Small differences in the effects of stearic acid, oleic acid, and linoleic acid on the serum lipoprotein profile of humans[1–3]

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ABSTRACT

Background: Studies have suggested that oleic and stearic acids, as well as oleic and linoleic acids, have comparable effects on the serum lipoprotein profile. If so, then substituting these three 18-carbon fatty acids for each other would result in similar effects on the serum lipoprotein profile.

Objective: The aim of this study was to compare simultaneously the effects of stearic, oleic, and linoleic acids on the serum lipoprotein profile of healthy subjects.

Design: Forty-five subjects (27 women and 18 men) consumed in random order 3 experimental diets, each for 5 wk. The diets provided 38% of energy from fat, of which 60% was supplied by the experimental fats. The dietary compositions of the diets were the same, except for 7% of energy, which was provided by stearic, oleic, or linoleic acid. At the end of each intervention period, serum lipid and lipoprotein concentrations were measured. In addition, LDL, HDL, and VLDL particle sizes and particle concentrations of lipoprotein subclasses were analyzed by nuclear magnetic resonance spectroscopy.

Results: No significant diet-induced changes in serum lipids and lipoproteins were found. Mean (±SD) serum LDL-cholesterol concentrations were 3.79 ± 0.91, 3.71 ± 0.79, and 3.65 ± 0.91 mmol/L with the high–stearic acid, high–oleic acid, and high–linoleic acid diets, respectively ($P = 0.137$ for diet effects). Mean (±SD) HDL-cholesterol concentrations were 1.45 ± 0.43, 1.46 ± 0.45, and 1.46 ± 0.44 mmol/L ($P = 0.866$). LDL, HDL, and VLDL particle sizes and lipoprotein subclass distributions also did not differ significantly between the 3 diets.

Conclusions: With realistic intakes of stearic, oleic, and linoleic acids, differences between their effects on the serum lipoprotein profile are small. *Am J Clin Nutr* 2005;82:510–6.

KEY WORDS Stearic acid, oleic acid, linoleic acid, total cholesterol, LDL cholesterol, HDL cholesterol, lipoprotein profile, humans

INTRODUCTION

It is well known that the various fatty acids in the diet exert different effects on serum lipid and lipoprotein concentrations. Saturated fatty acids are thought to increase cardiovascular disease risk because they elevate serum total and LDL-cholesterol concentrations relative to monounsaturated and polyunsaturated fatty acids. These effects have been quantified by earlier well-controlled dietary studies (1, 2). Relative to an isoenergetic amount of carbohydrates, a mixture of saturated fatty acids elevated serum total cholesterol concentrations, monounsaturated fatty acids had comparable effects, and polyunsaturated fatty acids were hypocholesterolemic. In contrast with the other saturated fatty acids, stearic acid—a saturated fatty acid with 18 carbon atoms—had no effects on serum total cholesterol concentrations (1, 2). These earlier studies, however, did not examine the effects of fatty acids on specific lipoproteins, which is important because of the opposing effects of LDL and HDL cholesterol on cardiovascular disease risk.

More recently, several studies have compared the effects of stearic acid on lipid and lipoprotein concentrations with those of unsaturated fatty acids. When stearic acid was substituted for oleic acid, effects on serum LDL- and HDL-cholesterol concentrations did not differ (3). Also, with realistic intakes of linoleic acid (<13% of energy), oleic and linoleic acids had similar effects on the serum lipoprotein profile (4, 5). If these findings are true (3-5), then the consequence is that the effects of stearic, oleic, and linoleic acids on serum lipid and lipoprotein concentrations would be comparable. To examine this hypothesis, we compared the effects of diets enriched in these three 18-carbon fatty acids on serum concentrations of triacylglycerol and total, LDL, and HDL cholesterol in a controlled crossover study in healthy subjects. In addition, we investigated the effects of these diets on LDL, HDL, and VLDL particle sizes and on the subclass distributions of these lipoprotein particles by nuclear magnetic resonance (NMR) spectroscopy.

SUBJECTS AND METHODS

Subjects

Healthy male and female nonsmoking subjects were recruited via advertisements in local newspapers and in a university hospital newsletter and via posters in university buildings. Persons who were interested were informed about the purposes and requirements of the study and had to give their written informed consent before entering the screening phase. At screening, 2 fasting blood samples were taken for the measurement of serum lipid and lipoprotein concentrations and hematologic variables,

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and blood pressure and urinary glucose and protein from a morning urine specimen were measured. Subjects were included in the study if they were aged 18–65 y, were healthy on the basis of a medical questionnaire, were not pregnant, were weight stable, had a body mass index (BMI; in kg/m^2) <32, had a diastolic blood pressure <95 mm Hg, had a systolic blood pressure <160 mm Hg, had a fasting serum total cholesterol concentration between 5.0 and 8.0 mmol/L, and had a serum triacylglycerol concentration <4.0 mmol/L. Subjects with a history of atherosclerotic disease, glycosuria, proteinuria, or anemia and who were taking medications known to affect blood lipids or hemostatic variables were excluded from the study. Fifty-eight persons met the eligibility criteria. Blood donation or participation in another biomedical trial was not allowed within 4 wk before the start of the study or during the study. The study protocol was approved by the Medical Ethics Committee of the Maastricht University.

Subjects withdrew mainly in the first 2 wk of the study, for reasons specifically related to the strict study protocol ($n = 4$ subjects), stressful personal or job circumstances ($n = 5$ subjects), and physical illness ($n = 2$ subjects in the first intervention period and 1 subject in the second intervention period). One subject was excluded after the first period, because he did not follow the protocol. Ultimately, 45 subjects (18 men and 27 women) aged 28–66 y ($\bar{x}\pm$ SD: 51 $\pm$ 10 y) completed the protocol. During the screening period, BMIs ranged from 18.0 to 29.8 (24.9 $\pm$ 2.7). The subjects' fasting serum lipid concentrations ranged from 4.97 to 7.76 mmol/L for total cholesterol (6.04 $\pm$ 0.75 mmol/L), from 0.83 to 3.60 mmol/L for HDL cholesterol (1.48 $\pm$ 0.54 mmol/L), and from 0.49 to 2.80 mmol/L for triacylglycerols (1.15 $\pm$ 0.55 mmol/L). Sixteen women were postmenopausal and 5 used oral contraceptives.

Experimental design and diets

The study had a randomized, multiple, crossover design and consisted of 3 consecutive periods. Each participant consumed each of the 3 different diets during three 5-wk periods. One diet was high in stearic acid (18:0), another was high in oleic acid (18:1), and the third was high in linoleic acid (18:2). Before the study started, the subjects were categorized according to sex and were then randomly divided into 6 groups. Each group received the diets in 1 of the 6 possible treatment orders. Between each 5-wk intervention period there was a washout period of ≥1 wk, during which time the participants consumed their habitual diets.

The prescribed nutrient composition of the diets did not differ, except for a 7% difference in energy intake provided by stearic acid, oleic acid, or linoleic acid. Before the participants started the study, their total energy intake was estimated with the Harris-Benedict equation (6). The diets were formulated to provide 16 different energy intakes ranging from 6 to 13.5 MJ/d. The experimental products supplied 60% of the total fat energy at a targeted total fat intake of 37% of energy. For the remaining 40% of the total daily fat intake, subjects were free to consume a certain amount of "free-choice" fat-containing products. Therefore, participants received a list of fat-containing products to which points had been assigned on the basis of fat content (1 point equals 1 g fat). These products had to be recorded in a diary. Furthermore, alcohol consumption, medications used, signs of illness, menstruation information, and any deviations from the study protocol were noted in this diary. The subjects were asked not to change their level of physical exercise or their use of alcohol, vitamins, or oral contraceptives during the study.

TABLE 1
Fatty acid composition of the 3 experimental fats[1]

Fatty acid	Stearic acid	Oleic acid	Linoleic acid
	% of total fatty acids by wt		
Saturated	57.0	22.6	23.0
Lauric acid (12:0)	0.1	0.1	0.1
Myristic acid (14:0)	0.2	0.3	0.3
Palmitic acid (16:0)	16.2	15.3	15.8
Stearic acid (18:0)	38.6	5.7	5.9
Monounsaturated	33.9	66.5	32.6
Palmitoleic acid (16:1n−7)	0.2	0.4	0.2
Oleic acid (18:1n−9)	33.0	64.9	31.3
Polyunsaturated	9.2	10.9	44.4
Total n−6	9.0	10.6	44.0
Linoleic acid (18:2n−6)	8.9	10.6	43.9
Total n−3	0.1	0.2	0.3
α-Linoleic acid (18:3n−3)	0.1	0.2	0.2

[1] Values were determined by gas-liquid chromatography of triplicate samples of the margarines.

During the study periods, the subjects visited the university at least once every week to receive a new supply of products and to be weighed. Any leftover products had to be returned and were weighed. Individual allowances were adjusted if weight changed >1.5 kg from the initial weight during the first week or >2 kg during the following weeks. At each visit, the diary was checked by a dietitian. In the last week of each intervention period, the subjects filled in a food-frequency questionnaire to estimate energy and nutrient intakes. These food-frequency questionnaires were immediately checked by a dietitian. Items were coded, and the composition of the diets was calculated according to a Dutch food-composition table (7).

Experimental fats

Experimental fats were produced by NIZO Food Research (Ede, Netherlands). The high–stearic acid fat was composed of 9.0% palm oil, 5.5% safflower oil, 5.0% olive oil, 33.5% cocoa butter, 18.0% high–oleic acid sunflower oil, and 29.0% glycerol tristearate. The high–oleic acid fat consisted of 19.5% palm oil, 26.0% olive oil, 7.5% cocoa butter, and 47.0% high–oleic acid sunflower oil. The high–linoleic acid fat was a mixture of 20.0% palm oil, 52.0% safflower oil, 7.0% olive oil, 9.0% cocoa butter, and 12.0% high–oleic acid sunflower oil. The fatty acid compositions of the experimental fats, as determined by gas–liquid chromatography, are shown in **Table 1**. From these fats, margarines were produced with a fat content (wt:wt) of 84%. The margarines were used to bake sponge cakes with a margarine content of 25% and bread with a margarine content of 10%. Products were labeled with a blue, orange, or yellow label to blind the subjects.

Blood sampling

Venous blood samples were obtained twice at the end of each period (weeks 4 and 5) while the subjects were in a recumbent position and after they had fasted overnight. Blood was collected with minimal stasis by using a 0.9-mm needle (PrecisionGlide; Becton-Dickinson Vacutainer systems, Plymouth, United Kingdom) in week 4 or with a 1.0-mm infusion needle (Microflex; Vygon, Ecouen, France) in week 5. All venipunctures were done

by the same person, in the same room, and mostly at the same time of the day.

For lipid and lipoprotein analyses, 10 mL blood was collected into a serum tube (Corvac; Becton Dickinson Vacutainer Systems). At least 1 h after venipuncture, serum was obtained by centrifugation at 3500 × *g* for 30 min at 4 °C and stored at −80 °C.

Lipids and apolipoproteins

Serum total cholesterol (ABX Diagnostics, Montpellier, France), HDL cholesterol (precipitation method; Roche Diagnostics Corporation, Indianapolis, IN), and triacylglycerol (Sigma Aldrich Chemie, Steinheim, Germany) concentrations were analyzed enzymatically. The within-run CVs were 1.3% for total cholesterol, 4.8% for HDL cholesterol, and 3.7% for triacylglycerols. LDL cholesterol was calculated by using the equation of Friedewald (8).

Apolipoprotein (apo) A-I and apo B were measured in serum by using an immunoturbidimetric method (ABX Diagnostics). The within-run CVs for apo A-I and apo B were 0.9% and 1.2%, respectively. All samples from one subject were analyzed within one run.

Serum concentrations of lipoprotein particles and their subclasses and particle sizes of lipoproteins were analyzed in a randomly chosen subset (stratified for sex) of 22 subjects (9 men and 13 women) by NMR spectroscopy (Liposcience, Raleigh, NC) as previously described (9). Before NMR analysis, serum samples from the end of each intervention period (weeks 4 and 5) were pooled.

Fatty acid composition

The fatty acid compositions of serum phospholipids in a pooled sample from weeks 4 and 5 and of the margarines were determined as previously described (10). Briefly, total lipids were extracted from 100 μL serum or 10 mg margarine according to the method of Bligh and Dyer (11). Aminopropyl-bonded silica columns (Varian, Harbor City, CA) were used to separate phospholipids from the total lipid extract of serum (12). The phospholipids from the serum and the triacylglycerols from the margarines were then saponified, and the resultant fatty acids were methylated into their corresponding fatty acid methyl esters (FAMEs) (13). Fatty acids were separated on an Autosystem (Perkin-Elmer, Norwalk, CT) gas chromatograph that was fitted with a silica-gel column (Cp-sil 88 for FAME, 50 m × 0.25 mm, 0.2-μm film thickness; Chrompack, Middelburg, Netherlands) with helium gas (130 kPa) as the carrier gas (10). A comparable protocol was used to separate the FAMEs from the triacylglycerols. For triacylglycerols, the injection and detection temperatures were both 300 °C. The starting temperature of the column was 160 °C. Ten minutes after injection, the temperature was increased up to 190 °C at a rate of 2.5 °C/min. After 20 min at 190 °C, the temperature was increased up to 230 °C at a rate of 4 °C/min. The final temperature of 230 °C was maintained for 10 min.

Data were analyzed by using CHROMCARD software (version 1.21; CE Instruments, Milan, Italy). The fatty acid compositions of the margarines and serum phospholipids are expressed in relative amounts (% of total fatty acids identified; wt:wt).

Statistics

For serum lipids and lipoproteins, the results of the 2 serum samples from weeks 4 and 5 were averaged before the statistical analyses. The statistical power to detect a true difference in total cholesterol of 0.21 mmol/L, in LDL cholesterol of 0.17 mmol/L, and in HDL cholesterol of 0.06 mmol/L was >80%. The data were analyzed with the general linear model procedure of the SPSS 11 for MacIntosh OS X package. A P value < 0.05 was considered statistically significant. Differences in effects on lipid and lipoprotein concentrations were examined with diet and period as fixed factors and subject number as a random factor. To analyze whether the effects of diet were modified by sex or BMI, the diet × sex or diet × BMI interaction terms were added to the model as fixed factors. To examine the effects of BMI, the subjects were divided into 2 groups. One group consisted of subjects with a BMI < 25 ($n = 25$) and the other group of subjects with a BMI ≥ 25 ($n = 20$). When the analyses indicated a significant effect of diet, the diets were compared pairwise. When the interaction terms diet × sex or diet × BMI were significant, the diets were compared pairwise for the 2 sex or BMI groups separately. Between-diets comparisons were corrected for 3-group comparisons by the Bonferroni correction; 95% CIs were calculated for the differences between the diets. Values are presented as means ± SDs. Pearson's correlations were determined to examine linear relations between variables.

RESULTS

Diets and dietary adherence

The mean daily energy intake and the composition of the 3 diets, as determined by the food-frequency questionnaires (**Table 2**), agreed well with the prescribed composition of the diets. Intakes of test products (bread, cake, and margarines) did not differ between diets. Total fat intake, on average, was 38% of energy and did not differ between the 3 diets ($P = 0.701$). The nutrient composition of the diets also did not differ, except that 7% of energy was provided by different fats: stearic, oleic, or linoleic acid. Because of minor differences in the fatty acid composition of the experimental fats, the estimated intakes of α-linolenic acid were, respectively, 0.02% ($P = 0.214$) and 0.03% ($P = 0.004$) of energy higher with the oleic acid and linoleic acid diets than with the stearic acid diet. The mean amount of fat consumed as free-choice fat-containing products denoted in the subjects' diaries was 41.5% of total fat intake. This agreed well with the intended amount of 40%.

Mean body weights at the end of each dietary period did not differ significantly between the 3 diets ($P = 0.449$) and were 72.5 ± 13.0 kg with the stearic acid diet, 72.5 ± 13.2 kg with the oleic acid diet, and 72.7 ± 12.9 kg with the linoleic acid diet.

Dietary adherence was confirmed by the fatty acid compositions of serum phospholipids (**Table 3**). During the stearic acid diet, the proportion of stearic acid was increased mainly at the expense of oleic acid. Likewise, the proportion of oleic acid increased after consumption of the oleic acid diet, mainly at the expense of stearic acid. During the diet rich in linoleic acid, the proportion of linoleic acid increased, whereas those of α-linolenic acid, eicosapentaenoic acid, oleic acid, and stearic acid decreased.

TABLE 2
Mean nutrient composition of the 3 diets according to the food-frequency questionnaires[1]

	Stearic acid diet	Oleic acid diet	Linoleic acid diet	*P* for diet effects[2]
Energy				
(MJ/d)	8.4 ± 1.5	8.6 ± 1.7	8.6 ± 1.7	0.086
(Kcal/d)	1997 ± 348	2047 ± 400	2058 ± 406	0.086
Protein (% of energy)	14.0 ± 1.8	14.0 ± 2.1	13.8 ± 2.0	0.412
Fat (% of energy)	38.2 ± 5.1	37.7 ± 5.6	38.0 ± 5.3	0.701
Saturated fatty acids	18.0 ± 2.3[a]	11.0 ± 2.0[b]	11.2 ± 1.9[b]	0.001
Stearic acid (18:0)[3]	7.7 ± 1.1[a]	1.2 ± 0.2[b]	1.2 ± 0.2[b]	0.001
Monounsaturated fatty acids	12.9 ± 2.0[a]	19.1 ± 2.9[b]	12.5 ± 1.8[a]	0.001
Oleic acid (18:1)[3]	6.8 ± 1.0[a]	13.1 ± 2.0[b]	6.6 ± 1.0[a]	0.001
Polyunsaturated fatty acids	4.7 ± 1.2[a]	5.0 ± 1.1[a]	11.8 ± 1.8[b]	0.001
Linoleic acid (18:2)[3]	2.1 ± 0.3[a]	2.4 ± 0.3[a]	9.3 ± 1.3[b]	0.001
α-Linoleic acid (18:3)	0.2 ± 0.1[a]	0.2 ± 0.1[a,b]	0.2 ± 0.1[b]	0.006
Carbohydrates (% of energy)	45.8 ± 5.6	46.3 ± 6.6	46.3 ± 6.2	0.624
Alcohol (% of energy)	2.3 ± 2.4	2.2 ± 2.3	2.1 ± 2.3	0.418
Cholesterol (mg/MJ)	17.7 ± 3.2	17.4 ± 4.2	17.9 ± 3.3	0.502
Dietary fiber (g/MJ)	3.1 ± 0.6	3.1 ± 0.7	3.1 ± 0.7	0.686

[1] All values are $\bar{x}$ ± SD; $n = 45$ (18 men and 27 women). Values in a row with different superscript letters are significantly different, $P < 0.05$ (Bonferroni-corrected pairwise comparisons in general linear model).
[2] Calculated by using a general linear model with subject number as a random factor and diet and period as fixed factors.
[3] As provided by the experimental fats only.

Serum lipids and apolipoproteins

The effects of the 3 different diets on serum lipid and lipoprotein concentrations are given in **Table 4**. No statistically significant changes in serum concentrations of total ($P = 0.110$ for diet effects) and LDL ($P = 0.137$ for diet effects) cholesterol were found.

Effects on HDL cholesterol ($P = 0.866$) and triacylglycerol ($P = 0.670$) concentrations also did not differ between the 3 diets. With respect to the total to HDL cholesterol ratio, no significant differences existed between the 3 diets ($P = 0.303$). Changes in concentrations of apo B ($P = 0.122$) and A-I ($P = 0.534$) were also not statistically significant between the 3 diets and they paralleled those of LDL and HDL cholesterol, respectively. A statistically significant diet × BMI interaction effect ($P = 0.029$) was observed for apo B. In the high-BMI group ($P = 0.011$ for diet effects), the linoleic acid diet reduced apo B concentrations by 0.08 g/L relative to stearic acid ($P = 0.010$; 95% CI for the difference: 0.02, 0.15 g/L). In the low-BMI group, apo B concentrations did not differ between the 3 diets ($P = 0.689$). None of the dietary effects differed significantly between men and women (data not shown).

Lipoprotein particle concentrations and sizes

Changes in VLDL, LDL, and HDL particle sizes and subclass concentrations did not differ significantly between the 3 diets (**Table 5**). No sex-dependent diet effects were observed (data not shown). The diet × BMI interaction was significant for small

TABLE 3
Fatty acid composition of serum phospholipids during the 3 dietary periods[1]

Fatty acid	Stearic acid diet	Oleic acid diet	Linoleic acid diet	*P* for diet effects[2]
		% of total fatty acids by wt		
Saturated	46.5 ± 1.5[a]	45.6 ± 1.5[b]	46.2 ± 1.9[a]	0.001
Palmitic acid (16:0)	26.5 ± 1.6[a]	26.8 ± 1.4[a,b]	26.9 ± 1.6[b]	0.014
Stearic acid (18:0)	14.3 ± 1.2[a]	13.1 ± 1.1[b]	13.7 ± 1.3[c]	0.001
Monounsaturated	13.6 ± 1.1[a]	15.0 ± 1.3[b]	12.2 ± 0.9[c]	0.001
Oleic acid (18:1n−9)	9.3 ± 1.1[a]	10.5 ± 1.2[b]	7.7 ± 0.8[c]	0.001
Polyunsaturated	39.1 ± 1.6[a]	38.6 ± 1.7[a]	40.6 ± 2.1[b]	0.001
Total n−6	33.7 ± 2.0[a]	33.3 ± 1.9[a]	35.8 ± 2.2[b]	0.001
Linoleic acid (18:2n−6)	20.7 ± 1.8[a]	20.5 ± 2.0[a]	23.2 ± 2.4[b]	0.001
Arachidonic acid (20:4n−6)	8.9 ± 1.5	8.7 ± 1.5	8.6 ± 1.7	0.103
Total n−3	5.2 ± 1.2[a]	5.1 ± 1.0[a]	4.7 ± 1.0[b]	0.001
α-Linoleic acid (18:3n−3)	0.1 ± 0.0[a]	0.1 ± 0.0[a]	0.1 ± 0.0[b]	0.001
EPA (20:5n−3)	0.8 ± 0.4[a]	0.7 ± 0.3[a]	0.5 ± 0.3[b]	0.001
DHA (22:6n−3)	3.4 ± 0.9	3.3 ± 0.7	3.2 ± 0.7	0.063
trans	0.8 ± 0.3	0.8 ± 0.2	0.9 ± 0.2	0.060

[1] All values are $\bar{x}$ ± SD; $n = 45$ (18 men and 27 women). EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid. Values in a row with different superscript letters are significantly different, $P < 0.05$ (Bonferroni-corrected pairwise comparisons in general linear model).
[2] Calculated by using a general linear model with subject number as a random factor and diet and period as fixed factors.

TABLE 4
Fasting serum lipid and lipoprotein concentrations and the ratio of total to HDL cholesterol during consumption of diets enriched in stearic, oleic, and linoleic acids for 5 wk by healthy men and women[1]

	Stearic acid diet	Oleic acid diet	Linoleic acid diet	*P* for diet effects[2]
Total cholesterol (mmol/L)	5.81 ± 0.94	5.73 ± 0.81	5.66 ± 0.91	0.110
LDL cholesterol (mmol/L)	3.79 ± 0.91	3.71 ± 0.79	3.65 ± 0.91	0.137
HDL cholesterol (mmol/L)	1.45 ± 0.43	1.46 ± 0.45	1.46 ± 0.44	0.866
Triacylglycerols (mmol/L)	1.24 ± 0.55	1.22 ± 0.52	1.21 ± 0.60	0.670
Apolipoprotein A-I (g/L)	1.39 ± 0.23	1.41 ± 0.25	1.40 ± 0.24	0.534
Apolipoprotein B (g/L)	1.08 ± 0.20	1.06 ± 0.19	1.04 ± 0.17	0.122
Total:HDL cholesterol	4.31 ± 1.33	4.22 ± 1.23	4.19 ± 1.28	0.303

[1] All values are $\bar{x}$ ± SD; $n = 45$ (18 men and 27 women).
[2] There were no significant differences between the 3 diets (general linear model with subject number as a random factor and diet and period as fixed factors).

VLDL concentrations ($P = 0.030$). In the low-BMI group ($P = 0.043$ for diet effects), linoleic acid increased the small VLDL concentration by 9.7 nmol/L ($P = 0.042$; 95% CI for the difference: 0.3, 19.1 nmol/L) when compared with oleic acid. In the high-BMI group, diet effects were not statistically significant ($P = 0.189$). Concentrations of small VLDL particles were 19.9 nmol/L ($P = 0.002$; 95% CI: −31.2, −8.6 nmol/L), of intermediate-density lipoprotein (IDL) particles were 31.5 nmol/L ($P = 0.018$; 95% CI: −57.0, −5.9 nmol/L), of total LDL particles were 402 nmol/L ($P = 0.024$; 95% CI: −745, −59 nmol/L), of small LDL particles were 535 nmol/L ($P = 0.010$; 95% CI: −927, −143 nmol/L), of medium-small LDL particles were 108 nmol/L ($P = 0.016$; 95% CI: −193, −22 nmol/L), and of very small LDL particles were 427 nmol/L ($P = 0.009$; 95% CI: −734, −120 nmol/L) lower in women than in men. Large HDL-particle concentrations were 3.9 μmol/L ($P = 0.002$; 95% CI: 1.7, 6.1 μmol/L) higher in women. LDL and HDL particle sizes were 1.0-nm ($P = 0.005$; 95% CI: 0.3, 1.6 nm) and 0.6-nm ($P = 0.003$; 95% CI: 0.2, 0.9 nm) higher, respectively, in women than in men. With the high–oleic acid diet, BMI was significantly correlated with total LDL ($r = 0.491$, $P = 0.020$), IDL ($r = 0.431$, $P = 0.045$), small LDL ($r = 0.440$, $P = 0.040$), medium-small LDL ($r = 0.457$, $P = 0.032$), and very small LDL ($r = 0.435$, $P = 0.043$) particle concentrations and with HDL particle size ($r = -0.532$, $P = 0.011$). Age correlated with LDL ($r = 0.468$, $P = 0.028$) and IDL ($r = 0.486$, $P = 0.022$) particle concentrations. Comparable relations were observed when subjects consumed the high–stearic acid or high–linoleic acid diets.

TABLE 5
Particle concentrations of lipoprotein subclasses and lipoprotein particle sizes as measured by nuclear magnetic resonance spectroscopy during consumption of diets enriched in stearic, oleic, or linoleic acid for 5 wk by healthy men and women[1]

	Stearic acid diet	Oleic acid diet	Linoleic acid diet	*P* for diet effects[2]
Particle concentrations				
VLDL (nmol/L)				
Total	83.5 ± 29.1	82.1 ± 30.8	86.3 ± 33.2	0.560
Large and chylomicrons	2.6 ± 3.1	2.8 ± 3.2	2.1 ± 3.4	0.209
Medium	31.4 ± 15.7	32.8 ± 14.9	33.5 ± 20.7	0.716
Small	49.5 ± 16.3	46.6 ± 17.8	50.8 ± 18.4	0.332
IDL (nmol/L)	47.8 ± 43.6	44.5 ± 30.2	36.7 ± 33.0	0.215
LDL (nmol/L)				
Total	1305 ± 468	1244 ± 437	1262 ± 387	0.213
Large	561 ± 204	551 ± 221	567 ± 223	0.875
Small	696 ± 558	648 ± 542	658 ± 441	0.568
Medium small	133 ± 118	124 ± 108	130 ± 101	0.595
Very small	563 ± 441	524 ± 435	528 ± 342	0.550
HDL (μmol/L)				
Total	33.8 ± 4.3	33.4 ± 4.3	34.1 ± 4.5	0.545
Large	8.4 ± 3.6	8.3 ± 3.0	8.8 ± 3.3	0.468
Medium	3.2 ± 3.6	3.4 ± 3.9	3.4 ± 3.6	0.942
Small	22.2 ± 4.4	21.7 ± 4.8	21.9 ± 3.6	0.759
Lipoprotein particle size (nm)				
VLDL	45.4 ± 4.1	45.0 ± 4.4	46.2 ± 5.9	0.277
LDL	21.5 ± 0.9	21.5 ± 1.0	21.5 ± 0.8	0.985
HDL	9.1 ± 0.5	9.2 ± 0.5	9.2 ± 0.5	0.907

[1] All values are $\bar{x}$ ± SD; $n = 22$ (9 men and 13 women).
[2] There were no significant differences between the 3 diets (general linear model with subject number as a random factor and diet and period as fixed factors).

DISCUSSION

In this well-controlled crossover study of healthy subjects, we found that the differences in effects of stearic, oleic, and linoleic acids on the serum lipoprotein profile were less than expected. Although total and LDL-cholesterol concentrations tended to decrease with the increasing degree of unsaturation, the changes between the 3 diets were not significant. Based on the classic equations derived by Keys et al (1), a decrease of 0.21 mmol/L in total cholesterol concentrations is expected when 7% of energy from stearic acid or oleic acid in the diet is exchanged for linoleic acid. However, we found decreases of 0.15 and 0.07 mmol/L, respectively.

Until now, only a few studies have examined simultaneously the effects of stearic acid, oleic acid, and linoleic acid. Consistent with our results, Hunter et al (14) found no differences in the effects of these fatty acids on plasma total or LDL-cholesterol concentrations. However, only 6 healthy male subjects participated in that study, and the statistical power may have been too low to detect any changes. Kris-Etherton et al (15) examined in 19 young men the effects of natural edible fats and oils rich in stearic acid (cocoa butter), oleic acid (olive oil), or linoleic acid (soybean oil) on the serum lipoprotein profile. It was found that the diet rich in linoleic acid significantly lowered serum total cholesterol concentrations relative to stearic acid or oleic acid. In addition, the LDL-cholesterol concentration was lower with the diet rich in linoleic acid than with the diet rich in stearic acid. A possible explanation for these apparent discrepancies with our results might be that, in their study, ≈10% of energy from stearic acid and ≈16% of energy from oleic acid was exchanged for linoleic acid. The expected decreases in total and LDL-cholesterol concentrations were therefore greater. In that study (15), the high–oleic acid diet also decreased total and LDL-cholesterol concentrations significantly more than did the high–stearic acid diet. The difference in response between these 2 diets can at least partly be explained by the higher intake of palmitic acid from the diet rich in stearic acid. Palmitic acid is known to increase serum total and LDL-cholesterol concentrations relative to stearic or oleic acid (3, 16).

Our results agree with the many studies that compared stearic acid with oleic acid (3) or oleic acid with linoleic acid (4, 5, 17, 18) and also found no different effects on the serum lipoprotein profile. In one study, however, an exchange of 8% of energy from stearic acid for oleic acid significantly decreased serum LDL cholesterol by 0.15 mmol/L. Surprisingly, no effects on apo B concentrations were found (19). In addition, Zock and Katan (20) found that when 8% of energy from stearic acid was replaced by linoleic acid, the linoleic acid diet significantly decreased serum LDL cholesterol by 0.17 mmol/L. When expressed as a percentage of energy, however, their effects did not differ from those in our study.

In a recent meta-analysis, equations were developed to describe the effects of individual fatty acids on serum lipids and lipoproteins (21). On the basis of these equations, replacement of 7% of energy from stearic acid by oleic acid may result in a decrease in LDL-cholesterol concentrations of 0.04 mmol/L and a decrease of 0.11 mmol/L when replaced by linoleic acid. These estimates agree well with the observed differences in LDL-cholesterol concentrations of −0.08 mmol/L between the diets enriched in stearic and oleic acids and of −0.14 mmol/L between the diets high in stearic acid and linoleic acid. The power of our study to pick up this latter difference was 60%. Taken together, evidence continues to accumulate to suggest that the earlier formulas (1, 2) overestimate the effects of linoleic acid on serum total cholesterol concentrations.

On the basis of the earlier meta-analysis (21), decreases in HDL-cholesterol concentrations of 0.04 mmol/L were predicted when oleic acid was replaced by stearic acid, and of 0.03 mmol/L when stearic acid was exchanged for linoleic acid. In our study, however, decreases were slightly, but not significantly, lower when stearic acid replaced either oleic or linoleic acid. Other studies also reported no differential effects of oleic and linoleic acids on HDL-cholesterol concentrations (4, 5, 14, 15). In contrast, some studies have reported that linoleic acid decreases HDL-cholesterol concentrations relative to oleic acid (17, 18). Zock and Katan (20) reported a decrease in HDL cholesterol when linoleic acid was exchanged for stearic acid, whereas Judd et al (19) reported a decrease when oleic acid was replaced for stearic acid. A nonsignificant decrease was also observed by Bonanome and Grundy (3). Thus, these 3 studies suggest that stearic acid may lower HDL cholesterol relative to oleic and linoleic acids, which is not supported by our results or the studies that simultaneously compared stearic, oleic, and linoleic acids (14, 15). In the 3 other studies, stearic acid was largely provided by interesterified and hydrogenated synthetic fats (3, 19, 20). In these fats, stearic acid was not only located at the sn-1 and sn-3 positions, as is the case in natural fats, but also at the sn-2 position (22). Because of these stereospecific distributions, it is possible that the effects of natural fats rich in stearic acid on the serum lipoprotein profile are different from those of synthetic fats. This suggestion, however, requires further investigation.

No differential effects of stearic acid, oleic acid, or linoleic acid were found on lipoprotein particle sizes and concentrations. As is true for small, dense LDL particles (23, 24), small HDL particles (25, 26) are positively associated with increased cardiovascular disease risk. Therefore, we also examined the effects of stearic, oleic, and linoleic acids on LDL, HDL, and VLDL particle size and subclass particle concentrations by using NMR spectroscopy. Until now, only a few studies have examined the effects of the quality of dietary fat on lipoprotein particle sizes or subclass distributions of lipoprotein particles. Relative to saturated fat, monounsaturated and n−6 and n−3 polyunsaturated fatty acids slightly but significantly decreased LDL particle size (27). In contrast, in another study no significant changes in LDL particle size were observed when saturated fatty acids were exchanged for monounsaturated fatty acids (28). Unfortunately, no details about the individual saturated fatty acid composition of the diets were given. Observed differences in particle sizes and particle concentrations between men and women in our study agreed well with those of The Framingham Offspring Study, in which these variables were measured in a large group of 1574 men and 1692 women (9).

In summary, the effects of stearic acid, oleic acid, and linoleic acid on LDL-cholesterol concentrations were less than expected. Effects on HDL-cholesterol and triacylglycerol concentrations as well as the size and the concentration of the lipoprotein particles also did not differ significantly between diets. These findings, however, do not imply that these three 18-carbon fatty acids can be exchanged without affecting cardiovascular disease risk, because other cardiovascular disease risk markers (eg, hemostatic function, oxidative stress, and low-grade inflammation) are also influenced by the fatty acid composition of the diet.

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MAT and RPM were responsible for the design of the study and the collection and analysis of the data. Both authors wrote the manuscript. None of the authors had any conflict of interest in any company or organization sponsoring the research, including advisory board affiliations.

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