TNO Institute at the end of the dietary phase.

Samples of total diets were analyzed for crude protein (ISO 5983-1979), total fat (Pardun, 1969), fatty acids (AOCS Ce 1-62), total carbohydrates (Van de Kramer, 1941), mono- and disaccharides (Richter & Woelk, 1977), cholesterol and plant sterols (NEN 6328, 6364, 6365; Netherlands Normalisation Institute, Delft, Netherlands), dietary fibre (total and soluble) (Prosky *et al*, 1988), vitamin E (Speek *et al*, 1985), total carotenoids and β-carotene (Speek *et al*, 1986), and vitamin C (*Speek et al*, 1984).

Statistical analysis

Data are expressed as the mean $\pm$ standard deviation. Data which were not distributed normally were *ln* transformed before further statistical analysis. Differences between means were evaluated by a two-sided ANOVA, using the following factors: type of fat blend, energy level, gender, and repeated measure. All possible interactions were also evaluated. The null hypothesis was rejected at the 0.05 level of probability. The statistical analyses were performed using Genstad^{TM} 5 Release 3 (The Numerical Algorithms Group, Limited, Oxford, UK).

Results

The overall fatty acid profile, and that at the *sn*-2 position, of each of the two margarines were homogeneous (CV < 5%). Also the fat and water levels in the two margarines were homogeneous (CV < 3%). During the human trial, no complaints with regard to margarine taste were recorded.

The whole diets were analyzed as described and the results are given in Table 4. Apart from ($\sim$16%) lower cholesterol concentrations in both diets containing 8 en% of the fat blends, the four types of diet were very similar in their macro and micro nutrient compositions. The effect of this difference in dietary cholesterol level ($\sim$4 mg/MJ) would have caused an insignificant change of 0.03 mM in serum total cholesterol concentration (Hegsted *et al*, 1993).

During the dietary phase of the study, two female subjects discontinued for unknown reasons. To maintain a balanced design, two other subjects were excluded from the statistical analysis. Consequently, a total of 60 subjects was included for the calculations on all data and statistical analysis, as shown in Table 1.

Body weights dropped slightly but statistically significantly in the first and second three-week dietary period of the study (0.18 kg/week and 0.16 kg/week, respectively). However, body weight loss never exceeded the pre-set value of 1.5 kg. There were no differences in body weight between the types and levels of energy of the fat blends, suggesting that changes in body weight would not have interfered with the outcomes of this study.

No differences in the whole serum fatty acid distribution were observed between the two fat blends (Table 5).

Analysis of blood clinical chemical characteristics revealed no significant differences between the two fat blends (Table 6). Activity of γ-GT was higher after the 8 en% diets when compared with the 4 en% diets. Activities of AP, ALT, and AST as well as concentrations of bilirubin, creatinine, and urea were significantly lower in women than in men.

Neither the type of fat blend, nor the energy level at which the fat blends were consumed resulted in significant changes in blood lipid values (Table 7). Total and HDL-cholesterol concentrations and vitamin E levels were significantly higher in females. Free fatty acids were lower in men than in women in the 4 en% groups, whereas the opposite was seen in the 8 en% group.

No significant differences in hemostasis parameters were observed between fat blends or energy levels in the diet, except for D-dimers concentration (Table 8). Following logarithmic transformation, D-dimers levels were significantly lower after consumption of the interesterified fat blend. In addition, males had lower D-dimers levels than females.

Table 4 Analyzed mean daily intake of nutrients during the dietary periods

	Control fat blend		*Interesterified fat blend*	
Nutrient	*4 en%*	*8 en%*	*4 en%*	*8 en%*
Energy (MJ)	11.7	11.6	11.6	11.9
Carbohydrates (en%)	53.4	53.0	54.0	53.8
Mono-/disaccharides	30.5	29.3	31.2	29.9
Polysaccharides	22.9	23.6	22.8	23.9
Protein (en%)	12.1	12.1	12.5	12.3
Fat (en%)	34.2	34.4	33.6	34.1
Saturated fatty acids	15.9	15.4	15.8	15.6
Monounsaturated fatty acids	10.8	10.1	10.6	10.1
Polyunsaturated fatty acids	5.1	5.5	5.2	5.6
Cholesterol (mg/MJ)	21.1	18.7	24.1	18.9
Fibre (g/MJ)	2.4	2.5	2.5	2.3
Non-soluble	1.7	1.6	1.6	1.6
Soluble	0.7	0.9	0.8	0.7
Vitamin C (mg/MJ)	8.9	8.7	8.7	8.6
Carotenoids (mg/MJ)	0.9	0.6	0.6	0.7
β-carotene (mg/MJ)	0.18	0.18	0.15	0.16
Vitamin E (TE/MJ)[a]	1.1	1.1	1.1	1.5

[a] 1 mg α-tocopherol = 1 TE (Tocopherol-Equivalent); 1 mg β-tocopherol = 0.4 TE; 1 mg γ-tocopherol = 0.1 TE; 1 mg δ-tocopherol = 0.01 TE.

Table 5 Distribution of fatty acids in whole serum at the end of dietary periods

	Control fat blend				*Interesterified fat blend*			
	4 en%		*8 en%*		*4 en%*		*8 en%*	
Fatty acid[a]	*Male (n = 16)*	*Female (n = 16)*	*Male (n = 14)*	*Female (n = 14)*	*Male (n = 16)*	*Female (n = 16)*	*Male (n = 14)*	*Female (n = 14)*
				% by wt of total fatty acids				
C12:0	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1
C14:0	0.9 ± 0.5	1.1 ± 0.4	0.9 ± 0.3	0.9 ± 0.3	0.9 ± 0.3	1.0 ± 0.3	0.9 ± 0.2	0.9 ± 0.3
C16:0	20.7 ± 1.7	21.4 ± 2.0	22.0 ± 1.4	21.4 ± 1.1	20.9 ± 1.6	21.7 ± 1.9	21.5 ± 1.7	21.6 ± 1.4
C16:1	1.7 ± 0.6	2.3 ± 0.6	1.6 ± 0.4	2.0 ± 0.4	1.7 ± 0.4	2.3 ± 0.6	1.6 ± 0.3	2.1 ± 0.4
C18:0	7.2 ± 0.7	6.8 ± 0.6	7.4 ± 1.2	7.0 ± 0.4	7.1 ± 0.6	6.8 ± 0.6	6.9 ± 0.4	7.0 ± 0.7
C18:1 cg[b]	19.2 ± 2.7	19.6 ± 1.9	18.4 ± 2.1	17.8 ± 1.9	18.5 ± 3.7	19.9 ± 2.1	18.5 ± 1.3	18.2 ± 1.9
C18:1 c11	1.5 ± 0.2	1.7 ± 0.3	1.3 ± 0.2	1.6 ± 0.2	1.5 ± 0.3	1.6 ± 0.2	1.5 ± 0.1	1.4 ± 0.2
C18:1 t6 + t9	0.2 ± 0.1	0.3 ± 0.2	0.3 ± 0.6	0.2 ± 0.1	0.3 ± 0.2	0.3 ± 0.2	0.2 ± 0.1	0.2 ± 0.1
C18:2	36.1 ± 4.3	34.0 ± 2.7	35.7 ± 3.2	37.1 ± 3.3	35.8 ± 4.3	34.1 ± 2.7	37.1 ± 2.8	36.5 ± 3.2
C18:3 (n-6)	0.1 ± 0.1	0.2 ± 0.2	0.2 ± 0.3	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.2	0.2 ± 0.2	0.1 ± 0.2
C18:3 (n-3)	0.2 ± 0.2	0.4 ± 0.2	0.3 ± 0.2	0.4 ± 0.1	0.3 ± 0.2	0.3 ± 0.2	0.3 ± 0.2	0.3 ± 0.2
C20:2 (n-6)	0.6 ± 0.7	0.9 ± 0.8	0.9 ± 0.9	0.9 ± 0.6	0.6 ± 0.7	0.8 ± 0.7	0.3 ± 0.4	1.1 ± 0.7
C20:3 (n-6)	1.7 ± 0.3	1.8 ± 0.3	1.6 ± 0.2	1.8 ± 0.4	1.8 ± 0.2	1.7 ± 0.2	1.7 ± 0.4	1.8 ± 0.6
C20:4 (n-6)	7.3 ± 1.1	6.8 ± 1.4	6.8 ± 1.4	6.3 ± 1.2	7.2 ± 1.1	6.6 ± 1.5	6.9 ± 1.5	6.2 ± 1.1
C20:5 (n-3)	0.5 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.2
C22:6 (n-3)	1.5 ± 0.3	1.4 ± 0.3	1.2 ± 0.4	1.4 ± 0.3	1.5 ± 0.3	1.6 ± 0.3	1.3 ± 0.4	1.4 ± 0.3

[a] The following fatty acids were not detectable: C18:1 c6; C18:1 t11; C18:2 t9,12; C20:0; C20:1; C22:0; C22:1.
[b] c = *cis*-isomer; t = *trans*-isomer.

Table 6 Blood clinical chemical characteristics at the end of the dietary periods

	Control fat blend				*Interesterified fat blend*			
	4 en%		*8 en%*		*4 en%*		*8 en%*	
Characteristic	*Male (n = 16)*	*Female (n = 16)*	*Male (n = 14)*	*Female (n = 14)*	*Male (n = 16)*	*Female (n = 16)*	*Male (n = 14)*	*Female (n = 14)*
AP (U/L)	71 ± 20	62 ± 14	72 ± 25	54 ± 10	69 ± 16	64 ± 15	72 ± 27	53 ± 11
ALT (U/L)	16 ± 6	10 ± 2	16 ± 5	11 ± 2	16 ± 6	10 ± 3	16 ± 5	11 ± 3
AST (U/L)	23 ± 9	16 ± 3	23 ± 6	17 ± 5	23 ± 9	16 ± 3	21 ± 5	18 ± 5
γ-GT (U/L)	15 ± 4	12 ± 3	21 ± 14	16 ± 10	14 ± 5	12 ± 3	21 ± 14	16 ± 11
LDH (U/L)	328 ± 57	304 ± 36	325 ± 46	309 ± 38	326 ± 61	306 ± 34	307 ± 34	310 ± 33
α-HBDH (U/L)	132 ± 23	122 ± 13	129 ± 21	124 ± 16	133 ± 25	123 ± 13	124 ± 18	125 ± 13
Bilirubin (μM)	16 ± 15	10 ± 5	14 ± 11	8 ± 2	17 ± 15	9 ± 3	15 ± 8	8 ± 2
Protein (g/L)	73 ± 3	72 ± 3	73 ± 4	73 ± 3	72 ± 4	73 ± 3	74 ± 4	72 ± 3
Albumin (g/L)	52 ± 3	50 ± 2	52 ± 4	51 ± 3	52 ± 3	51 ± 2	52 ± 3	51 ± 3
CRP (mg/L)	2.4 ± 1.4	2.3 ± 1.0	2.2 ± 1.2	2.0 ± 0.8	1.8 ± 1.1	3.2 ± 3.2	2.0 ± 1.3	2.5 ± 1.7
Creatinine (μM)	74 ± 9	62 ± 5	76 ± 7	63 ± 8	72 ± 9	63 ± 6	78 ± 7	62 ± 10
Urea (mM)	5.0 ± 0.9	4.1 ± 0.7	5.4 ± 1.0	4.4 ± 1.0	4.9 ± 1.0	4.2 ± 0.6	5.1 ± 0.8	4.1 ± 0.9
Glucose (mM)	5.0 ± 0.3	5.0 ± 0.4	5.1 ± 0.4	5.0 ± 0.5	5.0 ± 0.3	5.0 ± 0.4	5.2 ± 0.4	4.9 ± 0.4

Table 7 Blood lipid parameters at the end of the dietary periods

	Control fat blend				*Interesterified fat blend*			
	4 en%		*8 en%*		*4 en%*		*8 en%*	
Lipid parameter	*Male (n = 16)*	*Female (n = 16)*	*Male (n = 14)*	*Female (n = 14)*	*Male (n = 16)*	*Female (n = 16)*	*Male (n = 14)*	*Female (n = 14)*
Total cholesterol (mM)	4.8 ± 1.1	5.6 ± 1.0	4.8 ± 1.1	5.3 ± 0.8	4.7 ± 0.9	5.6 ± 0.9	4.9 ± 1.2	5.3 ± 0.8
LDL cholesterol (mM)	3.1 ± 1.0	3.6 ± 0.9	3.0 ± 1.0	3.3 ± 0.9	3.0 ± 0.9	3.6 ± 0.8	3.2 ± 1.1	3.2 ± 0.8
HDL cholesterol (mM)	1.3 ± 0.2	1.5 ± 0.3	1.2 ± 0.2	1.6 ± 0.3	1.3 ± 0.2	1.5 ± 0.4	1.3 ± 0.2	1.6 ± 0.3
Triglyceride (mM)	0.9 ± 0.4	1.3 ± 0.5	1.1 ± 0.6	1.0 ± 0.3	1.0 ± 0.4	1.3 ± 0.5	1.1 ± 0.5	1.1 ± 0.3
Lp[a] (mg/L)	183 ± 246	268 ± 400	135 ± 187	245 ± 229	190 ± 296	279 ± 415	140 ± 176	256 ± 241
FFA (mM)	0.41 ± 0.15	0.65 ± 0.32	0.54 ± 0.20	0.49 ± 0.22	0.39 ± 0.25	0.61 ± 0.21	0.52 ± 0.19	0.45 ± 0.13
Vitamin E (μM)	22.9 ± 5.2	27.6 ± 5.8	23.9 ± 6.4	27.0 ± 4.5	23.7 ± 4.5	28.1 ± 5.0	24.1 ± 6.0	26.4 ± 4.0
LDL-oxidation lag time (min)	98.1 ± 6.8	98.6 ± 5.7	96.2 ± 10.4	97.5 ± 6.8	99.0 ± 7.0	99.4 ± 7.0	96.7 ± 9.7	98.2 ± 6.5
propagation rate (nmol/mg/min)	9.7 ± 1.1	9.4 ± 0.8	10.1 ± 0.6	9.5 ± 0.9	9.8 ± 1.0	9.3 ± 1.0	9.9 ± 0.9	9.7 ± 0.7

Table 8 Hemostasis parameters at the end of the dietary periods

	Control fat blend				*Interesterified fat blend*			
	4 en%		*8 en%*		*4 en%*		*8 en%*	
Hemostasis parameter	*Male (n = 16)*	*Female (n = 16)*	*Male (n = 14)*	*Female (n = 14)*	*Male (n = 16)*	*Female (n = 16)*	*Male (n = 14)*	*Female (n = 14)*
PAI-1 Ag (ng/mL)	28 ± 19	30 ± 21	29 ± 14	23 ± 17	25 ± 20	31 ± 24	27 ± 15	28 ± 23
t-PA Ag (ng/mL)	4.9 ± 3.0	4.8 ± 3.3	5.3 ± 3.0	4.1 ± 2.5	4.7 ± 2.6	5.6 ± 3.1	4.9 ± 3.0	5.7 ± 2.9
t-PA activity (U/mL)	0.54 ± 0.22	0.45 ± 0.32	0.48 ± 0.20	0.56 ± 0.24	0.49 ± 0.15	0.49 ± 0.20	0.56 ± 0.25	0.63 ± 0.27
vWF (%)[a]	110 ± 33	99 ± 21	96 ± 18	99 ± 27	111 ± 37	103 ± 24	93 ± 22	103 ± 24
Factor VIIa (mU/mL)	96 ± 56	100 ± 31	91 ± 46	100 ± 36	93 ± 45	102 ± 39	78 ± 29	102 ± 37
Fibrinogen (g/L)	2.0 ± 0.4	2.1 ± 0.3	1.9 ± 0.4	2.2 ± 0.3	2.0 ± 0.4	2.3 ± 0.4	2.0 ± 0.3	2.3 ± 0.3
D-dimers (ng/mL)	19 ± 20	21 ± 10	10 ± 5	24 ± 16	16 ± 20	21 ± 10	9 ± 4	20 ± 17

[a] Arbitrary unit; expressed relative to the concentration in a plasma pool of healthy humans, according to Kluft *et al.* (1976).

Discussion

In this study the effect of random interesterification of a blend of commonly used edible vegetable fats, consumed at realistic intake levels, on various parameters, including risk factors for Coronary Heart Disease (CHD), was evaluated. In this double-blind placebo-controlled trial with healthy volunteers, the interesterified fat was compared with the same fat blend, but not interesterified, at two levels of energy intake. The main purpose of the current study was to evaluate the nutritional safety of interesterified fats at realistic intake levels.

At the higher intake level of energy, both fat blends increased serum γ-GT activity. The physiological implication of this isolated finding is not clear. Interesterification of the fat blend did not lead to statistically significant effects on any of the measured parameters, except for ($\sim$10%) lower D-dimers concentrations. Increased levels of D-dimers have been positively associated with risk for CHD in patients with peripheral artery disease (Fowkes *et al*, 1993). Concentrations in the upper two quintiles increased the relative risk for fatal and non-fatal coronary events in these patients. Also in apparently healthy men, with baseline D-dimers concentrations exceeding the 95th percentile of the control distribution, the risk for coronary events was two times higher than that for individuals with lower levels (Ridker *et al*, 1994). The observed effect of D-dimers concentration in our study was relatively small, and changes in the D-dimers concentration were not accompanied by other changes in the coagulation or fibrinolytic factors measured. Thus, the observed decrease of D-dimers level after consumption of the margarine containing the interesterified fat blend was not considered to be meaningful.

Rat studies (De Fouw *et al*, 1994; Brink *et al*, 1995) indicating that palmitic and stearic acids are less well absorbed ($\sim$10% reduction) when occupying the *sn*-1,3 positions of triglycerides relative to the *sn*-2 position. This effect was enhanced, namely the absorption from the *sn*-1,3 positions was further decreased (up to $\sim$15% reduction), at high calcium intakes. Palmitic acid must be considered as hypercholesterolemic (Spady *et al*, 1993; Temme *et al*, 1996). However, no sigificant effects of the positional distribution of palmitic acid on plasma lipids was observed in rats (De Fouw *et al*, 1994; Renaud *et al*, 1995). In contrast, piglets fed structured triglyceride containing palmitic acid predominantly at the *sn*-2 position showed higher plasma total and HDL-cholesterol concentrations than those fed palm oil with palmitic acid predominantly at the *sn*-1,3 positions (Innis *et al*, 1993).

Several human studies have been performed using chemically interesterified fats to obtain specific fatty acid compositions. Although not designed to investigate the effect of interesterification of fats, these studies indicate that such fats affect blood lipids as would be expected from their gross fatty acid composition, for example Bonanome & Grundy, 1988; Tholstrup *et al*, 1994. A few human studies have been published, that directly compared very high levels of dietary triglycerides specifically differing in their positional distribution of palmitic versus oleic acid. Zock *et al* (1995) showed that an extreme contrast of 10 en% in these types of fatty acid at the *sn*-2 position had negligible effects on fasting blood lipoprotein levels in humans consuming the experimental fats at high concentrations. In addition, Zampelas *et al* (1994) using similar fats, concluded that the positional distribution of these fatty acids is not an important determinant of postprandial lipemia. Another study by Nestel *et al* (1995) demonstrated that random interesterification, leading to considerable enrichment of the *sn*-2 position by palmitic acid and a corresponding loss from this position of oleic and linoleic acids, did not affect fasting plasma lipoprotein levels.

Two examples of structured triglycerides, Caprenin™ (a randomized triglyceride primarily comprising caprylic, capric and behenic acids) and Salatrim™ (triglycerides composed of mixtures of long-chain saturated fatty acids, predominantly stearic acid, and the short chain fatty acids acetic, propionic, and/or butyric acids), have been claimed to be low-caloric as based on animal (Webb *et al*, 1991) and human studies (Peters *et al*, 1991; Finley *et al*, 1994a). Caprenin™ has been reported to be hypercholesterolemic in men (Wardlaw *et al*, 1995), whereas very limited data suggest that Salatrim™ does not influence blood cholesterol levels (Finley *et al*, 1994b).

The human studies performed so far, using very high concentrations of the hypercholesterolemic palmitic acid in experimental fat blends, did not support the hypothesis that dietary triglycerides enriched with saturated fatty acids at the *sn*-2 position elevate blood lipoprotein concentrations more than triglycerides containing these fatty acids at the *sn*-1,3 positions. We studied the effects of interesterification of a blend of commonly used vegetable oils at much lower and more realistic levels of human intake, in relatively young normocholesterolemic adults. Thus, as had been anticipated, the resulting moderate enrichment of the *sn*-2 position by longer-chain saturated fatty acids did not affect blood lipid levels. Thus the data available so far favour the conclusion that interesterification of dietary triglycerides as such does not adversely influence blood lipid concentrations. In addition to measuring no effects on

blood lipids, our study also showed no adverse effect of interesterification on various clinical chemical and hemostasis parameters. To the best of our knowledge, our study is the first to report on the effect of random interesterification of dietary fats on such parameters.

Conclusions

We conclude that interesterification of a blend of commonly used vegetable oils did not affect fasting blood lipids, clinical chemical and hemostasis parameters, including risk factors for CHD in healthy humans, differently from a fat blend with the same fatty acid composition. This observation is of importance in terms of nutritional safety, because interesterification of edible fats offers an alternative to the use of *trans* fatty acids for food industries.

Acknowledgements—We thank Dr J Lammers, OE Rosier, and CW Heeren for preparation of the fat blends and margarines; and Drs G Schaafsma and HFJ Hendriks for careful conduct of the dietary phase of the study.

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