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Positional distribution of fatty acids in dietary triglycerides: effects on fasting blood lipoprotein concentrations in humans[1–3]

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ABSTRACT We examined the effect of the positional distribution of fatty acids within dietary triglycerides on serum lipoproteins. Sixty subjects consumed two diets of equal fatty acid composition for 3 wk each. In the palm oil diet 82% of palmitic acid was attached to the outer two carbon atoms of glycerol, and 18% to the middle carbon. In the diet rich in enzymatically modified palm oil these figures were 35% and 65%, respectively. On the modified-fat diet, average lipoprotein concentrations showed nonsignificant ($P > 0.13$) increases of 0.06 mmol/L for total, 0.03 mmol/L for HDL, and 0.04 mmol/L for LDL cholesterol compared with palm oil. The small increases in total and LDL cholesterol were statistically significant in the men ($n = 23$) but not in the women ($n = 37$). The ratio of HDL to LDL cholesterol and serum triglyceride concentrations were unchanged. Thus, a large difference in dietary fatty acid configuration had little effect on lipoprotein concentrations in humans. *Am J Clin Nutr* 1995;61:48–55

KEY WORDS Humans, dietary fatty acids, lipids, lipoproteins, cholesterol, stereospecific structure, triglycerides, configuration

Introduction

Dietary fatty acids may differ from each other in four aspects: number of double bonds, chain length, configuration of the double bonds, and position of the fatty acid on the glycerol molecule (**Fig 1**). The number of double bonds modulates the effects of fatty acids on cholesterol concentrations; more specifically, saturated fatty acids (with no double bonds) raise serum cholesterol (1, 2). The cholesterol-raising effect of saturated fatty acids depends on their chain length (2–6). The geometry of the double bonds of unsaturated fatty acids, in particular the *trans* vs the *cis* configuration, also influences lipoprotein cholesterol concentrations (7–10).

However, little is known about the influence of the position of fatty acids within dietary triglycerides on lipoprotein concentrations and cholesterol metabolism. More than 20 y ago, McGandy et al (11) found that stearic acid (18:0) in synthetic fats, in which this saturate is randomly distributed over each of the three positions of glycerol, raised cholesterol concentrations. In contrast, the cholesterol-raising effect of stearic acid is less when fed as cocoa fat (1, 3), in which it is predominantly esterified to the 1 and 3 positions. In laboratory animals randomization of fats with specific fatty acid distributions, such as peanut oil, can alter their atherogenicity and cholesterolemic effect (12–14). This has raised interest in the influences of the fatty acid configuration of triglycerides on the biologic effects of dietary fat (14–17), but at present few data on this topic are available.

By itself it is not implausible that the positional distribution of dietary fatty acids could affect lipoprotein concentrations. Triglycerides are absorbed in the intestine after hydrolysis to *sn*-2-monoglycerides and fatty acids (18), and are then resynthesized into triacylglycerols and secreted in chylomicrons. The chylomicron triglycerides largely retain the original fatty acid in the 2 position (18, 19). Fatty acids attached to the *sn*-2 position might then be preferentially transported to the liver instead of to the extrahepatic organs because lipoprotein lipase, like pancreatic lipase, primarily attacks the 1 and 3 position of triglycerides (20). Because the hepatocyte is the major site of action of fatty acids on low-density-lipoprotein (LDL) metabolism, saturated fatty acids in the *sn*-2 position of dietary triglycerides might elevate LDL concentrations more than the same fatty acid in the *sn*-1 or 3 position (16).

Common dietary fats show large differences in the positional distribution of their constituent fatty acids (15). In palm oil and other vegetable fats, palmitic acid (16:0) is predominantly esterified at the 1 and 3 position, whereas pork fat contains palmitic acid mainly on the 2 position (21). The fatty acid configuration of dietary triglycerides can also be altered to produce confectionary and other fats with better texture or certain desired physical properties. Palmitic acid is the most abundant saturate in the diet, and it is thus important to know whether its position in the triglyceride molecule modulates its effects on total and LDL-cholesterol concentrations. In newborn piglets a synthesized fat with palmitic acid mainly in the

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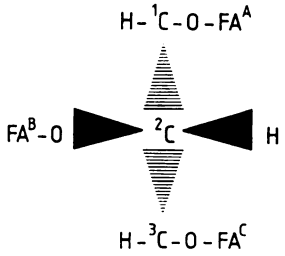

FIG 1. Structure of a triglyceride molecule showing the stereospecific numbering (*sn*) of the carbon atoms of glycerol. If the fatty acid on the middle carbon (*sn*-2-FA^{B}) is drawn to the left (above the plane of the page), then the top carbon is numbered 1 and the bottom carbon 3 (behind the plane of the page). Different fatty acids on the 1 and 3 positions make carbon atom 2 asymmetric, and then two optical isomers are possible: 1-FA^{A}, 2-FA^{B}, 3-FA^{C}-glycerol and 1-FA^{C}, 2-FA^{B}, 3-FA^{A}-glycerol.

2 position raised fasting total and high-density-lipoprotein (HDL) cholesterol concentrations as compared with palm oil (22). However, direct comparisons of the effects of palmitic acid in different triglyceride positions in humans are lacking. We therefore compared the effects of two dietary fats with equal amounts of individual fatty acids but different positional distributions on serum lipid and lipoprotein concentrations in healthy male and female volunteers.

Methods

Subjects

In response to announcements via local newspapers and posters in university and community buildings, 47 women and 28 men applied for enrollment in the study. They were invited to participate in a medical and dietary screening. Three women withdrew during the screening phase. Two men and one woman were excluded because their cholesterol concentrations exceeded 7.1 mmol/L (275 mg/dL) or their blood pressure was >140/90 mm Hg, one woman because she had low hemoglobin, and three women and one man because their food records revealed irregular dietary habits or an alcohol intake >10% of daily energy. Two men withdrew after the screening procedure because their spouses had been excluded for one of the reasons mentioned above. We accepted all 23 remaining men. Of the 39 eligible women, 2 were excluded by lot because only 60 subjects could be admitted to the trial.

All 23 men and 37 women completed the study. None suffered from anemia, glycosuria, or proteinuria, and they were apparently healthy, as indicated by a medical questionnaire. Preexperimental fasting total cholesterol ranged from 2.80 to 6.98 mmol/L (mean 4.62 mmol/L), HDL cholesterol from 0.76 to 2.42 mmol/L (mean 1.52 mmol/L), and triglycerides from 0.41 to 2.24 mmol/L (mean 0.92 mmol/L). The men were aged from 19 to 67 y (mean 29 y), they weighed between 63 and 108 kg (mean 79 kg), and their body mass indexes (in kg/m^{2}) ranged from 18.5 to 30.9 (mean 22.9). The women were aged between 20 and 67 y (mean 32 y), they weighed between 54 and 84 kg (mean 66 kg), and their body mass indexes ranged from 18.1 to 29.7 (mean 22.4). The habitual diet of the subjects as measured by self-recorded food intake on two working and one weekend day supplied on average 10.7 MJ/d (2560 kcal), of which 37% was fat, 13% was protein, 48% was carbohydrate, and 2% was alcohol. They consumed on average 280 mg cholesterol and 35 g dietary fiber daily. Nine women used oral contraceptives and five were postmenopausal. Nine women and four men smoked.

A majority of the subjects were university students and most of the others had college degrees. They lived in or around Wageningen, a small university town in the center of the Netherlands. The protocol and goals of the study were explained fully to the subjects, who gave their written consent. Before the study, they were thoroughly instructed about the daily routines during the trial and the necessity of full compliance, and they were strongly advised not to take part if not highly motivated or if they anticipated any problems in adhering to the protocol. No payment was given except for the study diets, which were free. The study had been approved by the Ethics Committee of the Department of Human Nutrition.

Design

The objective of the study was to investigate the effects of the positional distribution of fatty acids in the dietary triglyceride molecules on serum lipid and lipoprotein concentrations. The trial was designed to detect a significant ($P < 0.05$) effect of modified fat vs palm oil on total cholesterol with a power of 80% if the real population effect exceeded 0.13 mmol/L. This power calculation was based on the within-subject variation (SD) in cholesterol response to diet equal to 0.35 mmol/L observed in previous studies (6–8).

All subjects participated simultaneously from February 15 to March 29, 1993. The trial consisted of two consecutive 3-wk periods, during which each participant followed both diets, in different order (crossover). One diet was high in palm oil, with palmitic acid predominantly esterified at the *sn*-1 and *sn*-3 positions, and the other diet was high in an enzymatically modified palm oil (Betapol; Loders-Croklaan, Wormerveer, The Netherlands), which contains palmitic acid mainly in the *sn*-2 position.

Before the study began, subjects were categorized according to sex, and women also according to the use of oral contraceptives. Subjects within each subcategory were then randomly allocated to one of the two diet sequences, except members of a couple living together, who were assigned to the same sequence group. Both sequence groups had a nearly equal number of each category, and they were well-balanced for age, baseline total and HDL-cholesterol concentration, and body mass index. Use of this crossover design eliminated possible bias due to the order in which the diets were consumed or to drift of variables over time (23). As described earlier (6, 8), this experimental design also eliminates bias due to lipid variation during the hormonal cycle. This enables the measurement of the influence of diet on serum lipids in women, without confounding effects of menstrual cycle or use of oral contraceptives (6, 8). Foodstuffs and packages were color-coded so as to keep subjects unaware of the nature of their diets.

Diets

The diets consisted of conventional solid foods, and menus were changed daily during each 3-wk dietary period. The amount and type of foodstuffs were the same for the two diets. The differences in the positional distribution of the fatty acids were achieved by the use of special margarines and oils with

TABLE 1
Total, *sn*-2, and *sn*-1,3 fatty acid composition of the special oils and margarines[1]

Fatty acid	Palm oil			Modified fat		
	Total	*sn*-2	*sn*-1,3	Total	*sn*-2	*sn*-1,3
			% by wt			
Saturated	39.9	13.7	53.0	38.2	78.1	18.2
Lauric (12:0)	0.4	0.3	0.4	0.6	0.7	0.5
Myristic (14:0)	1.0	0.5	1.3	1.2	1.9	0.9
Palmitic (16:0)	28.8	6.4	40.0	28.9	66.9	9.9
Stearic (18:0)	9.1	6.5	10.4	7.1	8.4	6.5
Monounsaturated	47.8	63.5	39.9	49.5	18.5	65.0
Oleic (*cis*-18:1)	47.2	63.4	39.1	48.0	18.1	62.9
Polyunsaturated	12.0	22.4	6.8	10.5	3.3	14.1
Linoleic (18:2)	11.8	22.1	6.6	10.1	3.2	13.5
Unknown	0.3	0.4	0.3	1.8	0.1	2.7
Total	100	100	100	100	100	100

[1] Special oils and margarines provided 70% of dietary triglycerides and 28% of daily energy, which are weighted according to contribution to total dietary fat (oil provided 6%, margarine 64%); 33.3% of total fatty acids are in *sn*-2 position and 66.7% are in the *sn*-1 or *sn*-3 position.

highly distinct fatty acid configurations (**Table 1**). A fat rich in *sn*-1,3-palmitate was obtained by solvent fractionation of natural unprocessed palm oil. Subsequently, 87.5 parts of this fraction were blended with 11 parts of high-oleic acid sunflower oil and 1.5 parts of high-linoleic acid sunflower oil. This yielded an oil blend with palmitic acid predominantly in the 1 and 3 position. An oil with palmitic acid mainly in the 2 position was derived by *sn*-1,3 enzymatic interesterification of a palm oil fraction with sunflower fatty acids by using immobilized *Rhizomucor miehei* lipase (Novo Industries, Copenhagen), followed by solvent fractionation. The palm oil–based fat had a slightly higher melting point than the enzymatically modified fat, but both were liquid at 37 °C.

Margarines were made from a blend of 93 parts of these special oils with 7 parts of fully hydrogenated sunflower oil high in stearic acid. These margarines had similar textures and were easy to spread. They contained equal amounts of the various individual fatty acids, but differed substantially in the positional distribution of the fatty acids over the triglyceride molecules. Table 1 shows that the palm oil–rich margarine and oil contained mainly oleic and linoleic acid in the 2 position of the triglycerides, whereas the 2 position in the modified-fat product was predominantly occupied by palmitic acid. These margarines were used as spreads with the bread meals, in sauces and gravies, and for baking cookies and special bread containing on average 8% fat. The oils from which the margarines were made were also used to prepare salad dressings. The margarines provided 64% and the oils 6% of all triglycerides in the study diets.

The diets were formulated at 30 levels of energy intake, ranging from 5.5 to 20 MJ/d, so that each subject received a diet that met his or her energy needs. The energy requirements of each participant were estimated from 3-d food records kept by the subjects before the trial. As such food records underestimate actual energy requirements (24), we provided the subjects with an initial 10% more energy than his or her reported intake. Body weights were recorded twice weekly, and the energy level was adjusted when necessary to maintain stable weight. Over the 42 d of the trial, average body weight fell by 0.3 ± 1.1 kg (range −2.6–1.6 kg). Mean body weight at the end of the dietary treatments was 0.1 ± 0.8 kg (range −1.5 to 1.9 kg) higher on the modified-fat than on the palm oil diet.

All foodstuffs were weighed out for each individual subject. On weekdays at 1200, hot meals were served and eaten at the department. All other foods were supplied daily as a package. Foods for the weekends and guidelines for their preparation were provided on Fridays. In addition to the food supplied, the subjects were required to choose each day a limited number of fat- and cholesterol-free food items, which provided 8–9% of total daily energy. Subjects were urged not to change their selection of free-choice items between the dietary periods. They were also instructed to maintain their habitual pattern of physical activity and not to change their smoking habits, consumption of coffee, or use of oral contraceptives. The participants kept diaries in which they recorded their selection and amount of free-choice items, any sign of illness, all medications used, and any deviation from their diets. At the end of the trial, subjects completed an anonymous questionnaire relating to the blinding of the diets, problems, and noncompliance during the study.

Food composition

Duplicate portions of both study diets were collected on each of the 42 d for an imaginary participant with a daily energy intake of 11 MJ/d, stored at −20 °C, and pooled and analyzed after the study for protein (25), total fat (26), total fatty acid composition (27), dietary fiber (28), and cholesterol (29). Available carbohydrate was calculated by difference. *Sn*-2 fatty acid composition was determined enzymatically (30). After partial hydrolysis of triglycerides by *sn*-1,3-specific porcine pancreatic lipase (Type II; EC 3.1.1.3, Sigma, St Louis), *sn*-2-monoglycerides were isolated on aminopropyl columns (Bond Elut; Varian, Harbor City, CA) (31) and hydrolyzed, and the fatty acids were methylated and then separated by gas-liquid chromatography. This yielded the fatty acid composition of one-third of all dietary fatty acids, namely those esterified to the *sn*-2 position (Table 1 and **Table 2**). The composition of the *sn*-1,3 fatty acids, which made up the other two-thirds of dietary fatty acids (Tables 1 and 2), was calculated from the

TABLE 2
Total, *sn*-2, and *sn*-1,3 fatty acid composition of all triglycerides in the two diets[1]

Fatty acid	Palm oil diet			Modified-fat diet		
	Total	*sn*-2	*sn*-1,3	Total	*sn*-2	*sn*-1,3
		% by wt			% by wt	
Saturated	42.1	26.5	49.9	40.6	67.8	26.9
Lauric (12:0)	1.0	1.1	0.9	1.0	1.4	0.8
Myristic (14:0)	2.5	3.5	2.0	2.7	4.3	1.9
Palmitic (16:0)	27.7	14.6	34.2	27.4	53.5	14.3
Stearic (18:0)	9.2	6.2	10.8	8.1	6.9	8.6
Monounsaturated	42.8	52.0	38.1	44.4	22.3	55.5
Oleic (*cis*-18:1)	41.3	51.0	36.4	42.1	20.9	52.7
Polyunsaturated	13.2	20.6	9.6	12.3	8.4	14.2
Linoleic (18:2)	12.2	19.6	8.5	11.2	7.2	13.1
Unknown	1.9	0.9	2.4	2.7	1.5	3.3
Total	100	100	100	100	100	100

[1] Special oils and fats supplied 70% of total fat. The remaining 30% came largely from dairy products and meat; 33.3% of total fatty acids are in *sn*-2 position and 66.7% are in the *sn*-1 or *sn*-3 position.

total and the *sn*-2 fatty acid compositions. The following example illustrates this calculation:

sn-1,3 oleic acid = [3(total oleic acid) − *sn*-2 oleic acid]/2.

We also calculated percentages "horizontally." For instance, the amount of palmitic acid present on the 2 position as a percentage of all dietary palmitic acid was calculated from the data in Tables 1 and 2 as [*sn*-2 palmitic acid/(3 × total palmitic acid)] × 100%, and the percentage present on the 1 or 3 position as [100% − percentage on the 2 position].

The energy and nutrient contents of the free-choice items, which contained negligible amounts of fat, was calculated (32) and combined with the analyzed values of the foods supplied (**Table 3**).

TABLE 3
Mean daily intake of energy and nutrients of the 60 subjects on the two diets[1]

Energy or nutrient	Palm oil diet	Modified-fat diet
Energy		
(MJ/d)	12.4 ± 3.1[2]	12.3 ± 3.0
(kcal/d)	2958 ± 740	2941 ± 725
Protein (% of energy)	13.0	13.4
Fat (% of energy)	40.8	40.1
Saturated fatty acids	16.5	15.6
Lauric acid (12:0)	0.4	0.4
Myristic acid (14:0)	1.0	1.0
Palmitic acid (16:0)	10.8	10.5
Stearic acid (18:0)	3.6	3.1
Monounsaturated fatty acids	16.7	17.1
Oleic acid (*cis*-18:1)	16.2	16.2
Polyunsaturated fatty acids	5.3	4.8
Linoleic acid (*cis,cis*-18:2)	4.8	4.3
Carbohydrates (% of energy)	44.9	45.4
Alcohol (% of energy)	1.1	1.0
Cholesterol		
(mg/d)	368	367
(mg/MJ)	29.8	29.8
Dietary fiber		
(g/d)	44.6	43.0
(g/MJ)	3.6	3.5

[1] Values are based on chemical analyses of complete duplicate diets plus the calculated contribution of free-choice items. Each value represents the mean of two independent duplicates collected and pooled in the two different periods during which each diet was consumed by one-half of the subjects; variations between periods were negligible.
[2] $\bar{x}$ ± SD.

Blood sampling and analysis

Subjects were assigned a random number that was used for labeling blood and serum tubes. In this way, technicians were blinded to the subjects' diets. Blood was sampled after a 12-h fast on days 1, 17, and 21 (period 1); and on days 38 and 42 (period 2). All venipunctures of a particular subject were performed by the same technician, in the same room, and at the same time of the same days of the week for the two dietary periods. Serum was obtained by low-speed centrifugation within 1 h of venipuncture, stored at −80 °C, and analyzed enzymatically for total and HDL cholesterol and triglycerides (33–35). All samples from a particular subject were analyzed within one run. The CV within runs was 1.4% for total, 0.7% for HDL, and 1.3% for triglycerides. Mean bias with regard to the target values of serum pools provided by the Centers for Disease Control and Prevention (Atlanta) was −0.04 mmol/L for total cholesterol, 0.01 mmol/L for HDL cholesterol, and 0.12 mmol/L for triglycerides. LDL cholesterol was calculated by using the Friedewald equation (36).

Statistical analysis

The two lipid and lipoprotein values obtained for each subject at the end of each dietary period were averaged for statistical analyses. The data were analyzed with the *General Linear Models* (*GLM*) procedure of the *Statistical Analysis System* (37), by using a two-way analysis of variance (ANOVA) with subject and diet as class variables. This way of testing is equivalent to the paired *t* test (23). Carryover effects of previous diet were checked by introducing a diet-by-period interaction term in the model. Differences in responses between men and women or between women using or not using oral contraceptives were compared with unpaired *t* tests (37).

Results

Table 2 shows that the two diets contained equal amounts of each specific fatty acid, but differed markedly in their positional fatty acid distribution. In the modified-fat diet, 65% of the palmitic acid was on the 2 position, and the remaining 35% was on the 1 and 3 positions. In contrast, 82% of the palmitic acid in the palm oil diet was on the 1 and 3 positions, whereas only 18% was found on the 2 position. Sixty-eight percent of the *sn*-2 fatty acids in the modified-fat diet were saturated, mainly palmitic acid, whereas 73% of the *sn*-2 fatty acids in the palm oil diet were unsaturated, the majority being oleic acid (Table 2). Table 3 shows that the intake of energy and all other relevant food components was similar for both diets.

Inspection of the diaries and the anonymous questionnaires that were handed in at the end of the trial revealed only minute deviations from the study protocol. The diaries showed that consumption of foods outside the dietary regime provided at most 0.2 g fat/d. The magnitude and the frequency of deviations reported in the anonymous questionnaires were in line with those reported in the diaries. This indicated that compliance was high and that deviations probably did not materially affect the results. A two-choice question about the color-coding of the diets yielded 30 correct and 25 false answers ($P = 0.25$). Thus, blinding was successful.

Serum lipids and lipoproteins

The diets had little effect on serum lipoprotein cholesterol concentrations (**Fig 2**). Thirty-six participants showed higher total cholesterol values on the modified-fat diet than on the palm oil diet, 1 showed no change, and 23 participants had lower values with the modified fat (**Fig 3**). The changes in total cholesterol were not related to initial cholesterol concentration ($r = 0.13$, NS), age ($r = 0.07$, NS), or body mass index ($r = -0.11$, NS). The average rise in total cholesterol of 0.06 ± 0.32 mmol/L (2.3 ± 12.4 mg/dL) on modified fat relative to palm oil was not significant. The mean changes in HDL (0.03 mmol/L, or 1.2 mg/dL; $P = 0.13$), non-HDL (0.03 mmol/L, or 1.2 mg/dL; NS), and LDL cholesterol (0.04 mmol/L, or 1.5 mg/dL; NS) were also not statistically significant (**Table 4**). In addition, the HDL-LDL cholesterol ratio and the triglyceride concentrations did not differ significantly between the diets. The absence of significant diet-by-period interactions regarding the lipid and lipoprotein concentrations indicated that there were no important carryover effects from previous diet. CIs for changes in lipoprotein concentrations (Table 4) were narrow, and left little room for major effects.

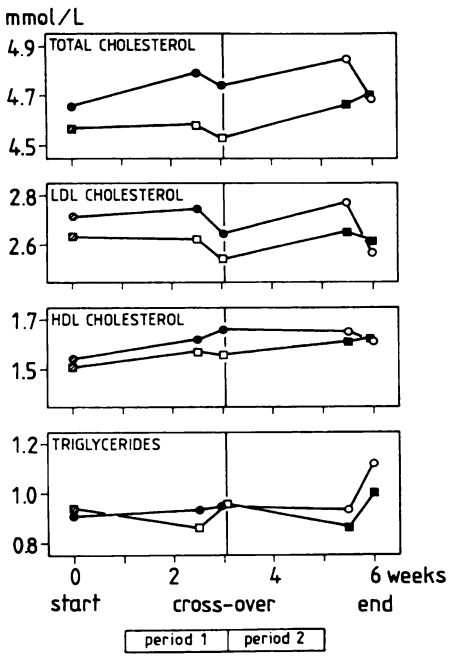

FIG 2. Mean concentrations of serum total, LDL, and HDL cholesterol and of serum triglycerides throughout the experiment. During the first 3 wk, 30 subjects consumed a diet rich in *sn*-2-saturated triglycerides (modified fat, ●) and in the subsequent 3 wk a diet rich in *sn*-2-unsaturated triglycerides (palm oil, ○). Thirty other subjects consumed these diets in reverse order (palm oil, □; modified fat, ■). The two diets had the same total fatty acid composition.

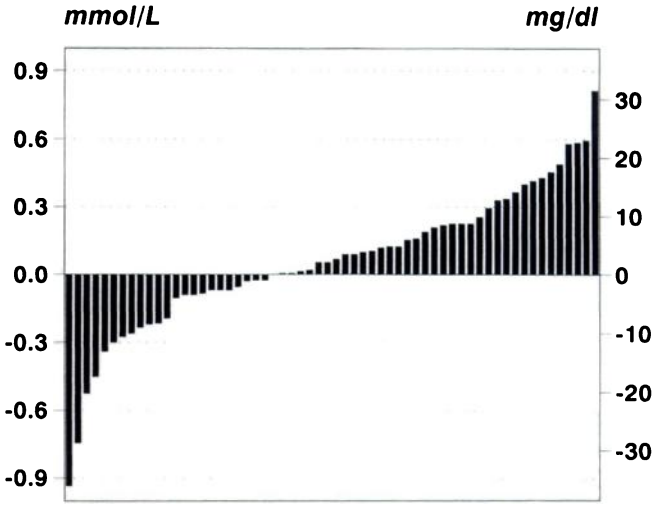

FIG 3. Individual differences in serum total cholesterol concentration between the end of the modified-fat diet and the end of the palm oil diet, sorted by magnitude. The average change was 0.06 ± 0.32 mmol/L ($P = 0.15$). The 60 subjects consumed both diets for 3 wk each.

The small increases in total (0.10 mmol/L) and LDL cholesterol (0.08 mmol/L) with the modified-fat diet were statistically significant in the men (Table 4). However, the men's non-HDL (or LDL + very-low-density-lipoprotein) cholesterol was not significantly different between the two diets. None of the responses in lipid and lipoprotein concentrations was significantly different between men and women. Also, no significant differences were detected between responses of women using or not using oral contraceptives.

Before the sera were analyzed, we identified five women who experienced events that probably influenced their lipoprotein concentrations: one received estrogen therapy during part of the trial, one had a sinus infection and was treated with antibiotics, one reported frequent deviations from the study diets, one suffered a knee injury and stopped daily vigorous exercise, and one lost >3 kg body weight during the trial. Statistical analysis without the data of these five women ($n = 55$ men and women) did not yield substantially different results. The mean effect of modified fat relative to palm oil for the remaining 32 women was somewhat, but not significantly, larger for total cholesterol (0.08 mmol/L, 95% CI −0.04 to

TABLE 4
Serum lipid and lipoprotein cholesterol concentrations in subjects consuming diets high in palm oil and an enzymatically modified palm oil analogue in which palmitic acid is in the *sn*-2 position instead of the *sn*-1,3 positions

	Palm oil diet	Modified-fat diet	Change (95% CI)
	mmol/L	*mmol/L*	
Total cholesterol			
All	4.66 ± 0.90[1]	4.72 ± 0.94	0.06(−0.02,0.14)
Women (n = 37)	4.89 ± 0.84	4.92 ± 0.88	0.03(−0.09,0.16)
Men (n = 23)	4.31 ± 0.89	4.41 ± 0.96	0.10(+0.02,0.18)[2]
HDL cholesterol			
All	1.60 ± 0.33	1.63 ± 0.37	0.03(−0.01,0.07)
Women	1.77 ± 0.24	1.79 ± 0.31	0.03(−0.04,0.09)
Men	1.33 ± 0.26	1.37 ± 0.29	0.04(−0.00,0.08)
Non-HDL cholesterol			
All	3.07 ± 0.85	3.09 ± 0.86	0.03(−0.04,0.09)
Women	3.12 ± 0.78	3.13 ± 0.79	0.01(−0.08,0.10)
Men	2.98 ± 0.94	3.04 ± 0.97	0.06(−0.02,0.13)
LDL cholesterol			
All	2.62 ± 0.78	2.66 ± 0.80	0.04(−0.03,0.10)
Women	2.69 ± 0.76	2.71 ± 0.76	0.01(−0.08,0.11)
Men	2.51 ± 0.81	2.59 ± 0.86	0.08(+0.00,0.15)[2]
HDL-LDL ratio			
All	0.67 ± 0.29	0.67 ± 0.30	0.00(−0.02,0.03)
Women	0.70 ± 0.20	0.70 ± 0.19	0.00(−0.04,0.04)
Men	0.62 ± 0.39	0.63 ± 0.41	0.00(−0.02,0.03)
Triglycerides			
All	0.97 ± 0.42	0.94 ± 0.40	−0.03(−0.08,0.03)
Women	0.92 ± 0.33	0.91 ± 0.38	−0.02(−0.08,0.05)
Men	1.04 ± 0.54	0.99 ± 0.44	−0.04(−0.16,0.07)

[1] $\bar{x}$ ± SD; n = 60. Subjects consumed each diet for 3 wk, in different order. To convert values for total, HDL, and LDL cholesterol to mg/dL, multiply by 38.67. To convert values for triglycerides to mg/dL, multiply by 88.54.
[2] Significantly different from zero, $P < 0.05$.

0.21 mmol/L) and for HDL cholesterol (0.06 mmol/L, 95% CI 0.01 to 0.12 mmol/L) than for the 37 women in the initial analysis (Table 4). Again, the responses in women and men were not significantly different. The mean effects of the modified-fat vs the palm oil diet in the 55 subjects who experienced no health problems or material changes in body weight were 0.09 mmol/L, or 3.5 mg/dL (95% CI 0.01 to 0.17 mmol/L) for total; 0.05 mmol/L, or 1.9 mg/dL (95% CI 0.02 to 0.09 mmol/L) for HDL; and 0.05 mmol/L, or 1.9 mg/dL (95% CI −0.02 to 0.12 mmol/L) for LDL cholesterol. As in the analysis with all 60 subjects, the HDL-LDL ratio was unchanged.

Discussion

Fasting serum lipids and lipoproteins

We found that two dietary fats with an extreme contrast in the type of fatty acid on the 2 position, specifically palmitic vs oleic acid, resulted in minimal differences in serum total or lipoprotein cholesterol concentrations. The only significant effects were observed in the men, who showed small increases in LDL and total cholesterol on the diet with palmitic acid on the *sn*-2 position. In view of the more modest differences in positional distribution that can be achieved in everyday diets, the influence on serum cholesterol concentrations in free-living people should be much smaller than the 0.1 mmol/L for men observed here. The design and the size of the trial provided high statistical power to detect real population differences. It is therefore unlikely that we failed to pick up any major effect through chance fluctuations. Also, most of the effect of dietary lipids on serum lipoproteins is established within 1 or 2 wk (3, 38–40); therefore, 3 wk should have been amply sufficient to observe an important effect if there had been one.

We used solvent fractionation of palm oil to increase the proportion of triglycerides with unsaturated fatty acids in the *sn*-2 position typical for vegetable oils. Solvent fractionation is a physical process that does not produce changes in molecular structures. Our results therefore suggest that fat with palmitic acid mainly on the *sn*-1,3 positions does not confer any major advantage in terms of effects on serum lipoproteins over other fats with the same fatty acid composition but a different positional distribution of fatty acids.

Innis et al (22) fed formulas containing palm oil or enzymatically modified fat to newborn piglets. At 18 d of age the modified-fat formula high in *sn*-2-palmitate triglycerides resulted in higher plasma total and HDL-cholesterol concentrations than the palm oil formula high in *sn*-2-oleate glycerides (22). These changes were in the same direction as, but much larger than those observed in the present study. However, the effects of dietary fat on lipid metabolism of newborn piglets are difficult to compare with those of adult humans, not only because of species differences, but also because in newborn animals and humans the absorption of palmitic acid in the 2 position might be more efficient than that of palmitic acid in the 1 and 3 positions (41–43).

Other potential effects of positional fatty acid distribution

Although dietary fatty acid configuration had no substantial effect on fasting lipoprotein concentrations it might still influence postprandial lipoprotein concentrations. Indeed, Mortimer et al (44) and Redgrave et al (45) injected chylomicrons or lipid emulsions into rats and found that hydrolysis of triglycerides by lipoprotein lipase and liver uptake of remnant particles were slower when the fatty acid in the 2 position was saturated than when it was unsaturated. If these findings can be extrapolated to humans, then eating *sn*-2-saturated triglycerides, such as lard, might result in elevated plasma remnant concentrations. Myher et al (46) found that in human volunteers fed a lard-rich breakfast the chylomicron triglycerides contained considerably more palmitic acid in the 2 position than those in subjects who consumed a control breakfast. This shows that in humans the *sn*-2 position of palmitic acid in lard is at least partly retained after hydrolysis by pancreatic lipase in the gut. Myher et al (46), however, did not report effects of lard on chylomicron concentrations. Recently, Zampelas et al (47) examined the effects of positional distribution of dietary palmitic acid on postprandial plasma lipids. Sixteen healthy men consumed the same dietary fats as studied in our trial and plasma total and chylomicron triglyceride concentrations were monitored over 6 h after the liquid test meals. No differences between the palm oil and the modified-fat meal were detected. The authors concluded that the positional distribution of fatty acids in dietary triglycerides is not an important determinant of postprandial lipemia (47).

Kritchevsky et al (12–14) found that randomization of fats with a specific fatty acid distribution such as peanut oil reduces

their atherogenicity in laboratory animals. Peanut oil contains 4–7% saturated 20:0, 22:0, and 24:0, almost all of it in the *sn*-3 position (48). Although these very-long-chain saturates can slow down chylomicron metabolism in rats (45), this cannot explain why randomized peanut oil, in which these fatty acids are present in the 2 position as well as in the 1 and 3 position, should be less atherogenic than native peanut oil. The atherogenesis in animals fed native peanut oil does not seem to be mediated by elevated plasma cholesterol concentrations (12, 13). It has been suggested that the presence of 20–24 saturates in the 3 position of peanut oil might render linoleic acid in the 2 position relatively unavailable, which could promote the atherogenicity of the oil (48).

Conclusion

The fatty acid configuration of food fats can be altered to produce confectionary and other fats with better texture or certain desired physical properties. Our study provides no evidence that changing the position of palmitic acid in this "structuring" process has important health consequences. However, future studies should also investigate the positional effects of other fatty acids.

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