Effect of butter, mono- and polyunsaturated fatty acid-enriched butter, *trans* fatty acid margarine, and zero *trans* fatty acid margarine on serum lipids and lipoproteins in healthy men

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Abstract The effect of diets containing 50% of fat calories from butter, butter enriched with mono- and polyunsaturated fatty acids, and margarines with and without *trans* fatty acids on the serum lipids of 38 healthy men in a free-living condition have been determined. Serum lipid responses to the high level of individual dietary fats were unexpectedly small. The butter diet produced a small, but significant rise (5%) in the total serum cholesterol and low density lipoprotein (LDL)-cholesterol, relative to all other diets. Enrichment of butter with either olive oil (50/50) or sunflower oil (50/50) failed to reduce serum lipid levels below habitual diet values. Hard margarine, containing 29% *trans* fatty acids, caused a decrease in apolipoprotein A-I and B levels, but did not change total serum cholesterol or LDL-cholesterol levels, relative to habitual diet values. A soft margarine, high in linoleate, with no *trans* fatty acids reduced total cholesterol, LDL-cholesterol, and apolipoprotein B significantly, relative to all diets. Soft margarine high density lipoprotein (HDL)-cholesterol levels remained unchanged, but apolipoprotein A-I values were decreased relative to habitual and butter diets. The quantities of saturated fatty acids and the sum of monounsaturated and polyunsaturated fatty acids consumed on the hard and soft margarines were equal; therefore, the different response of serum cholesterol and LDL-cholesterol between these two diets is attributable to the *trans* fatty acids in the hard margarine. The data indicate that *trans* fatty acids are not metabolically equivalent to the natural *cis* isomers and that they affect the serum lipid profile adversely.—**Wood, R., K. Kubena, B. O'Brien, S. Tseng, and G. Martin.** Effect of butter, mono- and polyunsaturated fatty acid-enriched butter, *trans* fatty acid margarine, and zero *trans* fatty acid margarine on serum lipids and lipoproteins of healthy men. *J. Lipid Res.* 1993. **34:** 1–11.

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The hypothesis that dietary fat can predictably alter serum lipid levels significantly continues to be an issue of considerable interest in the scientific literature and the popular press. The "lipid hypothesis" states that dietary fat intake can alter blood lipid levels which may initiate or exacerbate atherogenesis (1). Classical studies by Ahrens et al. (2), Keys, Anderson, and Grande (3), and Hegsted et al. (4), using hospitalized patients on liquid formula diets or institutionalized schizophrenic patients on solid foods, have provided the basis for the hypothesis. Over the years there have been, and continue to be, studies to examine the various components of "dietary fat" for their effect on serum lipids. Dietary cholesterol, first reported to have no effect on serum cholesterol (5), is now generally accepted to elevate serum cholesterol 4–5 mg/dl with each 100 mg increase in dietary cholesterol (6–8). Monounsaturated fatty acid effects were first considered as being neutral with regard to serum cholesterol (3), however the work of Grundy and colleagues (9–11) has shown that monounsaturated fatty acids are as effective as polyunsaturated fatty acids in lowering serum cholesterol. Initially, stearic acid, a saturated fatty acid, was considered to have no effect on serum cholesterol (12, 13), but it now has been reported to lower serum cholesterol (14). Myristic acid has been reported to be the strongest serum cholesterol-elevating fatty acid (4) while others contend that palmitic is the culprit (15). The early classical studies (2–4) indicated diets containing high levels of polyunsaturated fatty acids were effective in lowering serum cholesterol levels, however studies in recent years have demonstrated that dietary polyunsaturated fatty acids also lower high density lipoprotein (HDL) cholesterol (9, 16–20). More recently, the *trans* fatty acids resulting from the partial hydrogenation of vegetable oils, and their replacement of the saturated fatty acids in many processed foods, have been reported to produce undesirable serum lipoprotein

Abbreviations: LDL, low density lipoprotein; HDL, high density lipoprotein; TLC, thin-layer chromatography; GLC, gas-liquid chromatography.

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profiles: low density lipoprotein (LDL) cholesterol was raised and HDL cholesterol was lowered (21).

These examples of our changing views about the effects of dietary fat on serum lipids prompted the present study. The objectives of this study were: *1)* to re-examine the effect of butterfat on serum lipids, lipoproteins, and apolipoproteins of healthy men; *2)* to determine what effect the enrichment of butterfat with glycerides high in mono- and polyunsaturated fatty acids would have upon serum lipid, lipoprotein, and apolipoprotein levels; *3)* to determine the effects of a hard margarine containing *trans* fatty acids and a soft margarine with no *trans* fatty acids on the serum lipids, lipoproteins, and apolipoproteins of healthy men; and finally *4)* to compare the serum lipid, lipoprotein, and apolipoprotein data from the various diets and habitual diets.

Preliminary data from this study have been reported (22).

METHODS

Subjects

Forty healthy male Texas A&M University faculty and staff (no students) between the ages of 30 and 60 years were selected from 79 volunteers for the study. None of the participants had a history of coronary vascular disease or hypercholesterolemia or diabetes. Body weights were 80–120% of ideal body weight and all participants were normotensives. All participants' clinical chemistry tests and thyroid function values were within the normal range. The screening criteria also included a physical examination including electrocardiogram, family history, and personal conflicts. Although we did not screen against smokers, all participants were non-smokers except one who smoked an occasional cigar. Individuals requiring prescribed medication were excluded. Average body weight was 181 ± 21 pounds and the average age was 42 ± 8 years. Participants signed an informed consent form, were free-living, and reported any illnesses, abnormal activity, and extended travel for each diet period. The study was approved by the Texas A&M University Institutional Review Board for the use of human subjects in research. Subjects were requested not to eat organ meats and shell fish and to restrict their consumption of visible eggs to one every other day. Exercise and physical activities were not restricted, but participants were encouraged to maintain their normal lifestyle preferences. It was recommended that body weight be maintained within 5 pounds of study entry weight. This was monitored by weekly weight measurements at the time of blood collection and adjustments were made in the meal plan when necessary.

Experimental design

Five groups of eight participants each rotated through five diet periods of 6 weeks duration in a Latin square design. Each diet period was followed by a six-week habitual diet "wash out" period. A total of 12 weeks was required for each diet period cycle. No test diets were given between the Thanksgiving holidays and January 8th. Diet periods started 1/11, 4/4, 6/27, 9/19, and 1/9 the following year.

Test fats

The five test fats used are listed. A single lot of each test fat was prepared or purchased to cover the needs of the entire study.

1. Butter, 1.8% salt.
2. Butter-olive oil blend (50/50), 1.8% salt (Butter-olive).
3. Butter-sunflower oil blend (50/50), 1.8% salt (Butter-Sun.).
4. Hard margarine, Monarch Fine Foods, 3.0% salt (Hard Marg.).
5. Soft margarine, Monarch Fine Foods, 1.8% salt (Soft Marg.).

TABLE 1. Fatty acid composition of dietary test fats

	Percentages[a]							
Dietary Test Fats	10:0	12:0	14:0	16:0	18:0	*cis*[b] Mono.	*trans*[b] Mono.	18:2
Butter[c]	2.9	3.9	11.9	31.4	14.6	20.6	5.3	3.6
Butter-sunflower	0.9	1.5	5.5	18.6	10.9	18.5	2.6	36.5
Butter-olive	1.4	1.7	6.2	24.7	10.1	41.9	2.6	10.5
Hard margarine		0.5	0.7	13.9	6.6	43.4	29.0	3.5
Soft margarine	0.2	3.6	1.6	12.4	4.5	13.8	0	61.3

[a]Difference between the sum of any row and 100% represents the sum of minor amounts of other fatty acids not given in the table.

[b]*cis* and *trans* unsaturated fatty acid columns consisted predominately of the 18:1 chain length. The *cis* mono category usually contained measurable quantities of 16:1 of less than 1.5% whereas the *trans* mono fraction contained only traces of 16:1.

[c]C-4, C-6, and C-8 fatty acids that usually account for 5–8% of the total fatty acids in butter were not quantified and taken into account, therefore test fats containing butter have slightly elevated percentages of the fatty acids given in the table.

The fatty acid compositions of the dietary test fats are given in **Table 1.** Analyses were made on a 50 M × 0.25 mm fused silica capillary column containing a bonded 0.25 μ film of 007 CPS liquid phase. Temperature was programmed from 140–230°C at 2°C/min. This column resolves positional isomers with the same configuration (i.e., 18:1Δ9c and 18:1Δ11c) and *cis* and *trans* isomers with the double bond at the same position (i.e., 18:1Δ9c and 18:1Δ9t). Mixtures of *cis* and *trans* isomers consisting of numerous positional isomers, found in most monoene fractions resulting from the partial hydrogenation of fats and oils, are not resolved sufficiently for quantitation. This requires separation of the *cis* and *trans* monoene fractions by argentation thin-layer chromatography (TLC) before analysis by gas–liquid chromatography (GLC). Details of these techniques and procedures have been described (23). Only data on the geometrical isomers, predominately the octadecenoates, are given in Table 1. We did not attempt to quantify the C-4, C-6, or C-8 fatty acids that usually account for 5–8% of the total fatty acids in butter; therefore, the fatty acid percentages given for butter, butter-olive, and butter-sun test fats are slightly elevated. Cold pressed, unrefined sunflower oil (Arrowhead Mills, Hereford, Texas) contained 70% linoleic (18:2), 16% oleic (18:1), 5% stearic (18:0), and 6% palmitic (16:0) acids. The olive oil (Pompeian Inc., Baltimore, MD) contained 58% 18:1, 19% 18:2, 3% 18:0, and 18% 16:0 acids. Butter-olive and butter-sun test fats were prepared by blending equal parts of butter with olive oil and sunflower oil, respectively. The fatty acid composition of these test fat mixtures given in Table 1 agrees with expected values. The hard margarine consisted of partially hydrogenated vegetable oil. It contained 29% *trans* monoenes and a low level of 18:2 similar to butter. Butter contained approximately 5% *trans* fatty acids. The soft margarine contained no *trans* fatty acids and less than half the level of *cis* 18:1 monoenes found in the hard margarine and butter-olive test fats. Manufacturer's information on the soft margarine indicated it was composed of 87% sunflower oil and 13% of modified palm and palm kernel oils. The hard and soft margarines contained one-half and one-third as much saturated fatty acids as butter-olive and butter-sun and butter, respectively.

Food and diets

The test fats were supplied to the participants as spreads (one pound containers), cookies, ice cream, and milk where the test fats replaced the fats normally present. Cookies were made at the University Bakery. Butter, butter-olive, butter-sun, the modified fat ice cream, and milk were prepared at the University Creamery.

Subjects were supplied with food scales and instructions from a registered dietician on how to complete a 7-day dietary record and food frequency questionnaire to ascertain normal dietary patterns, food preferences, and usual energy intake for weight maintenance. Diets were designed to provide 40% energy from fat of which 60% would be from the test fat (24% energy) and were planned individually with the use of exchange lists developed for this study. Subjects were asked to avoid foods not included in the diet plan. Seven-day diet records and random 24-h recalls were also obtained for each diet period. The 24-h recalls were used as a crosscheck on food consumption, but were not used in the calculations of nutrient and energy intake. Diet records of food intake were analyzed for nutrient and energy content by Nutripractor 6000 nutritional analysis system (Practocare, Inc., San Diego, CA). Results are reported as average per day.

Thirty nine of 40 participants completed the study: one was removed for health reasons and the data of one participant were not used because of poor compliance. The average total caloric intake, distribution of energy intake from protein, carbohydrate, fat, and cholesterol content consumed daily by 38 participants on each of the five diets and baseline diets are given in **Table 2.** There were no significant differences in protein intake for any diet. Butter-olive and butter diets contained significantly less carbohydrate than the baseline diet. All test fat diets, ex-

TABLE 2. A comparison of the energy and composition of the various diets as determined from diet records

		Energy Percentages			
Diet/Test Fat	Energy (kcal)	Protein	CHO	Fat	Chol(mg)
Baseline	2358 ± 529	16.0 ± 2.0	47.8 ± 4.8	36.4 ± 4.8	374 ± 221
Butter-sunflower	2657 ± 581[a]	15.2 ± 1.8	46.5 ± 4.5	38.5 ± 4.4[a]	389 ± 168
Butter-olive	2581 ± 568	15.9 ± 2.1	45.2 ± 4.2[a]	39.0 ± 4.1[a]	371 ± 143
Butter	2628 ± 489[a]	15.7 ± 2.3	45.1 ± 4.4[a]	39.3 ± 4.1[a]	468 ± 126[a,b,c]
Hard margarine	2583 ± 621	15.3 ± 2.1	46.4 ± 4.7	38.4 ± 4.2[a]	296 ± 158[a,b,c,d]
Soft margarine	2577 ± 516	15.8 ± 1.9	46.9 ± 4.0	37.4 ± 3.5	273 ± 114[a,b,c,d]

Abbreviations are: CHO, carbohydrates; Chol, cholesterol.
[a]Significantly different from baseline ($P < 0.05$).
[b]Significantly different from butter-sun ($P < 0.05$).
[c]Significantly different from butter-olive ($P < 0.05$).
[d]Significantly different from butter ($P < 0.05$).

cept soft margarine, contained significantly more fat than habitual diet (baseline). Participants consumed significantly more calories on the butter and butter-sun diets, relative to baseline diet, but this did not cause a significant increase in body weight (discussed later). Approximately 100 mg more of dietary cholesterol was consumed on the butter diet and approximately 100 mg less of dietary cholesterol was consumed on the hard margarine and soft margarine diets than baseline, butter-olive and butter-sun diets.

The percentage distributions of saturated, monounsaturated, and polyunsaturated fatty acids in the test fat diets and habitual diets are given in **Table 3.** The values include both test fats and endogenous food fats. Because of the limited availability of information on the *trans* fatty acid content of many processed foods, no attempt was made to quantify this fraction. The *trans* fatty acid content is known for the test fats (Table 1) which represented approximately half of the dietary fat consumed. Table 3 values were obtained by summation of the individual fatty acid content of foods with reported values. Seventy five to 80% of the total fat in the test fat diets could be accounted for in the sum of the individual fatty acids. Actual accountability is approximately 10% higher because of glycerol, glycerol phosphoryl bases, sterols, etc. present in the total fat. The two margarine diets contained the lowest level of saturated fatty acids. The hard margarine diet contained the highest level of monounsaturated fatty acids, but a significant portion of this category was of *trans* fatty acids (Table 1). The soft margarine and butter-sun diets contained the highest percentage of polyunsaturated fatty acids. Variation in the level of polyunsaturated fatty acids between diets was up to fourfold, whereas the variation of saturated and monounsaturated fatty acids between diets was as much as twofold.

Blood collection and analysis

Weekly 12-ml samples of 12-h fasting blood were collected in Vacutubes, containing clotting factor, from all 40 participants starting 7 days before the diet period and at the start date. The latter two samples served as a baseline for each diet period and a 10-point baseline when combined. Blood from eight participants was collected each working day between 0730 and 0830 h. Requests for additional food and help with meal planning were handled at this time. Food records, when appropriate, were collected, body weights were recorded, and the results of the previous week's serum analyses were made available.

TABLE 3. Distribution of various types of fatty acids in the diets as determined from diet records

	Percentage of Fatty Acid Type		
Diet/Test Fat	Sat.	Mono.[a]	Poly.[a]
Baseline	34	46	21
Butter-sunflower	37	31	32
Butter-olive	39	46	15
Butter	57	32	11
Hard margarine	26	62	12
Soft margarine	28	26	46

[a]Contains both *cis* and *trans* isomers.

Within 1 h of collection, the serum was separated by centrifugation. Aliquots were removed for immediate analyses and future analyses of apolipoproteins, and the remainder was stored at −20°C. The nonlipid analyses performed by a robotic centrifugal COBAS FARA analyzer (Roche Diagnostic System, Montclair, NJ) on fresh serum weekly for each participant were: alanine aminotransferase, albumin, alkaline phosphatase, aspartate aminotransferase, bilirubin, calcium, creatinine, glucose, lactate dehydrogenase, magnesium, phosphorus, total protein, total blood urea nitrogen, uric acid, sodium, and potassium. These clinical tests were performed to establish that the participants remained healthy during the study. These analyses identified a health problem at an early stage in one individual who was removed from the study for treatment. Most assays were conducted with reagents, calibrator standards, and reference standards purchased from Roche Diagnostic Systems (Nutley, NJ) and Sigma (St. Louis, MO).

Lipid, lipoprotein, and apolipoprotein analyses

Lipid and apolipoprotein measurements made on the serum were: total serum cholesterol, high density lipoprotein cholesterol, triglycerides, apolipoprotein A-I, apolipoprotein B, and apolipoprotein E. Total cholesterol was analyzed with Roche reagents based upon a multienzyme system described by Allain et al. (24). High density lipoprotein cholesterol was determined with the same enzymatic assay after chylomicrons, very low density lipoproteins (VLDL), and LDL were precipitated with dextran sulfate and magnesium sulfate (25), (Seragen, Indianapolis, IN). Low density lipoprotein cholesterol was calculated using the formula reported by Friedewald, Levy, and Fredrickson (26). Triglycerides were analyzed with the totally enzymatic method reported by Bucolo and David (27) using Roche reagents. Total serum cholesterol, HDL cholesterol, and triglycerides were determined on fresh serum. Apolipoproteins A-I and B were determined by immunoturbidimetric methods (28, 29), (INCSTAR, formerly Atlantic Antibodies, Stillwater, MN). Apolipoprotein E was assayed immunoturbidimetrically according to the method of Rafai and Silverman (30) using goat antisera to human apoE (Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan). Apolipoprotein analyses were made at the end of the study with a single lot of reagents to eliminate lot to lot variation. A series of standards of varying concentrations and controls, based upon Centers of Disease Control reference material, were

run daily. Analyses were made on frozen serum that was thawed only once. All of the preceding analyses were made with a COBAS FARA centrifugal analyzer run at certified operating specifications.

Aliquots of serum (0.5–2.0 ml) were lyophilized and the lipids were extracted by the Bligh and Dyer procedure (31). After repeated evaporation and redissolving the sample with chloroform–methanol 2:1 (v/v) to remove the last traces of water, the samples were filtered through a fine porosity sintered glass funnel and evaporated under vacuum to a constant weight. Total lipid phosphorus was measured in duplicate by the procedure of Rouser, Siakotos, and Fleischer (32). Total phospholipid weight was calculated using 790 as the average molecular weight of a phospholipid. Total serum lipids were transesterified in acid-catalyzed anhydrous methanol; methyl esters were isolated by TLC and analyzed by GLC on 50 M capillary column as described earlier (see Test fats). Column temperature was programmed from 140–230°C at 2°C/min and the data were collected with an IBM model 9000 laboratory computer. Peak identification was established by co-chromatography with standard reference fatty acid mixtures. All the procedures used to extract, derivitize, and analyze the serum lipid methyl esters have been described in detail (23).

Statistical analysis

Statistical analyses were performed using SAS statistical programs (33). The data are expressed as mean ± standard deviation and significance at $P < 0.05$ unless specified otherwise. Analysis of variance was performed by using the general-linear-model procedure and Tukey test for multiple pairwise comparisons.

RESULTS

Cholesterol, lipoproteins, and apolipoproteins

Average lipid, lipoprotein and, apolipoprotein values using the mean of 5th and 6th week serum levels from 38 participants on five different dietary test fats and baseline diet values are compared in **Table 4.** Total serum cholesterol levels were significantly higher and significantly lower on butter and soft margarine diets, respectively, relative to baseline, butter-sun, butter-olive, and hard margarine diet values which were similar. HDL cholesterol levels were the highest on the butter and butter-olive diets, but the differences were not statistically significant at the 95% probability level. Soft margarine LDL cholesterol levels were significantly lower than all other diets while butter LDL cholesterol levels were significantly higher than all other diets. The various fat diets had little effect on triglyceride values: only the soft margarine diet exhibited lower values than the hard margarine diet which had the highest values. The soft and hard margarines had the same low apoA-I values which were significantly lower than butter and baseline diet levels. The soft margarine diet resulted in the lowest apoB level which was significantly different from all the other diets. The butter-sun diet, the diet containing the second most polyunsaturated fatty acids behind the soft margarine diet, had the next lowest apoB level which was significantly lower than baseline and butter diet values. Hard margarine and butter-olive diets exhibited significantly lower apoB levels than baseline diet values.

Total serum lipid and phospholipids

The average serum concentrations of baseline total lipids and phospholipids at the 5th and 6th weeks were 757 ± 130 and 167 ± 29 mg/dl, respectively. None of the diets, except soft margarine, resulted in values significantly different than the baseline values. The soft margarine diet serum total lipids and phospholipids were 684 ± 130 and 151 ± 29 mg/dl, respectively. These values were significantly lower than baseline and butter values.

Quantities of dietary fatty acids

The average daily consumption of the major dietary fatty acids found in the test fat diet and habitual diets is given in **Table 5.** The quantities given in the table for

TABLE 4. Average serum lipid and apolipoprotein levels of participants on baseline and test fat diets

	Dietary Test Fat Values (mg/dl)					
	Baseline	Butter-Sun.	Butter-Olive	Butter	Hard Marg.	Soft Marg.
Total chol.	202 ± 30[d,f]	198 ± 32[d,f]	203 ± 33[d,f]	211 ± 33[a,b,c,e,f]	198 ± 29[d,f]	187 ± 31[a,b,c,d,e]
HDL chol.	45 ± 8	46 ± 10	47 ± 11	47 ± 10	45 ± 9	45 ± 10
LDL chol.	139 ± 25[d,f]	135 ± 27[d,f]	139 ± 28[d,f]	146 ± 29[a,b,c,e,f]	134 ± 25[d,f]	126 ± 25[a,b,c,d,e]
Triglycerides	110 ± 44	107 ± 50	108 ± 46	109 ± 53	114 ± 44[f]	100 ± 38[e]
ApoA-I	110 ± 12[e,f]	107 ± 16	106 ± 15	108 ± 17[e,f]	103 ± 14[a,d]	103 ± 14[a,d]
ApoB	69 ± 11[b,c,e,f]	63 ± 13[a,d,f]	64 ± 13[a,f]	66 ± 12[b,f]	64 ± 13[a,f]	60 ± 12[a,b,c,d,e]
ApoE	3 ± 1	3 ± 1	3 ± 1	3 ± 1	3 ± 1	3 ± 1

Values bearing a superscription are significant at the 95% probability level or higher. The superscript codes indicate: [a], significantly different from baseline; [b], significantly different from butter-sun; [c], significantly different from butter-olive; [d], significantly different from butter; [e], significantly different from hard margarine; [f], significantly different from soft margarine.

TABLE 5. Minimal quantities of fatty acids consumed daily from test fats and food fats combined

Diet/Test Fat	Grams of Fatty Acid Consumed/Day					
	14:0	16:0	16:1	18:0	18:1	18:2
Baseline	2.2 ± 1.0	11.9 ± 4.7	1.4 ± .6	5.2 ± 2.0	26.1 ± 10.1	11.6 ± 6.1
Butter-Sunflower	4.2 ± 1.2[a]	16.6 ± 4.4[a]	1.1 ± .4	8.9 ± 2.4[a]	25.7 ± 7.7	26.9 ± 8.8[a]
Butter-olive	4.3 ± 1.4[a]	19.6 ± 6.5[a]	2.0 ± .6[a,b]	8.1 ± 2.2[a]	38.2 ± 10.5[a,b]	12.2 ± 3.8[b]
Butter	7.3 ± 2.5[a,b,c]	25.0 ± 6.9[a,b,c]	1.4 ± .4[c]	13.4 ± 4.3[a,b,c]	26.8 ± 5.1[c]	8.7 ± 3.0[b]
Hard margarine	1.1 ± .1[b,c,d]	13.3 ± 4.0[c,d]	0.8 ± .2[a,c,d]	7.0 ± 2.2[d]	51.8 ± 18.0[a,b,c,d]	9.3 ± 4.7[b]
Soft margarine	2.0 ± .7[b,c,d]	12.7 ± 3.4[c,d]	0.8 ± .3[a,c,d]	6.7 ± 2.5[b,d]	21.0 ± 5.2[c,e]	38.8 ± 12.[a,b,c,d,e]

[a], Significantly different from baseline; [b], significantly different from butter-sunflower; [c], significantly different from butter-olive; [d], significantly different from butter; [e], significantly different from hard margarine ($P < 0.001$).

each diet are minimal values. This is because the sum of the quantities in any row, except baseline, represents 75–85% of the fat consumed. The fatty acid of the food fats that were not available would likely elevate most fatty acid quantities slightly. Only the major fatty acids have been included in the table and only significant differences at the 99.9% probability level have been identified. Butter and butter blend diets contained two to four times more myristic acid than habitual or margarine fat diets. Margarine and habitual fat diets contained similar quantities of palmitic acid which was only one-half of the concentration found in the butter diet. The butter diet provided twice the level of stearate as both margarine and baseline diets. All diets provided more than 20 g/day of natural 18:1. The butter-olive diet provided the largest quantity of oleic acid despite the fact that nearly 52 g of 18:1 was provided by the hard margarine diet. The *trans* isomers from the hard margarine test fat alone amounted to 16 g. The soft margarine and butter-sun diets provided two to four times more polyunsaturated fatty acids than the other diets including the baseline.

Serum fatty acids

The average fatty acid compositions of the participants' serum lipids after 5 and 6 weeks on the various test fat diets are given in **Table 6** along with the baseline diet values. These baseline data, unlike the other baseline data, represent the mean of 38 individual participants whose baseline serums for each diet period were pooled before analysis. Serum from subjects on the butter diet contained the highest percentage of myristic acid whereas both margarine diets contained the lowest level. The two margarine diet serums also contained significantly less palmitic acid than the other diets, including the baseline, which were nearly identical. The hard margarine diet serum contained the lowest level of stearate of any diet. Serums from diets containing the highest levels of polyunsaturated fatty acids, butter-sun and soft margarine contained the highest level of 18:2 which was partially compensated for by a decrease in 18:1 percentages. All test fat diet serums contained a significantly higher level of 20:4 than the baseline diet.

Compliance

Subject compliance to research protocol is always a concern when studies are conducted under free-living conditions. Participants in full compliance would have consumed 24% of their calories from the test fats. The average compliance on all the diets was essentially the same; 19.0 ± 0.29% of calories from the test fats. The data of only one participant were excluded because of poor compliance. When total serum cholesterol values

TABLE 6. Mean fatty acid composition of total serum lipids from participants on the various diets

Diet/Test Fat	Fatty acid percentages						
	14:0	16:0	16:1	18:0	18:1	18:2	20:4
Baseline	1.2 ± .3	24.3 ± 2.2	2.6 ± .5	7.1 ± .8	19.2 ± 1.7	27.2 ± 2.9	4.1 ± .8
Butter-sunflower	1.3 ± .5	24.2 ± 3.1	2.5 ± .7	7.4 ± 1.0	16.8 ± 2.2[a]	31.6 ± 4.2[a]	5.2 ± 1.7[a]
Butter-olive	1.3 ± .3	24.2 ± 1.8	2.8 ± .6[b]	7.1 ± .9	19.9 ± 2.3[b]	28.2 ± 3.3[b]	5.1 ± 1.0[a]
Butter	1.6 ± .5[a,b,c]	24.2 ± 2.4	2.7 ± .5	7.2 ± .8	18.3 ± 2.1[b,c]	28.1 ± 3.9[b]	5.3 ± 1.2[a]
Hard margarine	1.1 ± .4[b,c,d]	22.5 ± 2.0[a,b,c,d]	3.0 ± .5[a,b,d]	6.2 ± .8[a,b,c,d]	21.2 ± 2.8[a,b,c,d]	27.6 ± 3.8[b]	4.7 ± 1.1[a,d]
Soft margarine	1.0 ± .3[b,c,d]	23.0 ± 3.0[a,b,c,d]	2.2 ± .6[a,c,d,e]	6.9 ± .7[b,e]	15.6 ± 2.7[a,b,c,d,e]	35.1 ± 4.3[a,b,c,d,e]	5.3 ± 1.2[a,e]

The difference between the sum of any row and 100 represents minor amounts of other fatty acids not seen in table.
[a], Significant difference from baseline; [b], significant difference from butter-sun.; [c], significant difference from butter-olive; [d], significant difference from butter; [e], significant difference from hard margarine.

were examined for all diets according to degree of compliance: >11%, >15%, with seven responders removed (responders were arbitrarily designated as those whose serum cholesterol increased and decreased 10 mg/dl on butter and soft margarine diets, respectively, relative to the baseline), and compared with values from all subjects, there were no significant differences. Food disappearance data were consistent with the indication of excellent compliance from diet records. The high level of compliance was probably the result of using faculty and staff. This type of participant is often involved in research and understands the importance of following a protocol. The high number of participants completing the study is also characteristic of dedicated participants.

Integration of test fats into diets

Another important consideration in the evaluation of data from human dietary studies is minimal perturbation of the habitual intake of the major nutrients. Protein content remained unchanged and carbohydrates were changed only marginally (Table 2). Fat accounted for 36.4% of the calories in the habitual diets and 37.4–39.3% of the calories in the test diets. The small, but significantly higher level of fat in the test fats was accompanied by a higher caloric intake on two diets (butter-sun and butter) relative to baseline diets. The additional 200–300 calories/day over a diet period of 42 days would have resulted in only a 2–3 pound gain in body fat if there had been a 100% efficient conversion. These small changes in body weight would have been indistinguishable from other causes of expected small weight fluctuations. The test fat diets contained different proportions of saturated, monounsaturated, and polyunsaturated fatty acids (Table 3) while the fat caloric content remained relatively unchanged. Although it is virtually impossible to incorporate approximately 20% of a participant's caloric intake from a single fat source without affecting his choice of food, we feel that allowing free choice of foods that contained the test fats minimized the perturbation.

DISCUSSION

We wish to remind the reader at this point that the levels of test fats consumed by the participants in this study far exceed the amounts of a single type of fat most people would consume on any dietary regimen. The extreme levels of a fat were used to elicit the largest possible change in serum lipids. If a fat produced little or no change in the serum lipids at this high level, it could be logically concluded that it would not be a health concern at lower concentrations. If, on the other hand, significant positive or negative changes in the serum lipids were observed, additional experiments could determine a response curve to concentrations. The data being discussed represents the mean of 5th and 6th weeks' values and the mean of 38 individual responses. The exception is where an occasional data point is missing.

One might question why the baseline or habitual diet data are included in the comparison with the test fat data, as many studies make comparisons only between dietary test fats. The primary reason is to answer the question: How will my serum lipids respond to a particular fat relative to the diet I now eat? Additionally, the baseline data from this population in the present study are very reliable. They represent the mean of 10 determinations on samples collected over more than a year's time for each participant. The composition of the baseline diet has also been determined. One might also question whether the dietary behavior of this educated population is typical of the public at large. It is more likely that many of the participants may have adjusted their fat intake to comply with the recommendations of The National Cholesterol Education Program (34) than the general population. If that assumption is correct, one might expect the response to butter to be greater than would be observed in the general population because the University population would have already eliminated butter from their habitual diet. Conversely, a smaller response to the soft margarine diet, high in polyunsaturated fatty acids, might be expected because the academic community participants would have already incorporated this type of fat in their diet. The opposite responses were observed.

Effects of dietary fats on serum lipids

The butter diet elevated total serum cholesterol levels significantly above baseline (6–9 mg/dl) and the other test fat diets (7–24 mg/dl). The degree of elevation of cholesterol was surprisingly low compared to results with institutionalized subjects or patients on liquid formula diets (2–4, 35, 36). The rise we observed was also approximately one-half that observed for a group of men, selected from the top quartile for serum cholesterol values, who consumed >31% of their energy from butter for 2 weeks (37). If one takes into account that the butter diet provided 100 mg more cholesterol/day than the baseline, butter-sun, and butter-olive diets (Table 2) and that an additional 100 mg/day of cholesterol at these dietary levels can be expected to elevate serum cholesterol 4 mg/dl (7), a rather small rise in serum cholesterol is left to be attributed to the saturated fatty acids in the butter diet. The level of polyunsaturated fatty acids in the butter diet is probably an important contributing factor to the marginal serum cholesterol response observed. If dietary foods had not contributed more polyunsaturated fatty acids than butter (Table 1) the results would probably have been more in line with previous data. Normocholesterolemic men under free-living conditions include food in their diets that contains significant quantities of polyunsaturated fatty acids. Subjects on the butter diet consumed

39.3% of their calories as fat (Table 2) of which butter represented 19.1% of the total energy. The polyunsaturated fatty acids in butter accounted for only 0.7% of the calories whereas polyunsaturates in the whole diet accounted for 4.5% of the total calories. Although this level is lower than the current average population intake of 7% of calories and the recommended level by the Committee on Diet and Health of the Food Nutritional Board (38), it is well above the adequate level of essential fatty acids. As more studies are conducted, where the level of polyunsaturated fatty acids is controlled, we may find that the hypercholesterolemic effect of some saturated fatty acids is minimized when the level of polyunsaturated fatty acids reaches some minimal percentage of the caloric intake. The point appears to lie between 3 and 6% of calories and should not be confused with the high levels of polyunsaturated fatty acids used to derive the Keys equation (39). Not all studies have shown that high levels of butter in the diet elevate serum cholesterol so dramatically. Flynn et al. (40) reported that normocholesterolemic and hypercholesterolemic males consuming 5–6% of their calories from butter and two eggs per day did not show a significant rise in serum cholesterol levels after 12 weeks relative to entry values. Both a butter and egg diet and a margarine and egg diet produced a significant rise in serum cholesterol of normocholesterolemic females. Serum HDL cholesterol levels in subjects on the butter and butter-olive diets were the highest of any diet, but did not reach a level of significance at the 95% probability level. Serum LDL cholesterol was significantly higher on the butter diet than all other diets. Similar observations were made earlier (35, 37). Butter diet apoA-I, the major protein of HDL (41), was similar in concentration to baseline levels which was the highest of all diets and significantly higher than the two margarine diets.

When sunflower oil and olive oil were blended with butter in a 50/50 mixture and incorporated into the diet (butter-sun and butter-olive), the resulting serum lipid responses were similar: apoB levels were reduced and total serum cholesterol and LDL-cholesterol levels were reduced significantly relative to the butter diet values (Table 4). Although the response was significant, one might have expected this degree of response from the 50% reduction of butter in these diets alone, without enrichment with large amounts of monounsaturated and polyunsaturated. This may indicate that the factor or factors that produce the butter serum lipid profile are well above saturation levels and the effect is not easily diluted. It may also indicate that the interacting effect between various fats is more complicated than the sum of individual parts. Interestingly, the fatty acid composition of the butter-sun diet (Table 3) had nearly equal percentages of polyunsaturated, monounsaturated, and saturated fatty acids, similar to the Step 1 diet recommended by an expert panel for lowering serum cholesterol (34). The difference in this study is that the butter-sun fat represented 38.5% of the calories and not the 30% recommended.

The soft margarine diet, which contained a high level of polyunsaturated fatty acids and no *trans* fatty acids, produced the most dramatic changes in serum lipids. Total cholesterol, LDL-cholesterol, triglycerides, and apoB were reduced significantly relative to all the other diets including the baseline. The only negative effect on the serum lipid profile was a significant decrease in apoA-I levels relative to the habitual and butter diet values. A most unusual aspect of this dietary fat is that HDL cholesterol levels did not fall, as often observed with dietary fats containing high levels of polyunsaturated fatty acids (9, 16–20). Perhaps a ratio of 1:1:2 of saturated, monounsaturated, and polyunsaturated fatty acids in the diet (Table 3) can maintain HDL cholesterol levels.

The hard margarine, made from partially hydrogenated fat, did not change total serum cholesterol relative to butter-sun, butter-olive, and baseline diet levels, but values were significantly lower than butter and significantly higher than the soft margarine diets (Table 4). Likewise, LDL cholesterol levels were not changed relative to butter-sun, butter-olive, and baseline diet values, but were significantly lower than the butter diet and significantly higher than the soft margarine diet levels. ApoB levels were reduced significantly, relative to baseline, but were significantly higher than soft margarine diet concentrations. The lack of significant LDL cholesterol lowering coupled with a significant decrease in apoA-I, raises the question of the wisdom of the widespread use of partially hydrogenated fats to replace saturated fats. Early studies (2, 42) questioned the use of partially hydrogenated fats, while other studies (43–45) indicated that the effect of partially hydrogenated fats on serum cholesterol differed little from the natural fat.

Effect of dietary fatty acids on serum lipids

The experiments were designed to compare the effects of the types and sources of dietary fats on serum lipids, but we can also look at the results, to a limited extent, based upon the fatty acids present in the diet. The minimal quantities of the major fatty acids consumed daily shown in Table 5 will serve as the data base. Excluding stearate, which has no effect on serum lipids (12, 13), the quantities of saturates (14:0 + 16:0) consumed on the soft margarine, hard margarine, and baseline diets are similar. This leaves any differences between the three diets attributable to the monounsaturated and polyunsaturated fatty acid content. Because the quantities of 18:2 for the baseline and hard margarine diets given in Table 5 are also similar, it would be easy to conclude that the differences in the response of serum lipids (decreased apoA-I and B relative to baseline) are due to the monounsaturated fatty acids, specifically the *trans* fatty acids. This may well be the correct conclusion, but the validity of the

comparison is weakened by the lower percentage accountability of the fatty acids in the baseline diet (58.4 vs. 83 g). If the unaccounted-for fatty acids were not all monounsaturated, which is unlikely, the saturated and polyunsaturated would no longer be similar. The comparison between the hard margarine and soft margarine, where fatty acid accountability is much higher and equal (83 vs. 82 g), is stronger. The sum of the 18:1 and 18:2 fatty acid quantities consumed was similar for the hard and soft margarine diets, but the quantities of 18:1 and 18:2 are dramatically different. The soft margarine diet contained four times the level of 18:2 as the hard margarine diet; whereas the hard margarine diet had more than double the level of 18:1 of the soft margarine diet. As it has been shown that natural *cis* 18:1 and 18:2 affect total serum cholesterol equally (9–11), any difference in response must be attributed to the presence of the *trans* fatty acid in the hard margarine diet. Based upon the level of compliance (19.0% of calories) and the percentage of *trans* fatty acid in the hard margarine (29%, Table 1) the minimal quantities of 18:1 in Table 5 can be divided into 16 g of *trans* and 35.8 g of *cis*. This is a low value for the *trans* fraction because the *trans* fatty acid content from food fats has not been included. Because they were probably present to the same extent in the food of all the diets, the omission is probably not important. The more than 20 mg/dl difference between the total serum cholesterol levels of the same participants on the hard and soft margarines can be attributed to the *trans* fatty acids. It is most unlikely that the difference in total serum cholesterol cannot be attributed to a dilution effect caused by neutral *trans* fatty acids replacing 30% of the natural *cis* monounsaturated fatty acids. The data strongly suggest that dietary *trans* fatty acids have an elevating effect on serum cholesterol. Our data are in agreement with those of Mensink and Katan (21) who showed that both male and female participants consuming a diet high in *trans* monounsaturated fatty acids had higher total serum cholesterol and LDL cholesterol and lower HDL cholesterol and apoA-I levels than a diet high in *cis* monounsaturated fatty acids. We observed a significantly lower level of apoA-I in both the hard and soft margarine diets, relative to baseline and butter diets. We did not see a decrease in HDL cholesterol levels relative to any diet, but LDL cholesterol levels were significantly higher on the hard margarine diet containing the *trans* fatty acids than the soft margarine diet. Flynn et al. (40) have also reported that total serum cholesterol increased and HDL cholesterol values decreased relative to entry levels in normocholesterolemic males and females that consumed a self-selected diet containing 5–6% hard margarine energy plus two eggs a day. Additional studies are warranted regarding dietary *trans* fatty acids, but presently we appear to have enough data to suggest that *trans* fatty acids should not be considered metabolically neutral or equivalent to the natural *cis* monounsaturated fatty acids.

The three remaining diets, butter, butter-sun, and butter-olive, all contain higher levels of saturated fatty acid (14:0 + 16:0) than the baseline and soft and hard margarine diets just discussed. Butter-sun and butter-olive diets contain similar quantities of saturated fatty acids and cholesterol and the sums of the 18:1 and 18:2 levels are nearly equal (Table 5). Consistent with the now-recognized equivalency of effect of natural *cis* monounsaturated and polyunsaturated fatty acids, serum lipid profiles on the butter-sun and butter-olive diets were similar and total serum cholesterol and LDL cholesterol were significantly lower than butter values.

Effect of dietary fatty acids on serum fatty acid composition

The mechanism by which dietary fatty acids affect serum lipid and lipoprotein levels is unknown. Generally, it is assumed that the serum lipid fatty acid composition reflects the fatty acid composition of the diet, which affects serum cholesterol, triglyceride, lipoprotein, and apolipoprotein levels. This scenario becomes questionable when one sees how little the serum lipid composition is affected by dietary fatty acids. The contrast between the quantities of the dietary fatty acids consumed daily and the composition of the total serum lipids after 5–6 weeks on a diet is shown in Tables 5 and 6. Twenty five grams of palmitic acid were consumed daily on the butter diet, twice as much as baseline, hard margarine, and soft margarine diets, yet the percentage of 16:0 in the serum is either identical or only differs by one or two percent. Stearate levels in the butter diet were not reflected in the serum fatty acid composition. Likewise, linoleate content of the diet, precursor of arachidonate, did not reflect the arachidonate composition of the serum. *Trans* fatty acids, which represented 5.5% of the calories in the hard margarine diet, were present in only trace amounts of serum lipid fatty acids examined by capillary GLC. The level of some fatty acids in the serum can be influenced by the quantities in the diet, but changes are not dramatic. The soft margarine diet provided over 38 g of linoleate daily, more than three times as much as baseline, butter-olive, butter, and hard margarine diets, but serum linoleate levels were increased only 20%. Increases in the dietary monounsaturated fatty acids increased serum monounsaturated, but changes were less than observed for dietary linoleate. Other investigators (46, 47) have previously reported the inability of diet to change the plasma fatty acid composition except for small changes in linoleate and oleate. When plasma phospholipids from subjects on different diets were examined, only significant changes in C-18 monenes and dienes were observed (35). Analyses of lipid classes from LDL have revealed some changes in the

fatty acid composition of sterol esters and triglycerides, but diet had little effect on the plasma phospholipids (48). These observations suggest that the fatty acid composition of the serum lipids is more tightly controlled and resistant to changes from dietary fat intake in most healthy individuals than previously thought.

Impact on dietary choices

This study indicates that the extreme measure of replacing half of the fat in the diet with a specific type of fat is possible, but the effect on the serum lipid profile of normal men between the ages of 30 and 60 is not dramatic when compared to their normal or habitual diets. Butter elevated serum cholesterol and LDL cholesterol significantly, but a 5% increase when nearly 20% of the energy was supplied by this one fat alone was surprisingly low. The results indicate that the replacement of butter in the normal diet is not likely to have a significant effect on healthy individuals. Adding large amounts of mono- and polyunsaturated fatty acid-containing glycerides to butter cannot be expected to reduce serum cholesterol below normal diet levels. Likewise, the use of a hard margarine dietary fat containing *trans* fatty acids does not improve the serum lipid profile over the normal diet. This may be because the normal diet already contains a significant level of partially hydrogenated fat. The serum lipid profile from subjects on the soft margarine diet that contained no *trans* fatty acids, but was otherwise similar in saturated and unsaturated fatty acid content to the hard margarine diet, was superior to the profile from all other diets. These data suggest that *trans* fatty acids in partially hydrogenated fats and oils are not metabolically equivalent to the natural *cis* unsaturated fatty acids and that they are not neutral with regard to their effect on serum lipids. Concerned individuals who wish to improve their serum lipid profile have the best chance by using a dietary fat that contains all natural *cis* unsaturated fatty acids; however, not every individual will experience the same response. The heterogeneity of individual responses to dietary fats is the subject of a separate publication.

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