Effects of margarine compared with those of butter on blood lipid profiles related to cardiovascular disease risk factors in normolipemic adults fed controlled diets1–3

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ABSTRACT  Effects of butter and 2 types of margarine on blood lipid and lipoprotein concentrations were compared in a controlled diet study with 23 men and 23 women. Table spreads, added to a common basal diet, provided 8.3% of energy as fat. Diets averaged 34.6% of energy as fat and 15.5% as protein. Each diet was fed for 5 wk in a 3 × 3 Latin-square design. One margarine (TFA-M) approximated the average trans fatty acid–containing margarines in the United States (17% trans fatty acids by dry wt). The other margarine (PUFA-M) was free of trans unsaturated fatty acids; it contained approximately twice the polyunsaturated fatty acid content of TFA-M (49% compared with 27% polyunsaturated fatty acids). The tub-type margarines had similar physical properties at ambient temperature. Fasting blood lipids and lipoproteins were determined in 2 samples taken from the subjects during the fifth week of each dietary treatment. Compared with butter, total cholesterol was 3.5% lower (P = 0.009) after consumption of TFA-M and 5.4% lower (P < 0.001) after consumption of PUFA-M. Similarly, LDL cholesterol was 4.9% lower (P = 0.005) and 6.7% lower (P < 0.001) after consumption of TFA-M and PUFA-M, respectively. Neither margarine differed from butter in its effect on HDL cholesterol or triacylglycerols. Thus, consumption of TFA-M or PUFA-M improved blood lipid profiles for the major lipoproteins associated with cardiovascular risk when compared with butter, with a greater improvement with PUFA-M than with TFA-M. Am J Clin Nutr 1998;68:768–77.

KEY WORDS  Margarine, butter, table spreads, cardiovascular disease risk factors, polyunsaturated fatty acids, trans unsaturated fatty acids, blood lipids, lipoproteins, humans

INTRODUCTION  It is well established that saturated fatty acids (SFAs) raise plasma total and LDL-cholesterol concentrations compared with n−6 polyunsaturated and n−9 monounsaturated fatty acids (MUFAs) (1–3). Therefore, recommendations to reduce the risk of cardiovascular disease usually stress the importance of reducing intake of SFAs (4). In practical terms, this advice frequently implies restricting the intake of products rich in SFAs and cholesterol, such as butter, and replacing them in part with equivalent products lower in cholesterol and SFAs but higher in unsaturated fatty acids, such as margarines.

Over the past 7–8 yr, evidence has shown that, in addition to SFAs, trans unsaturated fatty acids (TFAs) also raise plasma total cholesterol and LDL cholesterol and may lower plasma HDL cholesterol concentrations (5–8). In our laboratory, the hypercholesterolemic effects of dietary TFAs were between those of cis unsaturated fatty acids and 12–16-carbon SFAs (8). Some (9, 10), but not all (11), studies indicated that high intakes of TFAs increase the risk of cardiovascular disease, which agrees with the observed effects of TFAs on blood lipids.

The major source of TFAs in the diet are products containing partially hydrogenated vegetable oils. Margarines are important contributors to the intake of dietary TFAs in the United States. Therefore, the issue arises as to whether there are benefits to replacing a product rich in SFAs, such as butter, with a product lower in SFAs but higher in cis- and TFAs, such as margarine. A recent meta-analysis of dietary trials that directly compared the effects of butter and margarine on blood lipids concluded that replacement of butter by low-TFA soft margarines favorably affects the blood lipoprotein profile, whereas high-TFA hard margarines probably do not confer a benefit over use of butter (12).

It is now feasible to manufacture margarines that have no TFAs and have high amounts of unsaturated fatty acids without major increases in SFAs. In the current trial, we compared such a novel margarine with butter and with a margarine containing a TFA con-
TABLE SPREADS AND PLASMA LIPIDS 769

concentration typical of most margarines consumed in the United States to determine effects on plasma lipids, lipoprotein profiles, vitamin E, and lipid hydroperoxide concentrations.

SUBJECTS AND METHODS

Study design

A controlled, crossover feeding trial was conducted at the Beltsville Human Nutrition Research Center with 24 men and 24 women. The study was conducted from October 1995 to February 1996. All participants consumed 3 different diets for 5 wk. Diet assignments were determined according to a 3 × 3 Latin-square design. This design was chosen to ensure complete balance of the number of diets administered in each study period as well as the number of occurrences of each diet sequence (13). The random sequence of diet assignments was also balanced with respect to sex and baseline plasma LDL-cholesterol concentration. During the fifth week of each period, duplicate blood samples were collected. Subjects were switched from one diet to the next without a washout between periods. Because the timing of the study included the Christmas and New Year’s Day holidays, the study periods were timed to complete 2 study periods before Christmas. Subjects were maintained on the period 2 diet during the holiday period, and then switched to the period 3 diet after January 2.

Blinding of study results

Menus, menu food items and portions, and dietary treatments were color coded during the study. Although study participants easily recognized the butter diet, differences in appearance, taste, and other characteristics between the 2 margarines were not apparent. All samples were coded to blind analysts to treatments. Analytic data were blinded to those who performed the controlled feeding and sample collection phases of the investigation. After all data for an analyte were entered and the database was locked, the treatments were decoded by the statistician performing statistical analysis of the data.

Subject selection

Volunteers were recruited by advertisement in the area of the Beltsville Agricultural Research Center, Beltsville, MD. Men and women of all races between the ages of 25 and 65 y were recruited regardless of smoking habits. From the 601 respondents, 69 met the eligibility criteria described below; 48 (24 men and 24 women) were selected and entered the study.

Minimum eligibility criteria were based on general health, eating habits, age, body mass index (BMI), and fasting plasma HDL-cholesterol, LDL-cholesterol, and triacylglycerol concentrations. Volunteers were required to be within 85–120% of sex-specific ideal BMI specified by life insurance reference tables (14). Volunteers who reported taking lipid-lowering drugs, blood pressure medications, or dietary supplements, or who had eating habits inconsistent with the study protocol (eg, those consuming vegetarian or low-fat diets) were excluded. Volunteers were evaluated by a physician and determined to be in good health, with no signs or symptoms of hypertension, hyperlipemia, diabetes, peripheral vascular disease, gout, liver or kidney disease, or endocrine disorders. Subjects selected for the study were required to have fasting plasma HDL-cholesterol concentrations >0.65 mmol/L (25 mg/dL) for men and >0.91 mmol/L (35 mg/dL) for women, and fasting plasma triacylglycerol concentrations <3.39 mmol/L (300 mg/dL). From the volunteers who met all other selection criteria, those selected to participate had plasma LDL-cholesterol concentrations between the 25th and 75th percentile (mean: 3.51 mmol/L, 63rd percentile) of those screened.

Women taking hormones for postmenopausal replacement therapy (n = 10) or birth control (n = 5) were accepted as subjects with the provision that they continue their normal regimen (type of hormone, schedule, and dose) for the duration of the study and that they record their medication on their daily questionnaire. Exercise was not controlled but subjects were encouraged to maintain their normal exercise patterns (type of exercise, duration, and frequency) throughout the study and were required to record exercise on their daily questionnaire. Smoking was not disallowed, but only one of the participants smoked. The number of cigarettes smoked was reported on the daily questionnaire. This female subject smoked a maximum of 10 cigarettes/d, and on most days smoked <10.

Volunteers were fully informed of study requirements. They were required to read and sign a consent form detailing the study objectives, risks, and benefits before final selection as subjects for the study. All procedures were approved by the Johns Hopkins University Committee on Human Research.

Basal diet and table spreads

All spreads were fed in amounts similar to those of the US diet. Spreads were added to a basal diet to ensure that total dietary intake of macronutrients and major fatty acids was similar to a typical American diet with respect to fat and fatty acids. A basal diet with a 7-d menu cycle was designed so that, when the table spread was included, the percentage of energy from fat would be ≈37%, that from protein 15%, and that from carbohydrate 48%. Data from the US Department of Agriculture Handbook no. 8 series (revised series 1–21) (15), together with analyzed values for the table spreads, were used to formulate the diets.

One of 3 different table spreads (described below) was fed along with the basal diet and in an amount that would provide 8 en% from fat. This amount of table spread was selected to approximate the 90th percentile estimate for both butter and margarine consumption in the American diet for men and women 35–60 y of age who reported that they consumed butter and margarine in the Nationwide Food Consumption Survey, 1977–78 (16). More recent surveys do not list butter and margarine consumption as separate categories. However, on the basis of trends in per capita consumption of these spreads (17), the 90th percentile estimate for current consumption should be approximately the same.

Monday through Friday, all subjects consumed breakfast and dinner at the Beltsville Human Nutrition Research Center’s Human Study Facility under the supervision of a dietitian. At breakfast, each subject was provided with a carryout lunch to be consumed that day. Snack items were included in the daily menu, and subjects were provided the option of consuming the snacks at dinner or later in the evening. Table spreads were provided only at breakfast and dinner. Meals for the weekend were packaged for consumption at home and provided to the subjects, with written instructions, after dinner on Friday. Unlimited amounts of coffee, tea, and diet sodas were allowed but all additives (sugar and milk) for coffee and tea were provided with the meals. Only foods provided by the Human Study Facility were allowed to be consumed during the study.
Each morning, Monday through Friday, subjects were weighed before breakfast when they arrived at the facility. Energy intake was adjusted in 840- or 1680-kJ (200- or 400-kcal) increments to maintain initial body weight. Subjects were fed the same items and the same proportions of each item relative to total dietary energy. Therefore, the relative amounts of all nutrients, other than those provided by the table spread, were constant for all subjects. Each day, subjects completed a questionnaire detailing beverage intake, factors related to dietary compliance, exercise, medications, and illnesses. The questionnaires were reviewed routinely by a study investigator and all problems identified were discussed with the subject during the next meal.

Each table spread was from a single production lot prepared specifically for the study before it began. Two tub-type margarines were prepared by Lipton, Baltimore, to meet criteria established for the spreads to be used in the investigation. All table spreads were stored under refrigeration for the duration of the study. Butter was obtained from a commercial creamery (Sommer Made Creamery, Doylestown, PA). The fatty acid composition of the margarine containing TFAs (TFA-M) was based on the market share weighted-average fatty acid composition of all forms of the 21 margarines that comprised 69% of the US market share during the 12-wk period ending in July 1995 (Nielson Scantrack Data, AC Nielsen Co, Northbrook, IL). The distribution of 18:1 isomers in TFA-M was typical of US margarines based on partially hydrogenated soybean oil. The margarine high in polyunsaturated fatty acids (PUFA-M) was produced by blending liquid sunflower oil with completely hydrogenated soybean and canola oils. The product was produced with as much linoleic acid and as little SFAs as possible while maintaining the desired physical characteristics, and it contained virtually no TFAs. The sensory and physical characteristics of the 2 margarines were similar and were comparable with commercially available tub-type spreads.

Chemical analysis of diets and table spreads

During the first feeding period, 2 composites of the 7 diets were collected at 2 energy levels. The food was prepared as though it were to be consumed and then was mixed in a blender with ice added to prevent heat buildup. The blended samples were freeze-dried in preweighed containers and then reweighed. The samples were then pulverized and weekly composites were prepared by mixing 15% of each day’s dry weight. Composite samples of the table spreads were collected from 10 randomly selected containers and blended together. Thus, 4 weekly composites and duplicate table spreads were stored under refrigeration for the duration of the study. Butter was obtained from a commercial creamery (Sommer Made Creamery, Doylestown, PA). The fatty acid composition of the margarine containing TFAs (TFA-M) was based on the market share weighted-average fatty acid composition of all forms of the 21 margarines that comprised 69% of the US market share during the 12-wk period ending in July 1995 (Nielson Scantrack Data, AC Nielsen Co, Northbrook, IL). The distribution of 18:1 isomers in TFA-M was typical of US margarines based on partially hydrogenated soybean oil. The margarine high in polyunsaturated fatty acids (PUFA-M) was produced by blending liquid sunflower oil with completely hydrogenated soybean and canola oils. The product was produced with as much linoleic acid and as little SFAs as possible while maintaining the desired physical characteristics, and it contained virtually no TFAs. The sensory and physical characteristics of the 2 margarines were similar and were comparable with commercially available tub-type spreads.

Blood sample collection and analysis

Baseline blood samples were collected during the week before initiation of the controlled feeding. Subsequently, samples were collected at 5-wk intervals. The 48 subjects were randomly divided into 2 groups. One group had samples drawn on Mondays and Wednesdays and the other on Tuesdays and Thursdays.

Procedures for blood sampling and processing were those described in the protocol for the Lipid Research Clinics Program (18). Blood samples were drawn after an overnight fast (minimum of 12 h), immediately before breakfast. Samples for blood lipid, tocopherol, and lipid hydroperoxide analyses were collected by venipuncture using a 19-gauge butterfly needle into evacuated tubes containing disodium EDTA. After being mixed gently by inversion, the samples were placed immediately on wet ice. Within 30 min of collection, plasma was separated by centrifugation at 1400 × g for 20 min at 4°C.

Plasma was removed from the tubes, divided into samples, and stored in cryogenic vials at −80°C. Before storage, propyl gallate was added to the samples used for tocopherol analyses, and the sample to be used for HDL and HDL₃ determination was precipitated by using the sequential precipitation procedure of Gidez et al (19). Supernates from the HDL precipitation were stored at −80°C for later analysis of cholesterol. Analyses for cholesterol, triacylglycerols, apolipoproteins, and other blood components were performed after the final blood collection. All analyses of the samples from each subject were performed in the same analytic run.

Lipid analyses (cholesterol, triacylglycerols, and HDL cholesterol) were performed at the Lipid Research Clinic Laboratory, The George Washington University Medical Center, which maintains standardization with the Centers for Disease Control and Prevention, US Department of Health and Human Services. Plasma total cholesterol, HDL cholesterol, HDL₃ cholesterol, and triacylglycerols were determined enzymatically with commercial kits (Sigma Chemical Company, St Louis) on an Abbott VP analyzer (Abbott Laboratories, Chicago). HDL₂ cholesterol was determined as the difference between HDL cholesterol and HDL₃ cholesterol. LDL cholesterol was calculated by using the Friedewald equation (20). Plasma apolipoprotein (apo) A-I and B concentrations were determined by rate nephelometry (Beckman ICS Immunochemical analyzer; Beckman Instruments, Fullerton, CA). Apo A-II was determined by radial immunodiffusion. Plasma lipoprotein(a) [Lp(a)] was analyzed as described previously (21–23) by using a commercially available enzyme-linked immunosorbent assay (Strategic Diagnostics, Newark, DE).

α- and γ-Tocopherols were determined by using HPLC. Samples were extracted with isopropanol containing propyl gallate, and distilled water and hexane containing butylated hydroxytoluene (BHT). BHT and an internal standard (tocol) were added. After vortex mixing, the mixture was allowed to stand and an aliquot removed from the hexane layer. This aliquot was dried under nitrogen gas and the residue was redissolved in 120 μL ethanol:methylene chloride (90:10, by vol) and propyl gallate. Fifty microliters was injected onto a reversed-phase column (Microsorb-MV C₁₈, 4.6 mm internal diameter × 25 cm, Rainin Instrument Co, Inc, Woburn, MA) with a mobile phase of methanol:acetonitrile:methylene chloride (75:20:5) at a flow rate of 1.5 mL/min. Tocol and tocopherols were detected by spectrofluorescence at an excitation wavelength of 294 nm and an emission wavelength of 324 nm.

Lipid hydroperoxides were determined by using a commercially available kit (Kamiya Biomedical Company, Thousand Oaks, CA). The samples were dissolved in isopropanol and water containing triton X-100. The assays were performed on a microtiter plate. LDL size was determined by nuclear magnetic resonance spectroscopy (24).

Statistical analysis

All analyses were performed by using SAS for Windows version 6.11 or S-Plus (SAS Institute, Cary, NC). The analytic plan
was designed a priori and described a mixed-effects model for analysis of the data (25). For each variable, the average of 2 sample measurements taken during week 5 of each feeding period was analyzed by using an analysis of variance model that included terms for sex, period, and carryover of a diet from one period to the next. Because the investigators predicted that the interaction terms would account for only a small amount of the variation in the data, the analytic plan specified that an interaction term would be included in the final model if it were significant at the nominal 0.15 level in the presence of the other terms. The contrasts between diets were tested by an F test for differences between groups.

RESULTS

Subjects

Twenty-four men and 24 women completed the screening process and began the controlled feeding. One man withdrew because of a personal conflict and one woman withdrew because of travel that interfered with the feeding protocol. Thus, 23 men and 23 women completed the feeding phase of the study. Data were analyzed statistically only for subjects who completed all 3 feeding periods. Characteristics of the participants at baseline are presented in Table 1. The age range of subjects who completed the study was 28–65 y with a mean of 46.9 y for men and 46.7 y for women. Baseline BMI (in kg/m²) for men was 25.7 and mean calculated metabolizable energy intake during the feeding phase was 12.66 MJ/d (3025 kcal/d). For women, baseline BMI was 24.8 and mean metabolizable energy intake was 46.7 MJ/d (1113 kcal/d). The age range of subjects who completed the study was 25.7 ± 2.34 kg/m² for men and 24.8 ± 2.15 kg/m² for women. Baseline HDL cholesterol (mmol/L) was 1.08 ± 0.09 mmol/L for men and 1.04 ± 0.14 mmol/L for women. Baseline LDL cholesterol (mmol/L) was 5.11 ± 0.11 mmol/L for men and 5.14 ± 0.15 mmol/L for women. Baseline total cholesterol (mmol/L) was 5.11 ± 0.11 mmol/L for men and 5.14 ± 0.15 mmol/L for women. Baseline triacylglycerol concentrations were higher at baseline than after any of the diets with table spread added.

Diets and table spreads

Analyzed macronutrient and fatty acid compositions of the basal diet and table spreads are presented in Table 2. The 2 margarines differed in oil blend but had similar product characteristics (taste, hardness, mouth feel, spreadability, and melting behavior). The oil blend in TFA-M was liquid soybean and partially hydrogenated soybean oil; the oil blend in PUFA-M was liquid sunflower oil and completely hydrogenated soybean and canola oils. On a dry weight basis, the SFA content of TFA-M was 16% and 13% of SFAs, respectively, whereas the margarines contained relatively small amounts of myristic acid and only trace amounts of lauric acid. Although the absolute amount

![TABLE 1](https://academic.oup.com/ajcn/article-abstract/68/4/768/4648618)

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Analyzed composition of basal diet and table spreads fed to subjects for 5 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Basal diet</strong></td>
<td><strong>Butter</strong></td>
</tr>
<tr>
<td>Protein (%)</td>
<td>19.3</td>
</tr>
<tr>
<td>Total carbohydrate (%)</td>
<td>62.19</td>
</tr>
<tr>
<td>Total dietary fiber (%)</td>
<td>4.03</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>14.91</td>
</tr>
<tr>
<td>α-Tocopherol (µg/g)</td>
<td>—</td>
</tr>
<tr>
<td>γ-Tocopherol (µg/g)</td>
<td>—</td>
</tr>
<tr>
<td>α-Tocopherol equivalents (µg/g)</td>
<td>—</td>
</tr>
<tr>
<td>Short-chain fatty acids, 4–10 carbons (%)</td>
<td>0.05</td>
</tr>
<tr>
<td>Total saturated fatty acids, 12–24 carbons (%)</td>
<td>3.64</td>
</tr>
<tr>
<td>Lauric acid</td>
<td>0.03</td>
</tr>
<tr>
<td>Myristic acid</td>
<td>0.16</td>
</tr>
<tr>
<td>Palmitic acid</td>
<td>1.97</td>
</tr>
<tr>
<td>Stearic acid</td>
<td>1.22</td>
</tr>
<tr>
<td>Total monounsaturated fatty acids, 14:1–20:1 (%)</td>
<td>6.35</td>
</tr>
<tr>
<td>Oleic (cis 18:1)</td>
<td>4.81</td>
</tr>
<tr>
<td>trans Monoenones (trans 18:1)</td>
<td>1.33</td>
</tr>
<tr>
<td>Total polyunsaturated fatty acids (%)</td>
<td>3.74</td>
</tr>
<tr>
<td>Linoleic</td>
<td>3.22</td>
</tr>
<tr>
<td>Linolenic</td>
<td>0.33</td>
</tr>
</tbody>
</table>

¹Dry weight basis. TFA-M, margarine containing trans fatty acids; PUFA-M, margarine containing polyunsaturated fatty acids.
²None detected.
³Trace detected.

![TABLE SPREADS AND PLASMA LIPIDS](https://academic.oup.com/ajcn/article-abstract/68/4/768/4648618)
of SFA in PUFA-M was higher than that in TFA-M, the ratio of stearic acid to the sum of lauric, myristic, and palmitic acids was higher for PUFA-M than for TFA-M (≈2.3:1 and 0.9:1 for PUFA-M and TFA-M, respectively). Oleic acid (cis-18:1n−9) was 33% lower in PUFA-M than in TFA-M. The oleic acid content of butter was between that of PUFA-M and TFA-M.

Contributions to total dietary energy from the fat and fatty acids in the basal diet and each of the table spreads are shown in Table 3. Differences in the fatty acid composition among the 3 diets were not as great as among the 3 table spreads. All diets contained similar amounts of MUFA. The 2 margarine diets had comparable energy from SFAs. The major differences among the 3 diets were not as great as among the 3 table spreads. All diets contained similar amounts of MUFA. The 2 margarine diets had comparable energy from SFAs. The major differences among the 3 diets were not as great as among the 3 table spreads.

The basal diet provided ≈0.065 mmol cholesterol/MJ (25.1 mg/MJ). Because the margarines contained no cholesterol, the diets with margarine provided cholesterol at the level in the basal diet. Butter added to the basal diet provided an additional 0.015 mmol/MJ (5.8 mg/MJ) for a diet total of 0.079 mmol cholesterol/MJ (30.5 mg/MJ).

Energy from fat in all diets as fed, ie, basal plus table spread, was ≈34.6 en%. This is about that reported now for a typical American diet (26). It is, however, slightly lower than our targeted 37% of energy from fat. Fat from table spreads contributed 8.2–8.4% of energy as compared with our targeted value of 8% of energy. Because these differences from the targeted values occurred in all diets, they should not affect interpretation of the relative effects of the spreads on plasma lipids and other variables.

The basal diet contributed slightly more than 3 times the energy from fat as did the table spreads (ie, 26.3% compared with 8.3% of energy; Table 3). This diluted the effect of fatty acids from the spreads on biological responses to the total diet. However, this represents how table spreads are typically consumed and allows evaluation of their biological effects in experimental diets when they are fed at average intakes.

Macronutrient and fatty acid intakes from the 3 diets for men and women are presented in Table 4. Mean daily intake of table spreads for men was 35 g for butter, 41 g for TFA-M, and 41 g for PUFA-M. These amounts of spreads each provided 28 g fat. For women, intake was 25 g for butter, 30 g for TFA-M, and 30 g for PUFA-M, which provided 20 g fat each. TFA intakes from the 3 diets were 2.4 en% (PUFA-M), 2.7 en% (butter), and 3.9 en% (TFA-M), which is similar to estimates for intake of TFAs in the US diet of 3–6 en% (8). Total dietary fiber was supplied by the basal diet and was therefore constant across all treatments. Mean daily total dietary fiber intake was 24 g for men and 17 g for women.

The α-tocopherol concentrations of butter and TFA-M were similar. The α-tocopherol concentration of PUFA-M was 5 times greater than that of the other table spreads. The γ-tocopherol concentration was ≈3 times greater in PUFA-M than in the butter diet and 2 times greater in TFA-M than in PUFA-M (Table 2). Vitamin E activity expressed as RRR-α-tocopherol equivalents was calculated as α-tocopherol + 0.1 × γ-tocopherol (in μg), and was 80, 104, and 410 as RRR-α-tocopherol equivalents/g for butter, TFA-M, and PUFA-M, respectively (Table 2).

### Biological responses to diets

**Sex differences**

As expected from documented, inherent sex differences, women had higher concentrations of HDL cholesterol, HDL₂ cholesterol, HDL₃ cholesterol, apo A-I, and apo A-II and lower concentrations of LDL cholesterol and apo B than did men. The responses to all table spreads were, however, similar for men and women.

### Table 3

**Composition of the test diets**

<table>
<thead>
<tr>
<th>Saturated fatty acids (12–24 carbons)</th>
<th>Butter</th>
<th>TFA-M</th>
<th>PUFA-M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>15.5 ± 0.16</td>
<td>15.5 ± 0.16</td>
<td>15.5 ± 0.16</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>50.0 ± 0.15</td>
<td>50.0 ± 0.15</td>
<td>49.9 ± 0.15</td>
</tr>
<tr>
<td>Fat</td>
<td>34.5 ± 0.09</td>
<td>34.6 ± 0.09</td>
<td>34.6 ± 0.09</td>
</tr>
<tr>
<td>Saturated fatty acids (12–24 carbons)</td>
<td>11.2 ± 0.03</td>
<td>7.9 ± 0.01</td>
<td>8.3 ± 0.01</td>
</tr>
<tr>
<td>Stearic acid</td>
<td>3.5 ± 0.01</td>
<td>2.8 ± 0.00</td>
<td>3.3 ± 0.01</td>
</tr>
<tr>
<td>cis Monoenes</td>
<td>10.8 ± 0.01</td>
<td>11.2 ± 0.01</td>
<td>10.4 ± 0.01</td>
</tr>
<tr>
<td>trans Monoenes</td>
<td>2.7 ± 0.00</td>
<td>3.9 ± 0.01</td>
<td>2.4 ± 0.00</td>
</tr>
<tr>
<td>Polysaturated fatty acids</td>
<td>7.2 ± 0.00</td>
<td>9.0 ± 0.01</td>
<td>10.8 ± 0.01</td>
</tr>
</tbody>
</table>

### Table 4

**Daily nutrient intake of men and women, determined by using analytical data on the diets with table spreads added**

<table>
<thead>
<tr>
<th>Saturated fatty acids (12–24 carbons)</th>
<th>Butter</th>
<th>TFA-M</th>
<th>PUFA-M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>117</td>
<td>84</td>
<td></td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>378</td>
<td>272</td>
<td></td>
</tr>
<tr>
<td>Fat</td>
<td>116</td>
<td>84</td>
<td></td>
</tr>
<tr>
<td>Saturated fatty acids (12–24 carbons)</td>
<td>38</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>Stearic acid</td>
<td>12</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>cis Monoenes</td>
<td>36</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>trans Monoenes</td>
<td>9</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Polysaturated fatty acids</td>
<td>24</td>
<td>17</td>
<td></td>
</tr>
</tbody>
</table>

1Table values are means. Mean (± SD) energy intake for men (n = 23) was 12.66 ± 1.27 MJ/d (3025 ± 304 kcal/d) and for women (n = 23) was 9.10 ± 0.89 MJ/d (2175 ± 212 kcal/d). TFA-M, margarine containing trans fatty acids; PUFA-M, margarine containing polysaturated fatty acids.
women; therefore, the overall least-square means are reported for the sexes combined (Table 5).

**Lipids and lipoproteins**

Concentrations of plasma triacylglycerol, lipoprotein cholesterol, and apolipoproteins for all subjects are presented in Table 5. There were no diet-by-period interactions detected for any variables. Contrasts were used to estimate differences between diets containing butter and PUFA-M, butter and TFA-M, and PUFA-M and TFA-M. Because $P$ values were calculated sequentially, the significance of the effects of diet on the measured variables were corrected for subject, sex, period, and carryover.

After consumption of the butter diet, mean LDL-cholesterol concentration was 5.2% (0.17 mmol/L) higher than after consumption of the TFA-M diet ($P = 0.005$) and 7.2% (0.23 mmol/L) higher than after consumption of the PUFA-M diet ($P < 0.001$). There was an average 1.8% (0.06 mmol/L) decrease in LDL cholesterol after consumption of the PUFA-M diet compared with the TFA-M diet ($P = 0.017$).

Total cholesterol followed a pattern similar to that of LDL cholesterol. After consumption of the butter diet, mean total cholesterol was 3.6% (0.18 mmol/L) higher than after consumption of TFA-M ($P = 0.009$) and 5.7% (0.28 mmol/L) higher than after consumption of PUFA-M ($P < 0.001$). Mean total cholesterol decreased 2.0% (0.10 mmol/L) after consumption of PUFA-M compared with TFA-M ($P = 0.010$).

There were no significant differences among the diets for HDL cholesterol, HDL$_2$, and HDL$_3$ cholesterol fractions, and triacylglycerols (Table 5). The ratio of total to HDL cholesterol decreased by an average of 3.9% ($P = 0.032$) after consumption of the PUFA-M diet compared with the butter diet (Table 5). There were no significant differences in this ratio between the butter diet and the TFA-M diet or between the PUFA-M and TFA-M diets. A similar significant decrease was observed in the ratio of LDL to HDL cholesterol after consumption of the PUFA-M diet compared with the TFA-M diet ($P = 0.017$).

There were no significant differences among the diets for apo A-II concentrations. Apo A-I was significantly lower after consumption of TFA-M compared with the butter diet ($6.1\%$, $P = 0.023$). There were no significant differences among the diets for apo A-II concentrations. Apo A-I was significantly lower after consumption of PUFA-M than after TFA-M (Table 5). There was no difference in apo B concentration after consumption of the butter diet compared with the TFA-M diet; however, apo B was lower after PUFA-M than after both TFA-M ($P = 0.002$) and the butter diet ($P < 0.001$).

Differences in LDL particle size after consumption of the spreads were <0.5%, and were considered to be biologically unimportant. The largest LDL particles were associated with the butter diet ($21.0 \pm 0.06$ nm). LDL particle size for both TFA-M and PUFA-M diets was $20.9 \pm 0.06$ nm.

**Lipoprotein(a)**

Eight of the subjects who completed the study had baseline Lp(a) concentrations $\leq 10$ mg/L. All of these showed no response to diet. Because these observations were at the lowest detectable level for the assay and thus deflate the estimated variance appropriately, we considered them missing for the purpose of statistical analysis. Data for all subjects having Lp(a) concentrations $> 10$ mg/L are included in Table 5. For the 38 subjects with baseline Lp(a) concentrations $> 10$ mg/L, there was a highly significant response to diet (Table 5). Compared with butter, consumption of TFA-M resulted in an average 8.6% increase in Lp(a) ($P < 0.001$) whereas consumption of PUFA-M resulted in an average 5.9% increase ($P = 0.008$). There was no significant difference in Lp(a) concentration between the margarine treatments ($P = 0.08$).

Lp(a) response to diet was examined statistically for subjects grouped by plasma concentrations at baseline in a way similar to the procedure reported from a previous study in our laboratory (27). Subjects with baseline concentrations $\leq 50$ mg/L ($n = 11$) showed no response to diet. Subjects with medium baseline concentrations, between 50 and 200 mg/L, and high baseline concentrations, $> 200$ mg/L, showed significant responses to diet. The response pattern was similar for the medium and high Lp(a) groups. Analysis of the data based on this grouping still showed no significant difference in response to the 2 margarine treatments.

**Tocopherols and lipid hydroperoxides**

Plasma $\alpha$-tocopherol concentration was higher after consumption of PUFA-M than after consumption of both the TFA-M and butter diets; however, there was no difference in $\alpha$-tocopherol concentration after consumption of TFA-M compared with the butter diet (Table 5). $\gamma$-Tocopherol concentration was higher after consumption of TFA-M than after consumption of the butter diet and higher after consumption of the butter diet than after PUFA-M. There was a carryover effect of diet for $\gamma$- but not for $\alpha$-tocopherol concentration. There were no significant differences in plasma lipid hydroperoxide concentrations among the diets with the spreads added.

**DISCUSSION**

This investigation was a large dietary trial comparing the effects of butter and margarines on plasma lipids, lipoproteins, and antioxidant status. Two margarines were used: a margarine high in PUFA made with liquid sunflower and completely hydrogenated soybean and canola oils, and a margarine made with liquid soybean and partially hydrogenated soybean oils and having TFAs approximating that in an average margarine on the US
market. The margarines were prepared so that they would have very similar physical and sensory characteristics and were constituted to compare favorably with commercial tub-type margarines available on the open market. Compliance with the diets was judged by direct observation of consumption of weekday meals, weight being maintained with only infrequent changes in the energy provided in the meals, and by evaluation of the daily questionnaires completed by the subjects. Dietary compliance was excellent for the subjects who consumed all 3 diets and whose data are included in the results.

Estimates for the daily consumption of margarine and butter are 11 and 6 g/person, respectively, in the United States (17), 22 and 3 g/person in Netherlands (28), and 12 and 7 g/person in Britain (29). Because per capita estimates include both those who consume and do not consume butter or margarine, as well as children, who generally consume less than adults, in the investigation reported here the table spreads were fed in amounts based on adult consumers of the products. The table spreads were fed in amounts comparable with the 90th percentile estimate for US intake of butter or margarine by male and female consumers of table spreads in the age range of participants in this study (16). The spreads were included in an experimental diet that approximated a typical US diet with respect to fat and fatty acid contents.

Protein, carbohydrate, and fat in our diets provided 15.5%, 50.0%, and 34.6% of energy, respectively, whereas intake estimates in the survey data for 1994 (26) were 15.5%, 51.4%, and 33.1% of energy, respectively. The energy from fat was, however, slightly lower than the targeted 37% that was calculated on the basis of US Department of Agriculture Handbook no. 8 data (15). Although no attempt to determine the reason for this difference has yet been made, it is not unlikely that fat values in the handbook are higher than they should be for some foods in view of recent trends toward less fat in many food products. This may be especially true for meats and meat products.

SFAs provided from 7.9% (TFA-M) to 11.2% (butter) of energy in our diets compared with 11.4% of energy in the survey (26). The diet with butter provided 7.2% of energy as SFAs and 13.5% of energy as MUFAs; these values were 6.5% and 12.6%, respectively, in the survey. By design, both margarines were higher in PUFAs and MUFAs than was butter.

There were substantial improvements in plasma LDL-cholesterol concentrations after consumption of both margarine diets compared with the butter diet (on average, concentrations were 4.9–6.7% lower). In addition, the LDL-cholesterol average was 1.8% lower after consumption of PUFA-M than after consumption of TFA-M. Apo B, the major apolipoprotein in LDL, was lower after consumption of both margarine diets than after the butter diet, but the difference was significant only after PUFA-M. Although a predominance of small, dense LDL particles was indicated to be an important determinant of cardiovascular disease risk (30–35), we observed no difference in particle size associated with the type of table spread consumed.

There were no differences in HDL cholesterol, HDL<sub>2</sub>- and HDL<sub>3</sub>-cholesterol fractions, or apo A-II resulting from the type of table spread consumed. Apo A-I concentration was significantly lower after consumption of PUFA-M than after TFA-M. High intakes of PUFAs have been shown to lower HDL-cholesterol concentrations, but such effects are usually observed when PUFAs exceed ~20% of energy in the diet (36). These effects would not be expected for PUFA intakes of 11% of energy, as in the PUFA-M diet fed here. Studies in our laboratory showed that SFAs raise HDL- as well as LDL-cholesterol concentrations (37). Because the diet with butter was considerably higher in SFAs, a similar effect on HDL cholesterol may have occurred in this study. However, this effect, if present, was not great enough to produce a significant elevation in HDL cholesterol with the butter diet as compared with the 2 margarine diets.

In the 1990 report of strategies for blood cholesterol reduction from the National Cholesterol Education Program (38), it was estimated that for every 1% reduction in cholesterol concentration, the risk of cardiovascular disease decreased by an average of 2%. Application of this prediction to results of the present study would indicate an average reduction in risk for cardiovascular disease of 7% for TFA-M compared with butter and 11% for PUFA-M compared with butter. Individuals consuming a smaller or larger amount of butter than in this study would expect smaller or larger changes in blood cholesterol and risk for cardiovascular disease when switching from butter to margarine. In addition, the prediction equation that relates reductions in blood cholesterol concentrations to reduction in risk for cardiovascular disease is not likely to be valid for every person; it will underestimate risk for some individuals while overestimating risk for others.

Lp(a), a particle similar to LDL in lipid composition, but with 2 major proteins, apo B-100 and apo(a) (21), may be a risk factor for the development of cardiovascular disease (21, 22). Although the role of Lp(a) in promoting heart disease has not been definitively elucidated, the particle was reported to be more atherogenic than LDL (39, 40). Although Lp(a) concentrations are thought to be largely under genetic control, there have been reports that dietary SFAs can raise (41, 42) and SFAs can lower (7, 27) Lp(a). In this investigation, Lp(a) concentrations were significantly lower after the butter diet than after both margarine diets. This is probably because of the higher concentration of SFAs in butter than in either margarine. Although SFAs have been reported to raise Lp(a) (41, 42), no apparent effect of dietary SFAs on Lp(a) was evident while comparing a margarine containing no SFAs and high amounts of PUFAs with a margarine made with partially hydrogenated vegetable oil. Our observation of lower Lp(a) concentration with butter as compared with TFA-M is consistent with that of Almendingen et al (7), who found that butter consumption resulted in lower concentrations of Lp(a) than did consumption of partially hydrogenated fish or vegetable oils. Although there may be some positive effect of dietary SFAs in lowering Lp(a) concentrations, there is much stronger evidence that saturates raise LDL cholesterol, the major risk factor for cardiovascular disease associated with diet in most people. Until stronger evidence for effects of diet on Lp(a) and the risk of heart disease is available, the effects of dietary fat on LDL cholesterol must remain the major concern.

The hardness of a spread depends directly on its content of SFAs, SFAs, or both. Margarines vary greatly in their hardness, with harder margarines containing more SFAs or SFAs. This study takes a practical approach to comparing butter and tub-type spreads on an isoenenergetic basis within the range of normal consumption in the United States. Because the spreads differed considerably in fatty acid profiles, this study does not directly compare intake of individual fatty acids on an isoenenergetic basis. Neither can these results be generalized for all commercial margarines because they vary widely in fatty acid composition. Compared with butter, both types of spreads used in this study
had lower amounts of SFAs + TFAs, and both produced improved profiles for the major lipoproteins. In the margarine designated PUFA-M, the concentration of SFAs was less than the concentration of SFAs + TFAs in TFA-M; PUFA-M produced the most desirable blood lipid profile.

Changes in the ratios of total or LDL cholesterol to HDL cholesterol may be better predictors of risk for cardiovascular disease than are changes in LDL cholesterol alone (43, 44). In this study, there was an improvement (lowering) in the ratio of total to HDL cholesterol after consumption of PUFA-M compared with after consumption of butter that was not observed after consumption of TFA-M. Thus, margarines with reasonably high concentrations of PUFAs may offer additional benefits for risk reduction. In contrast, lowering of HDL by TFAs was reported (5), but at TFA intakes greatly in excess of those that would be expected from tub-type soft margarines like those used in this study. Although we did not observe an effect of TFAs on HDL cholesterol or on the ratio of total or LDL cholesterol to HDL cholesterol in this study, such effects at higher TFA concentrations cannot be ruled out. High PUFAs concentrations may, however, be present in margarines containing liquid vegetable oils even when they are hardened with partially hydrogenated fats containing TFAs. There was no difference in the ratios after consumption of PUFA-M compared with after consumption of TFA-M. However, effects on the ratios resulting from consumption of margarines with high amounts of PUFAs with and without TFAs over the broad range of fatty acid composition in commercial margarines remain to be determined.

Hegsted et al (2) used data from published studies of the effects of dietary fatty acids and cholesterol on lipoprotein cholesterol to develop an equation for predicting changes in blood cholesterol concentrations. According to this prediction equation, decreases in dietary cholesterol and SFAs and increases in PUFAs of the magnitudes seen in the current investigation can be predicted to result in changes in plasma total and LDL cholesterol of a magnitude similar to that observed. Predicted and observed changes, respectively, in total and LDL cholesterol, based on changes in dietary fatty acid and cholesterol intakes for the diets fed in this study are, in mmol/L (mg/dL): total cholesterol for butter compared with PUFA-M, 0.31 and 0.28 (12 and 11), and LDL cholesterol for butter compared with PUFA-M, 0.23 and 0.23 (9 and 9); total cholesterol for butter compared with TFA-M, 0.26 and 0.18 (10 and 7), and LDL cholesterol for butter compared with TFA-M, 0.21 and 0.18 (8 and 7). Changes predicted because of changes in dietary fatty acid intake with PUFA-M compared with TFA-M when using the Hegsted equation (2) were not as close to observed changes as they were for the change from butter to margarine. Predicted compared with observed lipid concentrations for PUFA-M compared with TFA-M were 0.04 and 0.1 mmol/L (1.5 and 4 mg/dL) for total cholesterol, and 0.05 and 0.08 mmol/L (2 and 3 mg/dL) for LDL cholesterol. Dietary TFAs were shown to be hypercholesterolemic compared with oleic acid (5, 8). Therefore, when TFAs are considered to be hypercholesterolemic and are added to SFAs, as reported by Lichtenstein et al (45), the prediction is closer to the observed change for PUFA-M compared with TFA-M, i.e., 0.13 compared with 0.10 mmol/L (5 compared with 4 mg/dL) for total cholesterol, and 0.08 compared with 0.08 mmol/L (3 compared with 3 mg/dL) for LDL cholesterol.

Most margarines are rich sources of tocopherols, both naturally occurring tocopherols in vegetable oils and tocopherols added to margarines as antioxidants during production. In this study, plasma α-tocopherol concentration but not γ-tocopherol concentration appeared to reflect the content of the table spreads. PUFA-M contained the greatest amount of α-tocopherol and TFA-M contained the greatest amount of γ-tocopherol. Butter and TFA-M contained similar amounts of α-tocopherol and plasma concentrations were similar as well. PUFA-M contained more α-tocopherol than did either butter or TFA-M, and plasma α-tocopherol concentrations were highest after consumption of PUFA-M. For γ-tocopherol, however, plasma concentrations were highest after consumption of TFA-M, which contained more γ-tocopherol than did the other 2 spreads. Although PUFA-M contained ~3 times more γ-tocopherol than did butter, plasma γ-tocopherol concentration was higher after butter was consumed. There was a significant carryover effect for γ-tocopherol but not for α-tocopherol, suggesting that the length of the dietary periods for this study might be shorter than the plasma half-life of tocopherols.

There was no difference in the effect of the 3 spreads on plasma lipid hydroperoxide concentrations even though the difference in lipid hydroperoxide concentrations of the spreads themselves was about 10-fold. Thus, there appear to be sufficient circulating amounts of tocopherols from all spreads to prevent changes in plasma lipid hydroperoxide concentrations.

Several studies reported plasma lipid responses to butter, margarine, or vegetable oils added to controlled diets (7, 12, 23, 46–48). However, in most of these studies, the amounts of butter, margarine, or vegetable oil were unrealistically higher than what might be reasonably consumed as part of a self-selected diet, which makes it difficult to extrapolate the results to what might be expected from a typical diet. Although inferences may be drawn from such studies of expected effects of margarine, the amounts of oils and fatty acid profiles of the oils may not be attainable in actual margarine-type products having acceptable physical and sensory properties.

In conclusion, we showed that within the range of consumption of table spreads in a typical American diet, the fatty acid profile of a margarine can be controlled in a product with excellent physical and sensory properties. When such a margarine is consumed in place of butter, and presumably in place of other fats high in SFAs, appreciable improvement in blood lipid profiles of the major lipoproteins can be expected for most people. Because table spreads represent a major portion of the visible fat in the diets of many countries, including the United States, United Kingdom, and Netherlands, selection of products with desirable fatty acid profiles can be an easy means to begin consuming a more healthful diet and reducing the risk of cardiovascular disease.

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