Dietary *cis* and *trans* Monounsaturated and Saturated FA and Plasma Lipids and Lipoproteins in Men

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ABSTRACT: *Trans* monounsaturated fatty acids (TFA) are hypercholesterolemic compared to oleic acid to a degree approaching or equivalent to saturated FA. However, it is unknown to what extent these effects may be due to cholesterol lowering by oleic acid rather than elevation by saturated FA and TFA. In order to better understand the impact of replacing TFA in foods, it is first necessary to know the relative lipid-modifying effects of the major FA that change as TFA are lowered or removed. For 5 wk, 50 normocholesterolemic men were fed controlled diets providing approximately 15% of energy from protein, 39% from fat, and 46% from carbohydrate in a randomized, 6 × 6, crossover design. Eight percent of energy was replaced across diets with the following: carbohydrate (CHO) (1:1 simple to complex); oleic acid (OL); TFA; stearic acid (STE); TFA|STE (4% of energy from each); carbon 12:0–16:0 saturated FA (LMP). LDL cholesterol concentrations (mmol/L) were as follows (different superscripts indicate significance at $P \leq 0.01$): OL 2.95[a]; CHO 3.05[a,b]; STE 3.10[b,c]; LMP 3.21[c,d]; TFA + STE 3.32[d,e]; and TFA 3.36[e]. HDL cholesterol concentrations (mmol/L) were as allows: STE 1.16[a]; TFA 1.16[a,b]; TFA|STE 1.17[a,b]; CHO 1.19[b]; OL 1.24[c]; and LMP 1.30[d]. Triacylglycerides were highest after STE (1.13) and lowest after OL (0.88) ($P < 0.001$). Thus, compared to the carbohydrate control diet, TFA raised LDL cholesterol at least equivalent to LMP but had no effect on HDL cholesterol; STE had no effect on LDL cholesterol but lowered HDL cholesterol; LMP raised both LDL cholesterol and HDL cholesterol; and oleic acid raised HDL cholesterol but had no effect on LDL cholesterol.

Paper no. L8859 in *Lipids 37*, 123–131 (February 2002).

In a study performed in our laboratory, dietary *trans* monounsaturated FA (TFA) were found to be intermediate between *cis* monounsaturated FA and long-chain saturated FA in their hypercholesterolemic effect on plasma total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C) concentrations in adult male and female volunteers (1). Recommendations to lower or replace TFA in the diet have been the subject of considerable discussion in both lay and scientific forums (2–5). In general, reviewers of TFA and cardiovascular disease (CVD) risk have concluded that, whereas consumption of TFA may raise plasma cholesterol concentrations, their benefit lies in their ability to substitute for saturated FA whose intake is of greater concern.

To fully understand the impact of replacing TFA in foods, it is first necessary to know the lipid-modifying effects of the major FA that replace TFA. This is difficult to determine because there is no energy-yielding, "neutral" dietary control to which FA effects on various components of the plasma lipid profile can be compared. To compare effects of TFA on LDL-C and high density lipoprotein cholesterol (HDL-C), the relative plasma lipid and lipoprotein-modifying effects of replacing equicaloric amounts of several major energy-yielding nutrients were assessed. In the current investigation, effects of equicaloric replacement of carbohydrate by stearic acid (STE), TFA, oleic acid (OL), and C12:0– C16:0 saturated FA were determined in men fed diets with carefully controlled FA profiles.

METHODS AND MATERIALS

Study design. A controlled feeding trial was conducted at the Beltsville Human Nutrition Research Center (BHNRC) with 50 men. The feeding period was divided into two phases with an 8-wk break between phases. Two blood samples were collected during the 4-d period immediately preceding initiation of the controlled diet for both phases, and values for plasma lipids and lipoproteins were compared to determine if the subjects returned to initial baseline levels during the break (Table 1). Participants consumed all six diets for 5 wk/diet. Diet assignments were determined according to a 6 × 6 Latin square crossover 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 (6). The random sequence of diet assignments was

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Abbreviations: apo, plasma apolipoprotein; BHNRC, Beltsville Human Nutrition Research Center; BMI, body mass index; CHO, LMP, OL, STE, and TFA, basal diet enriched with carbohydrate, saturated FA (as the sum lauric + myristic + palmitic acids), oleic acid, stearic acid, or *trans* monunsaturated FA, respectively; CVD, cardiovascular disease; en%, percentage of dietary energy; HDL-C, plasma high density lipoprotein cholesterol; LDL-C, plasma low density lipoprotein cholesterol; LMP, sum of lauric (L), myristic (M), and palmitic (P) FA; OL, oleic acid; STE, stearic acid; TC, total plasma cholesterol; TFA, *trans* monounsaturated FA; TFA|STE, basal diet plus 4% of energy (4 en%) added as *trans* fatty acids and 4 en% as stearic acid; TG, triacylglycerides.

TABLE 1
Baseline Characteristics of Men Who Completed All Diets (n = 50)[a]

	Phase 1	Phase 2	Difference (Phase 1 – Phase 2)	
	Observed sample mean ± SEM		Mean difference ± SEM	P-value[b]
Body weight (kg)	83.1± 1.7	82.7± 1.7	0.46 ± 0.32	0.153
		(mmol/L)		
Triacylglycerides	1.149 ± 0.081	1.157 ± 0.081	–0.008 ± 0.056	0.884
Total cholesterol	4.768 ± 0.092	4.781 ± 0.083	–0.013 ± 0.069	0.850
LDL cholesterol	3.082 ± 0.072	3.037 ± 0.072	0.045 ± 0.056	0.428
HDL cholesterol	1.160 ± 0.038	1.124 ± 0.043	–0.054 ± 0.016	0.002

[a]Mean age was 42 yr and mean body mass index was 26.2 kg/m^2.
[b]Probability of significant difference between phases calculated from a paired t-test.

balanced with respect to body mass index (BMI) and baseline plasma LDL-C concentration. During the fifth week of each period, replicate blood samples were collected on two different days, from one-half of the subjects on Tuesday and Thursday, and from the other one-half on Wednesday and Friday. Subjects were switched from one diet to the next without a washout between periods except as described between phases 1 and 2.

Blinding of study results. Dietary treatments including menus and menu food items were color-coded during the study. Although study participants could recognize differences in appearance, taste, and other characteristics between the different fats and foods prepared with the fats, they were unaware of the overall nutrient and FA profiles of the diets. For determination of plasma lipids, all samples were coded to blind analysts to treatments. Analytical data, blinded to those who performed the controlled feeding and sample collection phases of the investigation, were sent directly from the analytical laboratory to the statistician. After all data for plasma lipids and lipoproteins were in place and the database was locked, a preliminary statistical analysis was performed with the treatments coded; the treatments were then decoded by the statistician (M. Iwane), and final analyses of the data were performed.

Subjects. Volunteers were recruited by advertisement in the area of the Beltsville Agricultural Research Center (Beltsville, MD). Men of all races between the ages of 25 and 60 yr were recruited regardless of smoking habits. From the 207 respondents, 58 met the eligibility criteria, described next, and were selected to enter the study. Four of those selected dropped out before initiation of the first feeding period. Of the 54 subjects who started the diets, 50 completed all six diets, and only data from these 50 participants are included in the present report (Table 1). Samples from dropouts were not analyzed. Three dropped prior to the end of the first feeding period, and one was dropped for noncompliance during the third feeding period.

Minimum eligibility criteria were based on general health, eating habits, age, BMI, and fasting plasma LDL-C, HDL-C, and triacylglyceride (TG) concentrations. Volunteers were required to be within 85–120% of gender-specific ideal BMI specified by life insurance reference tables (7). Volunteers who reported taking lipid-lowering drugs, blood pressure medications, or dietary supplements or who had eating habits inconsistent with the study protocol (e.g., those on 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, hyperlipidemia, diabetes, peripheral vascular disease, gout, liver or kidney disease, or endocrine disorders. Subjects selected for the study were required to have fasting plasma HDL-C concentrations greater than 0.65 mmol/L (25 mg/dL) and TG concentrations less than 3.39 mmol/L (300 mg/dL). From the volunteers who met all other selection criteria, those selected to participate had plasma LDL-C concentrations between the 25th and 75th percentiles of those screened.

Exercise was not a selection criterion nor was it controlled during the study, but volunteers who reported participation in major physical activities such as routine jogging for long distances, weight lifting, or other strenuous exercise programs were not selected for the study. Otherwise, subjects were encouraged to maintain their normal exercise patterns (type, duration, and frequency) throughout the study and were required to record major departures from their normal pattern of exercise on a daily questionnaire.

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. Participants received monetary compensation commensurate with the effort required of them by the study. All procedures and payments were approved by the Johns Hopkins University Committee on Human Research.

Controlled feeding procedures. On Monday through Friday subjects consumed breakfast and dinner at the BHNRC's Human Study Facility under the supervision of a dietitian. At breakfast, each subject was provided with a carry-out 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. Meals for the weekend were packaged for home consumption and provided to the subjects with written instructions after dinner on Friday. Coffee and tea were allowed in unlimited amounts,

but all additives (sugar and milk) were provided with the meals. Diet sodas were provided free-choice to be consumed as part of the study meals for consumption away from the facility. Only foods provided by the Human Study Facility were allowed to be consumed during the study. Consequently, consumption of alcoholic beverages was not allowed.

Each morning, Monday through Friday, subjects were weighed before breakfast when they arrived at the facility. Energy intake was adjusted in 1.67 MJ (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, across subjects, the relative amounts of all nutrients were constant and directly proportional to energy required to maintain weight. Each day, subjects completed a questionnaire detailing beverage intake, factors related to dietary compliance, exercise, medications, illnesses, and questions or problems with the diets. The questionnaires were routinely reviewed by a study investigator, and all problems identified were discussed with the subject during the next meal.

Diets. Data from the USDA handbook No. 8 series (revised series 1–21) (8) together with analyzed values for other foods were used in initial formulation of the diets (Table 2). Five diets were planned to have 38.9 en% (percent of energy) fat, 15 en% protein, and 46.1 en% digestible carbohydrates with a 1:1 ratio of simple to complex carbohydrates. A sixth diet was planned to have 30.4 en% fat and 54.6 en% carbohydrate. The range in fat concentration in the diets was selected to include the average reported fat intake, 34 en%, for men in the United States (9). Thus, with a reported intake of 34 en% from fat, the five diets varying in FA had 38.9 en% from fat, whereas the diet carbohydrate diet had 30.4 en% from fat. The six diets were planned to vary by 8 en% as follows: diet CHO, 8.5 en% reduction in fat (approximately equivalent to 8 en% reduction in FA) replaced by digestible carbohydrate; diet OL, 8 en% enrichment in oleic acid; diet LMP, 8 en% enrichment with saturated FA as the sum of lauric (L), myristic (M), and palmitic acids (P) (LMP) with the ratio of L/M/P of 0.3:1.4:8.3; diet STE, 8 en% enrichment with stearic acid; diet TFA: 8 en% enrichment in TFA with a spectrum of *trans* 18:1 positional isomers similar to that in the U.S. food supply; and diet TFA|STE, a combined enrichment with 4 en% TFA and 4 en% STE. Dietary fatty acid levels prior to enrichment were targeted at levels within the range of typical U.S. diets with the exception that saturated FA were to be about 10 en% with STE excluded. Otherwise, the diets before enrichment were targeted to have 2.5 en% STE, 4 en% linoleic acid, 10 en% OL, 10 en% LMP FA, and 2.5 en% other FA.

TABLE 2
Analyzed Composition (% of energy) of the Diets[a]

	CHO	OL	TFA	TFA|STE	STE	LMP
Protein	15.0	15.1	14.9	14.9	14.9	15.0
Digestible CHO	**54.5**	46.6	45.7	45.5	45.2	45.4
Fat	30.5	38.2	39.4	39.6	39.9	39.7
FA						
Lauric (L)	2.7	2.5	1.4	1.9	1.8	5.4
Myristic (M)	1.5	1.5	1.0	1.2	1.3	2.7
Palmitic (P)	5.7	5.7	7.6	6.9	6.9	10.0
Stearic (STE)	2.9	2.9	2.8	**6.9**	**10.9**	2.7
Sum of L + M + P	9.8	9.7	10.1	10.0	10.0	**18.0**
Oleic (*cis* 18:1; OL)	10.5	**17.6**	10.6	10.6	10.5	10.5
trans 18:1 monounsaturates	0.2	0.1	**8.3**	**4.2**	0.3	0.2
Linoleic	3.8	3.8	4.0	4.3	4.4	4.2
Other	1.8	2.2	1.8	1.8	1.9	2.0

[a]Nutrient targeted for enrichment within a diet is in bold print.
[b]Other FA were not controlled in planning the diets. TFA, *trans* monounsaturated FA; TFA|STE, basal diet plus 4 energy percent (en%) added as TFA and 4 en% as STE; CHO, LMP, OL, STE, and TFA, basal diet enriched with carbohydrate, saturated FA (as the sum of L + M + P), OL, STE, or TFA.

Analysis of diets. During the feeding study, diets were composited across the menu cycle and analyzed as follows. During the first period of feeding, two composites of each of the six diets were collected at two 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. Diets were analyzed for dry matter, crude protein, crude fat, total dietary fiber ash, and cholesterol (Covance, Inc., Madison, WI). FA composition of food composites (Table 2) was determined by GC separation of FAME.

Foods and analyzed test fats used in the diets. All diets were similar in types of food and nutrient levels to those in the range of typical U.S. diets but were not intended to approximate an average U.S. diet. Foods prepared using fats obtained and analyzed for the study were added to a base menu whose foods were essentially constant across diets. A variety of test fats, i.e., vegetable fats, commercial fat products such as margarines and salad dressings, and butter oil, were obtained in amounts sufficient for the study and analyzed for FA including TFA. These were incorporated into foods such as baked goods, specially prepared margarines, icings, and salad dressings and were added to the basic menu foods in amounts that controlled total fat and FA profiles at the desired levels. The types and amounts of fats added to the diets are presented in Table 3.

In order to achieve dietary STE levels while maintaining balance of other FA, it was necessary to prepare a high-stearate fat. This fat was prepared from a randomized mixture of ethyl myristate, completely hydrogenated soybean oil, coconut oil, and canola oil and had 43% STE as the major FA (Table 3).

The amount of the high-stearate fat fed was minimized by preferential use of the other fats shown in Table 3. However, in the high-stearate diet (described below), 43.9% of the fat and 69% of STE was from this source. For the moderate stearate diet, 23% of the fat and 57% of STE was from the stearate test fat. Small amounts were used in other diets to adjust stearate levels to those shown in Table 2. In the current study we did not attempt to determine effects on blood lipids

TABLE 3
Sources and Relative Amounts (% of total fat) of Dietary Fats in the Mixed Diets

	Diets					
Fat source	CHO	OL	TFA	TFA| STE	STE	LMP
Butter oil, decholesterolized	1.2	2.0	1.7	0	0	2.3
Canola oil[a]	16.4	10.8	1.0	0.1	0	6.6
Cocoa butter	9.0	4.1	0.8	3.1	11.8	7.5
Coconut oil	15.2	13.2	4.3	5.3	0	18.0
Corn oil	0.4	0.2	0.0	0.1	0	2.3
Margarine (commercial)	0	0	21.3	16.1	0	0.0
Mayonnaise (commercial *trans*-free)	3.5	0	0.7	0	0	0.8
Palm stearin	12.0	6.7	5.7	8.4	8.5	25.9
Shortening (commercial)	0	0	30.9	11.3	0	0
Stearate test fat[b]	2.0	0.8	0.4	23.0	43.9	0.5
High-oleic sunflower oil, 86% oleic	5.3	31.0	0	0	0	0
Food fat from dairy and beef sources	17.7	13.5	12.6	13.0	14.4	13.3
Other food fat (unidentified sources)	28.2	17.9	20.5	19.6	21.4	22.9

[a]A major source of canola oil was "Hidden Valley Original Ranch Dressing" (HV Food Products Co., Oakland, CA), which was purchased and analyzed for the study.
[b]The stearate test fat had 58.3% saturated, 27% monounsaturated (all detected was oleic acid, *cis* 18:1n-9), and 14.7% polyunsaturated FA (12.8% linoleic acid, *cis,cis* 18:2n-6) and 1.9% *cis* 18:3n-3 isomers. The saturated FA were primarily STE (43.1%) and palmitic acid (12.5%). The STE glyceride distribution was 9.8% tristearin, 34.4% distearins, and 40.1% monostearins. For abbreviations see Table 2.

due to differences in stereospecific isomers of TG. However, there is some limited evidence that randomized fats, such as the stearate test fat used in this study, which has higher levels of saturated FA in the *sn*-2 position, may be absorbed and metabolized differently from natural fats having more OL in the *sn*-2 position (10).

Blood sample collection and analysis. Procedures for blood sampling and processing were those described in the protocol for the Lipid Research Clinics Program (11). Blood samples were drawn after an overnight fast (minimum 12 h) immediately before breakfast. Samples of blood were collected by venipuncture using a 19-gauge butterfly needle into evacuated tubes containing disodium EDTA. After gentle mixing by inversion, samples were promptly placed on ice (0°C). Within 30 min of collection, plasma was separated by centrifugation at 1400 × *g* for 20 min at 4°C.

Plasma was harvested from the tubes, divided into aliquots, and stored in cryogenic vials at –80°C. Before storage, the sample for HDL-C and HDL_3 cholesterol determination was precipitated by the sequential precipitation procedure of Gidez *et al.* (12). Supernatants from the HDL-C precipitation were stored at –80°C for later analysis of cholesterol. Analyses for cholesterol, TG, apolipoproteins, and other blood components were performed after the final blood collection. All analyses for an analyte on samples from one subject were performed in the same analytical run.

Lipid analyses (TC, TG, and HDL-C) 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, U.S. Department of Health and Human Services. Plasma TC, HDL-C, HDL_3 cholesterol, and TG were determined enzymatically with commercial kits (Sigma Chemical Company, St. Louis, MO) on an Abbott VP analyzer (Abbott Instruments, Fullerton, CA). HDL_2 cholesterol was calculated as the difference between HDL-C and HDL_3 cholesterol. LDL-C was calculated by the Friedewald equation (13). Plasma apolipoproteins AI (apoAI) and B (apoB) concentrations were determined by rate nephelometry (Beckman ICS Immunochemical analyzer).

Statistical analysis. All analyses were performed using SAS (Cary, NC) for Windows (version 6.11) or S-Plus. The analytic plan was designed *a priori* and described a mixed-effects model for analysis of the data (14). For each variable the average of two sample measurements taken during week 5 of each feeding period was analyzed by using an analysis of variance model that included terms for diet, period, subject, 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. For multiple comparisons the least significant difference for the study was set at probability level of 0.01. Mean differences between pairs of diets were adjusted for carryover and period effects. Contrasts between pairs of diets were tested by a *F*-test for differences between groups.

RESULTS

Subjects' ages, BMI, and baseline plasma lipids. Baseline blood lipid and lipoprotein concentrations are presented in Table 1 for phases 1 and 2 of the study. The average age of the participants was 42 yr and average BMI was 26.2 kg/m^2. Baseline plasma cholesterol concentrations from phases 1 and

2 were similar except HDL-C was lower than prestudy by 0.054 mmol/L ($P = 0.002$) at the start of phase 2.

Subject compliance. Compliance in consumption of the controlled diets was judged to be excellent based on observed consumption of the meals in the dining room of the Human Study Facility, review of responses to the daily questionnaires, weight maintenance at stable energy intake, and frequent interviews with the subjects throughout the study.

No adverse effects due to consumption of the diets were observed. The stearate test fat was, as would be expected based on its composition, hard at room temperature but was readily consumed after warming. Most of the test fats were served in hot foods. However, for margarine prepared from stearate and other test fats for consumption as part of the meals, subjects had the option of moderately heating the food in a microwave oven or eating it at room temperature. Observation of meal consumption and discussions with the subjects disclosed no problem with any of the diets that would have the potential for major effects on compliance.

Composition of the diets. Composition of the experimental diets is shown in Table 2. The relative amounts of fats used in formulation of the diets is presented in Table 3. A basal diet with a 7-d menu cycle was designed so that energy from carbohydrate, protein, fat, and FA was controlled to the desired level in the 7-d average of the menus. Variation across days was kept to a minimum so that the ranges of protein, fat, and carbohydrate across diets were 1 en% of the targeted values. Variation in FA composition across menu days was similarly small. Dietary cholesterol was constant at 31.1 mg/MJ/d across diets. Dietary fiber was 28.7 g/d for the high CHO diet, and 27.0 g/d for the other diets.

FA composition of the diets was close to the targeted values except for lauric acid, approximately 5% higher than targeted, and palmitic acid, 5% lower than targeted. Although there may be differences in the effects of lauric, myristic, and palmitic acids on blood lipids (15), changes in the ratio of the magnitude in the current study should not appreciably affect the overall cholesterolemic effect of the diet. The sum of C_{12} to C_{16} saturates was within ± 0.3 en% of the targeted value for all diets. The maximal deviation in other FA was 0.4 en% lower in OL and 0.4 en% higher in STE and TFA|STE than calculated from the nutrient database and data from analysis of fats used in planning the diets.

Dietary intake. Average intake of energy and nutrients from each diet for the 50 subjects who completed the study is presented in Table 4. Average daily energy intake was determined for each subject on each diet. Average energy intake varied from 12.9 MJ/d (3088 kcal/d) on CHO to 13.1 MJ/d (3122 kcal/d) on LMP. The range in average energy intake across diets was small, 1.3% of the mean of all diets. No further statistical treatment of variation among the diets appeared necessary or useful because the subjects' energy intakes were very stable at weight maintenance across study periods and diets. This also reflects well on compliance because major deviations from the diet would have lead to weight changes, up or down, that would have required frequent changes in energy intake from the study diets in order to maintain body weight in the face of noncompliance.

Intake of nutrients was calculated from a subject's daily energy intake and the analyzed composition of the composited diets presented in Table 2. As a result of this estimation procedure, the CV for individual nutrients is the same, within rounding errors, as for energy intake. The CV for energy intake among the subjects was 1.8% on OL and 1.9% on each of the other diets. The CV for BMI, a major determinant of energy requirement, was 1.8. No attempt was made to quantify factors affecting energy metabolism other than dietary intake.

Digestible carbohydrate intake was 65 g/d higher on CHO than on the other diets (Table 4). Using average energy factors of 4, 9, and 4 kcal/g for protein, fat, and carbohydrate, respectively, and a factor of 95% for FA in TG and 65 g/d

TABLE 4
Average Daily Energy (MJ/d) and Nutrient (g/d) Intake of 50 Men Who Completed All Diets[a]

	Diets					
	CHO	OL	TFA	TFA|STE	STE	LMP
Nutrients	Observed sample means ± SEM					
Energy	12.92 ± 0.24	13.09 ± 0.23	13.05 ± 0.24	12.97 ± 0.24	13.07 ± 0.25	13.10 ± 0.25
Protein	116 ± 2.2	118 ± 2.1	116 ± 2.2	115 ± 2.2	116 ± 2.2	117 ± 2.2
Digestible carbohydrate	421 ± 7.9	364 ± 6.4	356 ± 6.7	353 ± 6.6	353 ± 6.7	355 ± 6.7
Fat	105 ± 2.0	133 ± 2.3	137 ± 2.6	136 ± 2.6	138 ± 2.6	138 ± 2.6
FA						
L + M + P	33.6 ± 0.64	33.7 ± 0.60	35.0 ± 0.65	34.4 ± 0.65	34.7 ± 0.66	62.6 ± 1.18
STE	10.0 ± 0.19	10.1 ± 0.18	9.7 ± 0.18	23.8 ± 0.45	37.8 ± 0.72	9.4 ± 0.18
cis 18:1 (OL)	36.0 ± 0.68[b]	61.2 ± 1.08	36.7 ± 0.69	36.5 ± 0.68	36.4 ± 0.69	36.5 ± 0.69
Trans 18:1	0.7 ± 0.01[c]	0.3 ± 0.01	28.8 ± 0.54[d]	14.5 ± 0.27	1.0 ± 0.02	0.7 ± 0.01
Linoleic acid	13.0 ± 0.25	13.2 ± 0.23	13.9 ± 0.26	14.8 ± 0.28	15.3 ± 0.29	14.6 ± 0.28

[a]See Table 2 for abbreviations. Data are presented as observed sample means ± SEM.
[b]*Cis* 18:1 positional isomers in CHO, OL, STE, and LMP were almost totally n-9. In TFA and TFA|STE, *cis* 18:1n-9 was approximately 86%.
[c]TFA from natural food sources, e.g., beef and dairy products. Average positional *trans* isomer distribution in CHO, OL, STE, and LMP was: n-8, 14.5%; n-9, 17.6%; n-10, 33.0%; n-11, 24.7%; n-12, 10.3%.
[d]*Trans* isomers in TFA and TFA|STE were 97.6 and 95.3%, respectively. Average *trans* isomer distribution was: n-8, 18.3%; n-9, 18.9%; n-10, 25.0%; n-11, 21.2%; n-12, 16.8%. Note: *trans* n-12 was not completely separated from *cis* n-8.

digestible carbohydrate equates to about 8.4 en% fat or 8.0 en% FA. This was the targeted difference in carbohydrate compared to FA enrichment between CHO and the other diets. Fat intake was about 4 g lower than planned in OL. This fat was replaced by a 9 to 10 g increase in digestible carbohydrate. These differences are well within what would be expected when menus are formulated using tabulated nutrient data for foods. The lower fat level in the basal diet enriched in OL appeared to be mainly in OL, which was 0.4 en% less than the targeted value. These minor differences should be of little or no consequence in interpreting biological effects of the diets.

Plasma lipids and lipoproteins. Least square means ± standard error of the estimate for plasma lipids, lipoprotein cholesterol, and apolipoprotein concentrations and ratios of TC/HDL-C and LDL-C/HDL-C for the 50 men who completed all six diets are presented in Table 5. There were no significant interactions of diet and period (lowest *P*-value was 0.25), indicating that the responses to diet were the same in all periods. This term was dropped from the final statistical model. Likewise, there were no significant carryover effects for any of the variables (smallest *P*-value 0.12). However, because carryover was a fundamental component of the study randomization structure, this term was retained in the final model.

Three comparisons of responses (percent change) among the diets are shown in Table 6: (i) comparison of changes in plasma lipid and lipoprotein concentrations as 8 en% LMP is replaced by carbohydrate or different classes of FA, (ii) comparison of changes as CHO is replaced by FA other than LMP (compared above), and (iii) comparison of changes as STE is replaced by the remaining diets, OL, TFA, and TFA|STE. Only those values significant at the 0.01 probability level are shown in Table 6. Other comparisons that may be of interest can be derived from Table 5.

TC. Compared to LMP, TC was not significantly different after TFA or TFA|STE, but was significantly lower after CHO, OL, and STE. Compared to CHO, TC increased after TFA, TFA|STE, and LMP. There were no significant differences in TC when CHO was replaced with OL or STE. TC increased when STE was replaced by TFA, TFA|STE, and LMP. In contrast, TC decreased when 8 en% STE was replaced by OL.

LDL-C. LDL-C was 4.8% higher after TFA than after LMP (Table 6). LDL-C after TFA|STE was not statistically different than after LMP. When LMP was replaced by CHO and OL, there were statistically significant decreases in LDL-C of 4.8% for CHO and 8.1% for OL. LDL-C increased when TFA, TFA|STE, and LMP replaced CHO. There were no significant differences when CHO was replaced by OL or STE. As did TC, LDL-C decreased when STE was replaced by OL and increased when STE was replaced by TFA and TFA|STE. There was no significant difference in LDL-C when STE was replaced by CHO and LMP.

HDL-C. When LMP was replaced by CHO or other classes of FA, HDL-C was significantly higher for all diets (Table 5). HDL-C was significantly higher after OL than after all other diets except LMP. Compared to CHO, HDL-C increased after OL and decreased after STE. There were no significant differences in HDL-C when CHO was replaced by TFA, or TFA|STE. There were no significant differences in HDL-C concentrations between STE, TFA and TFA|STE. HDL-C increased when STE was replaced by OL. HDL-C increased when CHO replaced STE. Differences in both HDL_2 and HDL_3 cholesterol fractions mimicked those for HDL-C when LMP was replaced by other diets (Table 5).

Lipoprotein cholesterol ratio. Although LMP raised TC and LDL-C compared to CHO and OL, HDL-C also increased after LMP so that there was no difference in either the TC/HDL-C (Table 5) or LDL-C/HDL-C ratio (data not shown) compared to CHO or OL. The ratio of TC/HDL-C was higher after TFA than all other diets except TFA|STE. The higher

TABLE 5
Plasma Lipid, Lipoprotein Cholesterol, and Apolipoprotein Concentrations and Ratios of Total and LDL Cholesterol to HDL Cholesterol of 50 Adult Men After 5 wk Consumption of Each Diet[a]

	Diets					
	CHO	OL	TFA	TFA|STE	STE	LMP
	(mmol/L)					
TC	4.719 ± 0.088^{a,b}	4.593 ± 0.088^{a}	4.991 ± 0.088^{d}	4.981 ± 0.088^{d}	4.776 ± 0.088^{b}	4.957 ± 0.088^{c,d}
LDL	3.054 ± 0.083^{a,b}	2.948 ± 0.083^{a}	3.36 ± 0.08^{e}	3.320 ± 0.083^{d,e}	3.101 ± 0.083^{b,c}	3.209 ± 0.083^{c,d}
HDL	1.195 ± 0.041^{b}	1.241 ± 0.041^{c}	1.159 ± 0.041^{a,b}	1.174 ± 0.041^{a,b}	1.156 ± 0.041^{a}	1.303 ± 0.041^{d}
HDL_2	0.424 ± 0.031^{a,b}	0.437 ± 0.031^{b}	0.396 ± 0.031^{a}	0.403 ± 0.031^{a,b}	0.416 ± 0.031^{a,b}	0.481 ± 0.031^{c}
HDL_3	0.771 ± 0.023^{b,c}	0.807 ± 0.023^{c,d}	0.763 ± 0.023^{a,b}	0.771 ± 0.023^{a,b}	0.740 ± 0.023^{a}	0.822 ± 0.023^{d}
TG	1.025 ± 0.064^{b,c}	0.878 ± 0.064^{a}	1.024 ± 0.064^{b,c}	1.057 ± 0.065^{b,c}	1.134 ± 0.064^{c}	0.972 ± 0.064^{a,b}
	(g/L)					
ApoAI	1.23 ± 0.28^{a,b,c}	1.27 ± 0.028^{c,d}	1.21 ± 0.028^{a,b}	1.20 ± 0.028^{a}	1.21 ± 0.028^{a,b}	1.30 ± 0.028^{d}
ApoB	0.71 ± 0.019^{a}	0.68 ± 0.019^{a}	0.75 ± 0.019^{b}	0.75 ± 0.019^{b}	0.72 ± 0.019^{a}	0.71 ± 0.019^{a}
	(ratio)					
TC/HDL	4.1 ± 0.1^{b}	3.9 ± 0.1^{a}	4.5 ± 0.1^{d}	4.4 ± 0.1^{c,d}	4.3 ± 0.1^{c}	4.0 ± 0.1^{a,b}

[a]Least square means are adjusted for period and diet by period interaction but not for crossover. Data are presented as least square means ± standard error of the estimate. Diets labeled with different roman superscripts are significantly different at $P \leq 0.01$. *P*-values were obtained from tests of mean differences between pairs of diets adjusted for carryover and period effects. TC, total cholesterol; TG, triacylglycerides; ApoAI, apolipoprotein AI; apoB, apolipoprotein B. See Table 2 for other abbreviations.

TABLE 6
Changes (expressed as percent change) in Blood Lipids and Lipoproteins When 8 Percent of Energy from Hypercholesterolemic Saturated FA (LMP), Carbohydrate (CHO), or Stearic Acid (STE) Replace Each Other, Oleic Acid (OL), or *trans* Monounsaturated FA (TFA)[a]

Replacement of lauric + myristic + palmitic acids (LMP) with:					
	CHO	OL	TFA	TFA|STE	STE
Triacylglycerides	NSD	NSD	NSD	NSD	16.7
Total cholesterol	–4.8	–7.3	NSD	NSD	–3.7
LDL cholesterol	–4.8	–8.1	+4.8	NSD	NSD
HDL cholesterol	–8.3	–4.8	–11.1	–9.9	–11.3
Replacement of carbohydrate (CHO) with:					
		OL	TFA	TFA|STE	STE
Triacylglycerides		–14.3	NSD	NSD	+10.6
Total cholesterol		NSD	+5.8	+5.6	NSD
LDL cholesterol		NSD	+10.1	+8.7	NSD
HDL cholesterol		+3.8	NSD	NSD	–3.3
Replacement of stearic acid (STE) with:					
		OL	TFA	TFA|STE	
Triacylglycerides		–22.6	NSD	NSD	
Total cholesterol		–3.8	+4.5	+4.3	
LDL cholesterol		–4.9	+8.4	+7.1	
HDL cholesterol		+7.4	NSD	NSD	

[a]Percentage change = concentration of parameter for: [replacement diet – diet to which compared)/diet to which compared] × 100. Values shown are those where probability of a significant difference between diets was 0.01 or lower. NSD = no significant difference between diets at probability level of 0.01.

ratio when 8 en% TFA replaced LMP was due to an increase in LDL-C with no corresponding change in HDL-C. Conversely, when LMP was replaced by STE, the change in the ratio was due to a decrease in HDL-C with no significant change in LDL-C. HDL-C increased when OL was replaced by CHO; thus, the ratio of TC/HDL-C was significantly lower after OL than after all other diets except LMP (as noted above).

TG. There were no significant differences in TG between LMP and CHO, OL, TFA, and TFA|STE (Tables 5 and 6). Compared to LMP, TG increased significantly only after STE. When CHO was replaced by FA, there were no differences in TG for TFA, TFA|STE, or LMP. TG decreased after replacement of CHO with OL and increased after replacement with STE. There were no significant differences in TG when 8 en% STE was replaced by TFA or TFA|STE. When STE was replaced by OL, TG decreased significantly.

Apolipoproteins. There were no significant differences in apoB when CHO, OL, LMP, and STE replaced each other in the diets (Table 5). ApoB was highest when TFA or TFA|STE replaced CHO or other FA. ApoAI was significantly higher after LMP than after all other diets except OL. ApoAI after OL was higher than after TFA, TFA|STE, and STE but was not different from concentrations after CHO.

DISCUSSION

Several well-designed studies have compared plasma lipid and lipoprotein responses to changes in dietary *trans* and *cis* FA and have reached similar conclusions: TFA are hypercholesterolemic compared to *cis* FA (OL). However, the implications of such dietary comparisons are unclear because OL may have lowered LDL-C concentrations more than would be expected simply due to replacement of either saturated FA or TFA (1,16). Furthermore, high levels of dietary TFA have been reported to lower HDL-C (16). While equicaloric replacement of saturated FA with TFA results in lower HDL-C, it is not clear whether TFA actually lower HDL-C or whether the difference is due entirely to the HDL-C raising-effects of saturated FA.

LDL-C. Both *trans* FA and saturated FA raised LDL-C when they replaced either 8 en% carbohydrate or OL in the diets fed in the current study. The elevation in LDL-C was greater with TFA than with LMP. When 8 en% carbohydrate was replaced by LMP, LDL-C increased by 0.15 mmol/L or about 5%. When 8 en% carbohydrate was replaced by 8 en% TFA, LDL-C increased by 0.31 mmol/L or about 10%. In the lower *trans* FA diet where 4 en% TFA plus 4 en% STE replaced carbohydrate, LDL-C increased by 0.27 mmol/L, or about 9%. Because STE did not raise LDL-C when it replaced 8 en% carbohydrate, we can speculate that the increase in LDL-C after the TFA|STE diet was mostly due to TFA and not to stearate. On the basis of effects on LDL-C of different levels of TFA fed in several studies, other investigators (17) have postulated that the effects of TFA on LDL-C are linear. Since 4 and 8 en% TFA enrichment of the diets produced about the same effect on LDL-C, this is not supported in the present study.

The finding of higher LDL-C with TFA than with LMP FA in the current study suggests that TFA are actually the more hypercholesterolemic FA. This observation contrasts not only with our prior study (1) but also with that of Mensink and Katan (16), where LDL-C after the *trans*-FA-containing diets was intermediate to the OL and saturated FA diets. Thus, based on the current study as well as our previous study (1), we conclude that the LDL-C-raising effects of TFA are at least equal to, and perhaps greater than, those of the most hypercholesterolemic saturated FA, lauric, myristic, and palmitic acids. The diversity of responses of LDL-C to TFA and LMP FA cannot be readily explained by any of the current studies. This does not, however, affect the major conclusion that TFA, like saturates, raise LDL-C. Care should be taken in extrapolating this conclusion to all levels of dietary TFA intake, and especially to TFA at typical American intake levels of 2 to 3 en% (18), where few well-controlled studies have been done.

Mensink and Katan (16) reported that, compared to OL, apoB secretion increased after consumption of saturated FA and there was a further significant increase after TFA. In the current study, the secretion of apoB after LMP was not significantly different from OL. TFA, however, increased apoB secretion compared to OL, and both of the TFA containing diets (4 and 8 en%) increased apoB concentrations by the same amount. These differences may indicate that the plasma LDL-C-raising effects of TFA and saturates are induced, at least in part, by different mechanisms, such as differing

levels of LDL receptor activity with TFA as compared to saturates.

HDL-C. In the study by Mensink and Katan (16), replacement of 10 en% OL with TFA or with saturated FA resulted in lower HDL-C after the TFA diet than after both the OL and saturated FA diets. This led these investigators to conclude that TFA were at least as unfavorable as the cholesterol-raising saturated FA because they not only raised LDL-C levels but also lowered HDL-C levels. A similar pattern of HDL-C response to dietary OL, TFA, and saturates was observed in our prior study at 6 en% replacement of these FA across diets (1). When we compare the responses of the FA diets to that of the carbohydrate diet, replacement of 8 en% with OL or with 8 en% LMP FA raised HDL-C with the greatest increase after LMP. However, there was no difference in HDL-C when TFA replaced carbohydrate or STE in the diet. Based on the current study, we now conclude that TFA do not lower HDL-C, and the differences in HDL-C reported previously were due to HDL-C increases by both OL and LMP FA. However, since saturates raise HDL-C, their replacement in the diet with TFA will likely lead to lower plasma HDL-C.

TG. In general, plasma TG increase as dietary digestible carbohydrate increases and dietary fat decreases (19,20). The magnitude of such effects at the fat levels fed in this study, 30.5 and 38.9 en%, would not be expected to be large. Grundy (19) points out that effects of low-fat, high-carbohydrate diets on plasma lipids are more striking when total fat intake falls below 30% of total energy. In our study, we saw no major effect on TG that could be attributed to higher carbohydrate (lowered fat) in CHO compared with any of the other diets.

TG concentrations in our prior study (1) were higher after both levels of TFA than after the OL and saturated FA diets. In the current study, a similar pattern was observed in that after TFA and TFA|STE, TG concentrations were significantly elevated when compared to OL. However, compared to CHO, TG were not different after either of the TFA-containing diets in the current study. Similarly, TG after STE were higher than after OL or LMP. Why TFA and STE seem to follow carbohydrate rather than saturates in their effects on TG is not known.

In the present study, we have shown that TFA have plasma LDL-C-raising effects that are at least comparable to saturated FA. However, it is important to note that fats that are sources of TFA are also carriers of other dietary unsaturated FA, such as OL, linoleic acid, and linolenic acid, that beneficially affect risk of CVD. Some nutritionists are now recommending that food sources of TFA, such as partially hydrogenated vegetable oils, be limited or eliminated from the diet to achieve a reduction in blood cholesterol. For some popular foods, it is not feasible to eliminate fats containing TFA at the present time without substituting a fat high in saturated FA. Partially hydrogenated fats give many foods important functional and physical characteristics that affect texture and sensory properties as well as stability against rancidity. These properties are not mimicked by liquid vegetable oils. Inclusion of these foods in the diet admittedly will provide some dietary TFA, but food products containing partially hydrogenated fats can also provide beneficial *cis*-unsaturated FA. The favorable effects of consuming typical food products containing increasing ratios of linoleic/TFA on plasma LDL-C in a step I diet were amply demonstrated by Lichtenstein *et al.* (21). Furthermore, a vegetable fat that is solid at room temperature and has a relatively small amount of TFA (i.e., soft margarines that contain 0 to 5% TFA) is preferable to a solid fat high in saturated FA (i.e., butter, 60 to 65% saturates) because it is a carrier of *cis*-unsaturated FA and also is low in saturated FA (22).

The task force on TFA of the British Nutrition Foundation (2) concluded that there was no unequivocal evidence that TFA of the type and amount consumed in the United Kingdom posed a significant risk to human health because the average intake of saturated FA was 16% of dietary energy, whereas that from TFA was only 2%. Thus, the priority was to reduce intake of saturated FA even though there was convincing evidence that TFA had adverse effects on plasma LDL-C and HDL-C concentrations. The American Society of Clinical Nutrition/American Institute of Nutrition task force on TFA (3) concluded that, compared with saturated FA, the issue of TFA was less significant because the U.S. diet provides a smaller proportion of TFA, and current data on their biological effects are limited. However, they did recommend that the intake of major sources of TFA, such as hard margarine and shortening, be limited and that food manufactures make the effort to decrease TFA content of these products.

Decreasing the intake of TFA at the expense of increasing saturated fat intake cannot be justified based on current evidence even though there may be some advantage in increased HDL-C and a lower ratio of TC and LDL-C to HDL-C. Elevated LDL-C remains the major dietary risk factor for CVD. Although TFA are hypercholesterolemic and raise LDL-C as do saturated FA, the public health questions raised by this finding are complex. First, whereas TFA contribute, on the average, 2 to 3 en% in the U.S. diet (20), the intake of the hypercholesterolemic saturated FA, lauric, myristic and palmitic acids (23), contributes three to four times more toward the cholesterol-raising FA in the diet. Understanding the relative importance of dietary saturated FA and TFA may change as additional data on other risk factors for CVD, such as effects on inflammation and blood clotting, become available.

ACKNOWLEDGMENTS

We thank Evelyn Lashley and the staff of the BHNRC for preparing and feeding the controlled diets; the staff of the Lipid Research Clinic, The George Washington University, for lipid and lipoprotein analyses; Joseph Sampugna, University of Maryland, for FA analyses; Edward Emken, Midwest Research Consultants, for analysis of *cis* and *trans* FA positional isomers; the staff of the Diet and Human Performance Laboratory, BHNRC, for assistance in performance of the study; and Benjamin Caballero, Johns Hopkins School of Public Health for medical supervision of the study. We thank the following for donation of fats and oils and food products for the study; Kraft Foods, Inc.; Lipton; Nabisco Foods, Inc.; Hershey Foods Corp.; Procter & Gamble; Best Foods Division, CPC International, Inc.;

and Aarhus Oils, Inc. We also thank Peter Huth, Kraft Foods, Inc., for formulating and arranging for the preparation of the randomized stearate test fat and Shirley Chen, Lipton, for preparing specially manufactured margarines for the study. The assistance of the members of the Fatty Acid Technical Committee, ILSI, NA, in planning and obtaining funding for the study is appreciated. This study was supported in part by a research agreement with the Technical Committee on Dietary Lipids, International Life Sciences Institute, NA, Washington, DC.

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[Received June 13, 2001, and in revised form October 17, 2001; revision accepted November 28, 2001]