Effect of palm oil, margarine, butter, and sunflower oil on the serum lipids and lipoproteins of normocholesterolemic middle-aged men

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Twenty-nine healthy middle-aged men participated in a Latin square-designed study containing six dietary fats: butter; crude palm oil; hard margarine; refined palm oil; 80% refined palm oil + 20% sunflower oil blend; and sunflower oil. Each diet period was 6 weeks in duration followed by 6 weeks of habitual diet. Test fats were consumed in ice cream, milk, cookies, and as spreads and represented 50% of the total fat energy (38%) on all diets. Serum lipid responses to the high level of test fats in the diets were small relative to habitual diet values. Large changes in quantity and type of fatty acids consumed daily were not reflected in the fatty acid composition of the total serum lipids. Butter did not elevate total serum cholesterol or low density lipoprotein (LDL) cholesterol relative to habitual diet levels, but these values were significantly higher than sunflower oil-diet and palm oil-diet values. The sunflower oil diet produced the most dramatic changes: total serum cholesterol was reduced significantly relative to all diets except margarine, and apolipoprotein B values were the lowest of any diet. Unfortunately, the desirable high density lipoprotein (HDL) cholesterol and apolipoprotein A1 were also reduced on the sunflower oil diet. Diets containing either crude or refined palm oil did not elevate total serum cholesterol relative to habitual diet values or LDL cholesterol or apolipoprotein levels relative to any diet. Unexpectedly, the refined palm oil diet HDL-cholesterol and apolipoprotein A1 levels were the highest of all diets and significantly higher than sunflower oil diet values. The hard margarine diet, containing 26% trans *fatty acids, reduced apolipoprotein B values relative to habitual diet levels, but HDL cholesterol was reduced significantly relative to the refined palm oil diet values. Comparison of the diet's fatty acid compositions suggests the decrease in the HDL cholesterol on the hard margarine diet is attributable to the* trans *fatty acids. The data indicate that the* trans *fatty acids, produced during the partial hydrogenation of fats and oils, are not neutral and adversely affect serum lipid profile.*

Keywords: cholesterol; triglycerides; lipoproteins; apolipoproteins; palm oil; dietary fats; trans fatty acids

Introduction

Palm oil, as an oil, has never been available in the USA, except in a few culinary shops in major cities. It enters the food supply as a component of processed foods. Before 1970 palm oil was used very little by food processors because of its limited availability. Even now it represents only about 1.2% of the fat in the American food supply.[1] However, on a global scale palm oil production ranks number two behind soybean oil.[2] Production is still in the growth stage and palm oil could account for 25% of the world's supply of vegetable fats and oils by the mid-1990s.

Negative USA newspaper articles about palm oil in the past few years have had a major impact on the palm oil industry around the world from at least two points of view.[1] First, public pressure in the USA has forced food processors to use alternative sources of fat. Partially hydrogenated oils have been the principal replace-

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ment fat. Unlike the all-natural palm oil, the partially hydrogenated fats contain more than a dozen unnatural fatty acids, including the *trans* fatty acids, not present in the original oil. Several studies have indicated that dietary fats containing *trans* fatty acids had little or no effect on serum lipids[3]; however, a recent study by Mensink and Katan[4] concludes that the effect of *trans* fatty acids on serum lipoprotein profile "is at least as unfavorable as the cholesterol-raising saturated fatty acids." This conclusion has been confirmed by a second study that used a lower energy level of *trans* fatty acids,[5] and in a study similar to the one reported here we have concluded that *trans* fatty acids adversely affect the serum lipid profile when *trans* fatty acids represent 5.5–6.0% of the energy.[6] Secondly, the negative coverage in the USA newspapers have caused millions of people around the world, who depend upon palm oil as a source of nutrition, to question its effect on health.

Generally, the allegations against palm oil were made without a scientific basis. Palm oil has been used as a control or basis for comparison in a few studies that have examined the effect of dietary fat on serum lipids. These studies have been reviewed and none showed that serum cholesterol levels at the end of the palm oil diet periods were significantly higher than pre-trial habitual diet values.[1]

Because of the alleged health risk of palm oil the present study was conducted. The study was designed to compare the serum lipid, lipoprotein, and apolipoprotein profiles of 30 healthy middle-aged men that rotated through six dietary test fat periods. Refined palm oil, crude palm oil, and a palm oil-sunflower oil blend were compared with margarine, butter, and sunflower oil. Preliminary results of these studies have been presented at scientific meetings.[7–9]

Materials and methods

Subjects

Thirty healthy male Texas A&M University faculty and staff (no students) between the ages of 30 and 60 years were selected from 50 volunteers for the study. Screening criteria included clinical chemistries; blood pressure; body weight; cholesterol level <6.2 mmol/L; physical examination including electrocardiogram; medical history; personal conflicts; and interest in the study. Although we did not screen against smokers, all participants were non-smokers. Individuals requiring prescribed medication were excluded. Average body weight was 83.5 ± 10 kg and the average age was 41 ± 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 2.2 kg of study entry weight. This was monitored by weekly weight measurements at the time of blood collection.

Table 1 Fatty acid composition of dietary test fats

Dietary test fats	Percentages[a,b]				
	14:0	16:0	18:0	18:1	18:2
BU[c]	10.3	31.3	15.5	29.2	3.9
CPO	—	37.3	3.4	45.7	10.6
PAR	0.5	12.5	7.9	59.9[d]	14.1
RPO	—	42.0	4.5	40.6	8.9
SPO	—	29.5	4.6	33.0	24.9
SUN	—	7.9	4.9	17.5	65.2

[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]Fatty acids are designated by a shorthand nomenclature system. The first number represents the hydrocarbon chain length. The second number separated by a colon indicates the number of double bonds in the fatty acid. Abbreviations used are: BU = butter; CPO = crude palm oil; PAR = Parkay margarine; RPO = refined palm oil; SPO = 80% RPO and 20% sunflower oil; SUN = sunflower oil.
[c]Contained 2.4, 3.3, and 4.0% 10:0, 12:0, and *trans* monoenes, respectively.
[d]*Trans* octadecenoate fatty acids represented 26.2% of the total fatty acids.

Experimental design

Five groups of six participants rotated through six diet periods of 6 weeks in duration in a Latin square design. Each diet period was followed by a 6 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 holiday and January 8th. The six test fats used are (1) sweet butter, 1.8% salt (BU); (2) crude palm oil (CPO); (3) margarine, Parkay brand, Kraft Inc. (PAR); (4) refined palm oil (RPO); (5) 80% RPO + 20% SUN (SPO); (6) refined sunflower oil (SUN).

A single lot of each test fat was prepared or purchased to cover the needs of the entire study. The test fats were supplied to the participants as spreads (1 pound containers), cookies, ice cream, and milk when the test fats were used to replace the fats normally present. Cookies were made at the Texas A&M Bakery. Butter, butter blends, the modified fat ice cream, and milk were prepared at the Texas A&M Creamery.

The diets were designed to contain 40% fat energy of which 60% would be test fat. Full compliance participants were to get 24% of their total energy from the test fat. Participants were supplied with food scales and instructions from a registered dietician on how to prepare a 7 day habitual or customary diet record. This diet record was used to prepare an individualized meal plan based on the participants' likes and dislikes of the modified fat foods available. The subjects were provided exchange food lists and encouraged to consult with the dietician or have their wives call to discuss ways of substituting test fats into the diets. Seven day diet records and 24-hour recalls were also obtained for each diet period.

The fatty acid compositions of the dietary test fats 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 in the table for butter are slightly elevated. Butter contained 4% *trans* monounsaturated fatty acids, almost exclusively C-18 positional isomers. Stove brand 17 kg containers of crude palm oil were provided by The Palm Oil Research Institute of Malaysia (PORIM). This hybrid palm oil contained less than 40% palmitate and more than 45% oleate. A single lot of Parkay brand margarine, a product of Kraft Inc. (Glenview,

IL USA) was purchased from a local supermarket. The margarine contained nearly 60% monounsaturated fatty acids, but half was octadecenoate isomers resulting from partial hydrogenation. The *trans* monounsaturated fatty acids represented 26.2% of the margarine. Although this lot of margarine was not analyzed for *cis* positional isomers, analyses of margarine produced by partial hydrogenation of vegetable oil have shown that a significant percentage of the unnatural Δ8, Δ10, and Δ12 *cis* isomers is also present.[10,11] Labour brand 17 kg containers of refined palm oil were provided by PORIM. The refined palm oil contained more palmitate and less oleate than the crude hybrid palm oil. Refined sunflower oil was obtained from Arrowhead Mills in Hereford, TX USA. It contained more than 65% polyunsaturated fatty acids. The distribution of saturated, monounsaturated, and polyunsaturated fatty acids in SPO was near the equal amounts recommended by the expert panel.[12]

The description of the food items and quantities from the 7 day diet records were used to analyze food intake. A Nutripractor 6000 computer program (Practocare Inc., San Diego, CA USA) and USDA Agricultural handbooks 8, 8-4, 8-11, and 1989 supplements were used to calculate food composition and nutrient intake for each participant.

Blood collection and analysis

Approximately 12 mL of 12-hour fasting blood were collected in vacutubes containing clotting factor from all 30 participants weekly starting 7 days before the diet period and the start date. The latter two samples served as a baseline for each diet period and a 12-point baseline when combined with the other diet period baselines. An extra 7 mL of blood were collected in vacutubes containing EDTA for preparation of plasma at baselines and the 5th and 6th weeks for prostanoid analyses. Aspirin (0.01 mL of 0.4% solution) was added for each mL of blood. Twelve-hour fasting blood was collected between 07:30 and 08:30. Requests for additional food and help with meal planning were handled at this time. Food records, when appropriate, were collected, body weights recorded, and the results of the previous week's serum analyses were made available.

Within 1 hour of collection, the blood was centrifuged for 15 minutes in an IEC model CL centrifuge and the serum removed. Aliquots were removed for immediate analyses and future analyses of apolipoproteins, and the remainder was stored at −20° C. The non-lipid analyses made with a robotic centrifugal COBAS FARA analyzer (Roche Diagnostic System, Montclair, NJ USA) 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. Except for the electrolytes, which were measured with ion specific electrodes, most of the assays were enzyme based and conducted with reagents, calibrator standards, and reference standards purchased from Roche Diagnostic Systems, Nutley, NJ USA and Sigma, St. Louis, MO USA.

Lipids and lipoprotein analyses

Lipid measurements made on the serum were: total serum cholesterol; high density lipoprotein-cholesterol; triglycerides; apolipoprotein A1; apolipoprotein B; fatty acid profile of serum lipids; total lipid; and total phospholipid.

Total cholesterol was analyzed with Roche reagents based on a multi-enzyme system described by Allain et al.[13] High density lipoprotein (HDL) cholesterol was determined by the same enzymatic assay after chylomicrons, very low density lipoproteins (VLDL) and low density lipoproteins (LDL) were precipitated by dextran sulfate and magnesium sulfate[14] using Seragen reagents (Indianapolis, IN USA). LDL cholesterol was calculated using the formula reported by Friedewald et al.[15] Triglycerides were analyzed with the totally enzymatic method reported by Bucolo and David[16] using Roche reagents. Apolipoprotein A1 and B were determined by immunoturbidimetric methods[17–19] using INCSTAR reagents (Stillwater, MN USA). Total serum cholesterol, HDL cholesterol, and triglycerides were determined on fresh serum. Apolipoprotein determinations were made on frozen serum that was thawed once. All of the analyses described to this point were made with a COBAS FARA centrifugal analyzer run at certified operating specifications.

Aliquots of serum of known volume (0.5–2.0 mL) were lyophilized and the lipids extracted by the Bligh and Dyer procedure.[20] After repeated evaporation and redissolving the sample with chloroform and methanol (2:1, vol/vol) to remove the last traces of water, the samples were filtered through a fine porosity Büchner funnel and evaporated under vacuum to a constant weight. Total lipid phosphorus was measured in duplicate by the procedure of Rouser et al.[21] on total lipid aliquots. 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 isolated by thin layer chromatography (TLC) and analyzed by gas-liquid chromatography (GLC) on 50 M × 0.25 mm I.D. capillary column containing bonded 007 CPS phase. This column resolved positional isomers with the same configuration (i.e., 18:1Δ9c and 18:1Δ11c) normally present in biological tissues and fluids, but would not resolve a mixture of *cis* and *trans* isomers resulting from the partial hydrogenation of vegetable oils. The *cis* and *trans* isomers were first separated by argentation TLC before quantifying by GLC. Column temperature was programmed from 140 to 230° C at 2° C/minute and the data were collected with an IBM model 9000 laboratory computer (Danbury, CT USA). 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.[22]

Direct radioimmunoassay kits from Biotecx (Houston, TX USA) were used to measure thromboxane B_2 and 6-keto-prostaglandin $F_{1\alpha}$, stable metabolic products of thromboxane A_2 and prostacyclin. The amount of ^{125}I-labeled eicosanoid bound by antisera and precipitated is inversely proportionate to the concentration of the eicosanoids present in plasma.

Statistical analysis

Statistical analyses were performed using SAS (Cary, NC USA) statistical programs.[23] The data are expressed as mean ± a standard deviation and significance at $P < 0.05$ noted unless specified otherwise. Analysis of variance was performed by using the general linear models procedure and Tukey test for multiple pairwise comparisons.

Results

Diet energy and composition

The average protein, carbohydrate, fat, cholesterol content, and energy level consumed by 29 participants on

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

Diet/ test fat	Composition[a]				
	Energy (kJ)	Protein (g)	CHO (g)	Fat (g)	Chol. (mg)
Baseline	9,562 ± 2,334	91 ± 23	264 ± 70	96 ± 30	328 ± 132
BU	9,863 ± 1,513	87 ± 16	286 ± 48	96 ± 19	372 ± 73
CPO	10,240 ± 2,233	87 ± 21	302 ± 66[b]	99 ± 27	282 ± 93[c]
PAR	10,027 ± 2,020	91 ± 17	281 ± 66	101 ± 23	243 ± 74[b,c]
RPO	10,387 ± 1,605	92 ± 21	290 ± 47	106 ± 22	262 ± 90[b,c]
SPO	9,834 ± 2,166	88 ± 19	276 ± 67	99 ± 25	273 ± 139[c]
SUN	10,261 ± 2,154	93 ± 22	287 ± 57	103 ± 28	269 ± 113[b,c]

[a]See *Table 1* for abbreviations. Additional abbreviations are: CHO = carbohydrates; Chol. = cholesterol.
[b]Significantly different from baseline ($P < 0.05$).
[c]Significantly different from butter ($P < 0.05$).

Table 3 A comparison of the major nutrients in the various diets

Diet/ test fat	Energy percentages		
	Protein	Carbohydrates	Fat
Baseline	16.2 ± 2.8	46.4 ± 6.9[b]	37.4 ± 5.8
BU	14.7 ± 1.5[a]	48.7 ± 4.2	36.6 ± 3.8
CPO	14.3 ± 1.8[a]	49.6 ± 4.6	36.1 ± 3.8
PAR	15.4 ± 1.9	46.8 ± 3.1[b]	37.8 ± 2.7
RPO	14.9 ± 2.3[a]	46.9 ± 4.6[b]	38.2 ± 3.6
SPO	15.2 ± 2.4	46.9 ± 4.1[b]	37.9 ± 3.6
SUN	15.1 ± 1.6	47.2 ± 4.4	37.7 ± 3.7

[a]Significantly different from baseline ($P < 0.05$).
[b]Significantly different from CPO ($P < 0.05$).

each of the six diets and baseline diet are given in *Table 2*. There were no significant differences in protein, fat, or energy intake for any diet. Subjects consumed more carbohydrates on the CPO diet than the other diets. This was due to most participants trying to consume as much as possible of this fat from the more palatable cookies. As might be expected, CPO, PAR, RPO, SPO, and SUN diets contained less cholesterol than the habitual or butter diets.

The average percentages of energy contributed to each of the diets by protein, carbohydrate, and fat are given in *Table 3*. Dietary protein accounted for 14–16% of the energy in the diet. BU, CPO, and RPO contained slightly less protein than the baseline diet. Carbohydrates, the major source of energy in all diets, was elevated significantly in the CPO diet over baseline, PAR, RPO, and SPO diets. Fat accounted for 36–38% of the energy in all diets, slightly less than the 40% goal.

Although the USDA handbook and the data base of the Nutripractor system did not contain the fatty acid composition of all the food items in the diets, the sum of the fatty acids from these sources accounted for 78–82% of the total fat in the test fat diets and 63% of the total fat in the habitual fat diet. Actual accountability is approximately 10% higher because the fatty acid percentages do not include glycerol, glycerol phosphoryl bases, sterols, etc., present as components of the total fat. The average daily consumption of the major dietary fatty acids found in each test fat diet and habitual diet is given in *Table 4*. Butter and baseline diets contained significantly more myristic acid than the other diets. Baseline, PAR, and SUN diets contained half the quantities of palmitate (16:0) as diets containing palm oil (CPO, RPO, and SPO). All the diets provided more than 24 grams of octadecenoate monoenes daily. SPO and SUN provided 7% and 14% of the energy in the diet as polyunsaturated fatty acids whereas butter, CPO, PAR, and PRO diets provided 3–4%.

Serum lipids

The average concentration of total lipids and phospholipids in the serum of the subjects was 9.9 ± 2.5 and 2.4 ± 0.6 g/L, respectively, for habitual diets. Mean 5th and 6th week values for test fats ranged from 9.1 ± 2.4 for butter to 8.1 ± 1.2 g/L for SUN total lipids, and 1.8 ± 0.4 to 1.7 ± 0.4 g/L for phospholipids. Except for butter total lipids, all test fat diet average total lipid and phospholipid values were significantly lower than baseline values. Although our total lipid and phospholipid concentrations are somewhat higher than reported earlier by Shore et al,[24] our data are in agreement with theirs, showing a decrease in both fractions with an increased proportion of polyunsaturated fatty acids in the diet.

The average fatty acid compositions of the serum lipids after 5 and 6 weeks on the various test fat diets are given in *Table 5* along with the mean of 12 habitual diet values. Palmitate, a major serum lipid fatty acid, accounted for 22–26% of the fatty acids on all diets. Serum from all diets except PAR contained more palmitate than habitual diets. All diets except SUN produced a 19–20% C-18 monoene level in the serum. Likewise, the serum lipids from all subjects on all diets except SUN contained 26–28% linoleic acid. The SUN diet serum lipids contained a significantly higher percentage of linoleate than the serum from other diets.

Mean 5th and 6th week serum lipid, lipoprotein, and apolipoprotein values from participants on the various dietary fats were compared with the mean of two serum baseline values *(Table 6)*. Baseline data represent the mean of all participant values taken just prior to the start of a diet by the different groups of participants at

Table 4 Minimal quantities of the major fatty acids consumed daily for each diet as determined from diet records

Diet/ test fat*	Grams of fatty acid consumed/day					
	14:0	16:0	18:0	18:1 total†	18:1 *trans*‡	18:2
Baseline	2.2 ± 1.2	11.8 ± 5.2	5.3 ± 2.5[b]	27.0 ± 10.1		10.8 ± 4.5[f,g]
BU	7.1 ± 2.2[a]	20.9 ± 5.2[a]	8.4 ± 2.5	24.1 ± 5.0	0.6	7.7 ± 2.7[f,g]
CPO	0.9 ± 0.5[a,b]	24.3 ± 9.4[a]	4.4 ± 1.5[b]	36.1 ± 12.2[a,b]		10.7 ± 3.2[f,g]
PAR	1.2 ± 0.7[a,b]	12.3 ± 3.4[b,c]	6.6 ± 1.9[c]	43.8 ± 12.9[a,b]	7.9	12.6 ± 3.3[f,g]
RPO	1.0 ± 0.7[a,b]	29.9 ± 7.6[a,b,c,d]	5.4 ± 1.5[b]	37.2 ± 8.4[a,b]		10.8 ± 2.6[f,g]
SPO	0.9 ± 0.8[a,b]	20.9 ± 6.0[a,d,e]	4.9 ± 1.7[b]	30.0 ± 8.5		18.4 ± 5.0
SUN	1.0 ± 0.7[a,b]	10.9 ± 3.9[b,c,e,f]	5.5 ± 2.0[b]	24.3 ± 8.1[c,d,e]		38.0 ± 11.9[f]

[a]Significantly different from baseline.
[b]Significantly different from butter.
[c]Significantly different from CPO.
[d]Significantly different from Parkay.
[e]Significantly different from RPO.
[f]Significantly different from SPO.
[g]Significantly different from SUN.
$P < 0.001$.
*See text for abbreviations.
†Total of all 18:1 fatty acids including the *trans* isomers.
‡Quantities of 18:1 *trans* fatty acids consumed from the test fats in addition to the *trans* fatty acids in non-test fat foods common to all diets.

Table 5 Fatty acid composition of serum of pooled weeks 5 & 6 for the various dietary fats and 12-point baseline

Diet/ test fat†	Fatty acid percentages*						
	14:0	16:0	16:1	18:0	18:1	18:2	20:4
B-line	1.2 ± .3	22.6 ± 1.4	2.0 ± .4	6.0 ± .6	18.8 ± 1.8	28.0 ± 2.3	4.0 ± .8[b]
BU	1.3 ± .4	24.3 ± 2.0[a]	1.8 ± .6	6.9 ± .9[a]	19.6 ± 2.4	28.4 ± 2.9	4.7 ± 1.2
CPO	1.1 ± .3[b]	25.3 ± 2.7[a]	1.9 ± .5	6.4 ± 1.1[b]	20.6 ± 2.7[a]	26.6 ± 3.9	4.0 ± 1.3[b]
PAR	1.0 ± .3[a,b]	22.3 ± 2.6[b,c,d]	1.6 ± .5[a]	6.0 ± .9[b]	19.6 ± 2.4[d]	28.8 ± 3.6[c,d]	4.3 ± 1.0
RPO	1.0 ± .4[b]	26.0 ± 2.7[a,b]	1.7 ± .4	6.5 ± .8	20.9 ± 3.0[a]	26.6 ± 3.9	4.0 ± 1.0[b]
SPO	1.0 ± .3[a,b]	25.3 ± 2.8[a,e]	1.6 ± .4[a]	6.7 ± .9[a,e]	19.9 ± 2.8	28.7 ± 3.8[c,d]	4.1 ± 1.1[b]
SUN	1.1 ± .5	23.1 ± 2.3[c,d,f]	1.5 ± .5[a,b,c,d]	7.0 ± 1.1[a,c,d,e]	16.8 ± 2.4[a,b,c,d,e,f]	32.4 ± 4.6[a,b,c,d,e,f]	4.2 ± 1.0

*The difference between the sum of any rows and 100 represents minor amounts of other fatty acids not seen in table.
†See text for diet abbreviations.
[a]Significant difference from B-line.
[b]Significant difference from BU.
[c]Significant difference from CPO.
[d]Significant difference from RPO.
[e]Significant difference from PAR.
[f]Significant difference from SPO.

different times of the year. Total serum cholesterol was reduced significantly in the participants consuming the SUN diet, but unfortunately HDL cholesterol was also reduced significantly. Unexpectedly, butter did not significantly elevate total serum cholesterol relative to habitual baseline values. The CPO diet reduced LDL-cholesterol significantly. Triglyceride levels were not affected significantly by any dietary fat. Apolipoprotein A1 was reduced significantly on the SUN diet. Serum apolipoprotein B, the major apoprotein of the LDL fraction, was reduced significantly in BU, CPO, RPO, and SUN diets.

Averaged lipid, lipoprotein, and apolipoprotein values from the mean of 5th and 6th week serum levels from 29 participants on six different dietary test fats and an average of 12 habitual diet values are compared in *Table 7*. Participants on the SUN diet had their total serum cholesterol reduced significantly relative to all diets except PAR. The RPO diet elevated HDL-cholesterol significantly relative to PAR and SUN diets. SUN LDL-cholesterol values were significantly lower than butter and SPO levels. CPO triglycerides were elevated significantly (0.16–0.21 mmol/L) relative to baseline, butter, PAR, SPO, and SUN values. The butter diet reduced apolipoprotein A1 levels relative to baseline, CPO, RPO, and SPO diets. Apolipoprotein A1 values from the RPO diet were increased relative to the SUN diet. Both PAR and SUN diets reduced apolipoprotein B relative to baseline values.

The effect of the dietary test fats on plasma thromboxane B_2 (TXB_2) and 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) relative to baseline values is given in *Table 8*. Values represent the means of 9–12 individuals on each diet. No participants were tested for TXB_2 and 6-keto-$PGF_{1\alpha}$ levels on more than three dietary test fats. Only the SUN diet elevated TXB_2 level, but not significantly

Table 6 A comparison of mean fifth and sixth week serum lipid concentrations with two point-baseline values for various dietary fats consumed by free living males

Dietary test fats	Lipid class concentrations (mmol/L)				(g/L)[a,b]	
	Total Chol.	HDL Chol.	LDL Chol.	Trig.	Apo A-1	Apo B
Baseline	5.09 ± 0.85	1.06 ± 0.18	3.44 ± 0.78	1.33 ± 0.64	1.19 ± 0.16	0.73 ± 0.16
BU	5.17 ± 0.88	1.03 ± 0.18	3.52 ± 0.80	1.39 ± 0.71	1.15 ± 0.20	0.69 ± 0.13*
Baseline	5.22 ± 0.96	1.03 ± 0.21	3.49 ± 0.90	1.55 ± 0.82	1.23 ± 0.16	0.76 ± 0.14
CPO	5.15 ± 0.90	1.03 ± 0.21	3.36 ± 0.88*	1.61 ± 0.91	1.21 ± 0.18	0.71 ± 0.11*
Baseline	5.04 ± 0.75	1.03 ± 0.18	3.39 ± 0.67	1.44 ± 0.70	1.22 ± 0.19	0.71 ± 0.13
PAR	4.99 ± 0.72	1.00 ± 0.21	3.36 ± 0.63	1.40 ± 0.61	1.20 ± 0.17	0.68 ± 0.15
Baseline	5.15 ± 0.78	1.03 ± 0.21	3.47 ± 0.67	1.43 ± 0.60	1.25 ± 0.18	0.75 ± 0.14
RPO	5.15 ± 0.80	1.06 ± 0.23	3.41 ± 0.83	1.54 ± 0.88	1.24 ± 0.18	0.70 ± 0.13*
Baseline	5.04 ± 0.80	1.03 ± 0.21	3.41 ± 0.70	1.34 ± 0.61	1.23 ± 0.14	0.73 ± 0.13
SPO	5.12 ± 0.85	1.03 ± 0.21	3.41 ± 0.83	1.44 ± 0.77	1.23 ± 0.15	0.73 ± 0.17
Baseline	5.04 ± 0.88	1.06 ± 0.21	3.34 ± 0.75	1.41 ± 0.52	1.24 ± 0.17	0.73 ± 0.15
SUN	4.84 ± 0.72*	1.00 ± 0.18*	3.23 ± 0.65	1.33 ± 0.46	1.18 ± 0.15*	0.66 ± 0.13*

[a]See text for abbreviations.
[b]Values marked with an asterisk are significantly different from the corresponding baseline values at the 95% probability level or higher.

Table 7 A comparison of a twelve sample pooled baseline value and the mean of the fifth and sixth week serum lipid concentrations from normal free living males consuming various dietary fats

	Dietary test fat values*†						
	B-line	BU	CPO	PAR	RPO	SPO	SUN
Total Chol. (mmol/L)	5.07 ± 0.80	5.17 ± 0.88	5.15 ± 0.90	4.99 ± 0.72	5.15 ± 0.80	5.12 ± 0.85	4.84 ± 0.72[a,b,c,e,f]
HDL chol. (mmol/L)	1.03 ± 0.18	1.03 ± 0.18	1.03 ± 0.21	1.00 ± 0.21[e]	1.06 ± 0.23	1.03 ± 0.21	1.00 ± 0.18[e]
LDL chol. (mmol/L)	3.39 ± 0.70	3.52 ± 0.80[g]	3.36 ± 0.88	3.36 ± 0.65	3.41 ± 0.83	3.41 ± 0.83[g]	3.23 ± 0.65
Trig. (mmol/L)	1.40 ± 0.63[c]	1.39 ± 0.71[c]	1.61 ± 0.91	1.40 ± 0.61[c]	1.54 ± 0.88	1.44 ± 0.77[c]	1.33 ± 0.46[c,e]
Apo A-1 (g/L)	1.2 ± 0.13[b]	1.15 ± 0.20	1.21 ± 0.18[b]	1.20 ± 0.17	1.24 ± 0.18[b]	1.23 ± 0.15[b]	1.18 ± 0.15[e]
Apo B (g/L)	0.73 ± 0.13	0.69 ± 0.13	0.71 ± 0.12	0.68 ± 0.15[a]	0.70 ± 0.13	0.73 ± 0.17[d]	0.66 ± 0.13[a,c,f]

*See text for abbreviations.
†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 BU.
[c]Significantly different from CPO.
[d]Significantly different from PAR.
[e]Significantly different from RPO.
'Significantly different from SPO.
[g]Significantly different from SUN.

Table 8 Effect of dietary fat on plasma thromboxane and prostaglandin levels

Diet test fat	Concentration (pg/mL plasma)[a]			
	Thromboxane B_2		6-Keto-prostaglandin $F_1\alpha$	
	Baseline	Week 5 & 6	Baseline	Week 5 & 6
Butter	36 ± 12	35 ± 17	110 ± 18	89 ± 27[b]
CPO	39 ± 17	41 ± 22	108 ± 16	94 ± 22
PAR	38 ± 11	40 ± 19	111 ± 21	86 ± 18[b]
RPO	38 ± 15	40 ± 23	106 ± 14	87 ± 20[b]
SPO	37 ± 14	36 ± 13	117 ± 20	100 ± 23
SUN	37 ± 15	62 ± 37	110 ± 21	95 ± 21

[a]Values represent the mean of 9–12 participants. Each participant value is the mean of three baseline measurements and the mean of the 5th and 6th week diet determinations.
[b]Significantly different from baseline at 95% confidence level.

because of the large standard deviation. The observed increase in the level of TXB_2, an indicator of increased platelet aggregation, without a significant change in 6-keto-$PGF_{1\alpha}$ levels, an indicator of antiaggregatory activity, is not consistent with the increased clotting time observed in animals fed polyunsaturated fatty acids.[25] The significant decrease in 6-keto-$PGF_{1\alpha}$ levels on the butter, PAR, and RPO diets, without a significant decrease in TXB_2 levels, is consistent with animal studies showing that saturated dietary fatty acids reduce clotting time, but is inconsistent with results indicating palm oil acts more like unsaturated than saturated fatty acid.[25] The reduced level of 6-keto-$PGF_{1\alpha}$ on the margarine diet may be the result of the *trans* fatty acids present. Too much emphasis should not be placed on these results until additional experiments have been conducted under more controlled conditions. We observed large differences between baseline values and between 5th and 6th week values for both TXB_2 and 6-keto-$PGF_{1\alpha}$ determinations. TXA_2 and PGI_2 levels are apparently more sensitive to other influences than dietary fat. Agren et al.[26] described limitations in activity, avoidance of common pain drugs, and the need for sedentary blood collecting procedures required for reproducible results. Those restrictions were inconsistent with the unrestricted free-living conditions of our participants in this study and may have contributed to large variations encountered.

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 energy from the test fats. The average compliance for each diet was 18–20% ± 5–6% of energy. When total serum cholesterol concentrations were compared for various levels of compliance [>15%, >11%, all subjects, and minus responders (+0.25 mmol/L on butter and −0.25 mmol/L on SUN relative to baseline)] for each test fat diet, only those with a compliance of >15% of energy on only the SUN diet had significantly lower cholesterol values (4.63 mmol/L) from the other levels of compliance (4.81–4.91 mmol/L). The level of compliance and the removal of responders made little difference on the other diets, especially when compared with the baseline that showed some change. Disappearance data on subjects' test fat foods were consistent with the excellent compliance obtained from diet records. Compliance of those on crude palm oil was easy to evaluate as most participants' skin turned yellow from the high level of carotenoids in the oil. This was our least palatable diet, which was made more acceptable after generous use of lemon and orange flavor extracts. It must be concluded that participants this dedicated to the consumption of this diet would also have excellent compliance on the other test fat diets. We feel the high level of compliance and low drop-out figure (one participant) is attributable to the use of faculty and staff who understand the importance of following a protocol.

Incorporation of test fats into diets

The ability to substitute the test fats into the habitual diet without major changes in the basic nutrients is important in evaluating data from human dietary studies. Protein and carbohydrate percentages of the total energy intake were only slightly modified and the fat energy of all diets were not significantly different from habitual levels. Test fat levels represented 36–38% of diet energy, while 37% of the energy came from fat in the habitual diets *(Table 3)*. While the level of fat energy in the test diets remained unchanged from habitual fat levels, we were able to dramatically change the types of fat in the diet: an objective of the study.

The question regarding the fatty acid composition of the remaining 50% of the dietary fat after the incorporation of the test fats into the diets was examined. From the quantities of fat consumed on each diet *(Table 2)*, the quantities of test fat consumed (50% of the total), and the composition data of the test fats *(Table 1)*, the quantities of saturated, monounsaturated, and polyunsaturated fatty acids contributed by the test fats were calculated. The difference between the quantities of saturated, monounsaturated and polyunsaturated fatty acids, calculated from the percentages in the total diet *(Table 4)* (quantities given in Table 4 can not be used directly because of the differences in accountability between diets) and the quantities in the test fats gave an estimate of the saturated, monounsaturated, and polyunsaturated fatty acids contributed by the remainder of the diet. When these quantities were converted into percentages, the mean values of all six diets were 35.1 ± 5.5, 44.8 ± 5.2, and 20.1 ± 4.6% respectively, for the saturated, monounsaturated, and polyunsaturated fatty acids. The percentages agree well with the 34, 47, and 19% calculated for the saturated, monounsaturated, and polyunsaturated fatty acids from the baseline quantities given in *Table 4*. These comparisons indicate that the remaining fat in the diet not replaced by the test fats was similar to the fatty acid composition of the baseline or habitual diet. Calculations of data from a similar study, involving 40 participants and five dietary fats after 50% had been replaced with test fats, exhibited a distribution of saturated, monounsaturated, and polyunsaturated fatty acids similar to the habitual diet.[6]

Discussion

The level of test fat consumed by the participants (18–20% of total energy) in this study far exceeds the amount most individuals would consume from a single source on any dietary regimen. The extreme levels were used to elicit the largest possible change in serum lipids. Despite the high level of test fats consumed, the responses of serum lipids, lipoproteins, and apolipoproteins were unexpectedly small. The data discussed represents the mean of 5th and 6th weeks' values from 29 healthy participants that rotated through all diets. The exception is when an occasional data point is missing.

In addition to the usual comparisons between test

fats of most dietary fat studies, the present study allows comparisons to also be made with the customary or baseline diet. Seven-day diet records allowed the composition of the diet to be assessed, and the analyses prior to the start of each test diet period of two baseline serum samples, which had been preceded by 6 weeks of customary diet, provided reliable data for comparisons. The comparison of the test fat data with the baseline values provides the answer to an important question: how will my serum lipids respond to a particular type of fat relative to the diet I now eat? Each 12-point baseline value in *Table 7* represents nearly 350 determinations (12 each for 29 participants). The two-point baseline values in *Table 6* represent more than 50 determinations (two each for 29 participants). The two point baseline is a more refined comparison because the higher number of participants in the winter and spring seasons coupled with seasonal effects on serum lipid values are taken into account. The two-point baseline also takes into account changes that occurred nearest the time the experimental values were collected.

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. Current evidence suggests that one or more long-chain saturated fatty acids may decrease LDL receptor activity, which elevates LDL levels.[27,28] The assumption that serum lipid fatty acid composition reflects the composition of the dietary fat[29] becomes questionable when one sees how little the serum lipid concentration is affected by dietary fats. The contrast between the quantities of the dietary fatty acids consumed daily and the composition of the serum fatty acids after 5–6 weeks for various diets is shown in *Tables 4 and 5*. The level of palmitic acid in the diets ranged from 11 to 30 grams/day, but the percentage of palmitate in the serum lipid remained within a narrow range (22.3–26%). Likewise, the level of myristate, the suspected principal cholesterol-elevating saturated fatty acid in the diet,[30] varied up to seven fold between the diets, yet serum lipid levels were similar (1.0–1.3%). The percentage of unsaturated fatty acids in the serum is likewise resistant to change. The butter diet provided the lowest level of linoleate of any diet *(Table 4)*, yet serum lipid fatty acids from participants on this diet contained an equal or higher percentage of 18:2 than any diet except SUN. The SUN diet that provided three to four times more linoleate than the other diets elevated 18:2 percentages from 28 to 32.4%. However, despite the elevation of serum linoleate by the SUN diet, arachidonate levels were not elevated, whereas arachidonate percentages were the highest in serum lipids of participants on the butter diet, which had the lowest level of 18:2. The inability to change serum lipid by dietary manipulation, except small changes in monenes and dienes, has been noted by other investigators.[24,31,32] Recently Flynn et al.[29] reported that the characteristic fatty acid composition of serum triglycerides, cholesterol esters, and phospholipids were not changed significantly by diets containing 16% of total dietary fats as either butter or margarine. Analysis of lipid classes from LDL fractions has revealed some changes in the fatty acid composition of sterol esters and triglycerides, but diet had little effect on the fatty acid composition of phospholipids.[33]

Perhaps the most convincing evidence of the insensitivity of serum lipid fatty acid composition to diet is the lack of an effect by the *trans* fatty acids in the PAR diet. Based on our average compliance data, participants obtained 19.2% of their energy from the margarine. Because 26.2% of the fatty acids were *trans (Table 1)*, a minimum of 5% of the total energy in the diet was contributed by *trans* fatty acids. Careful inspection of the PAR serum lipid chromatograms from the capillary gas chromatograph, capable of resolving *trans* fatty acids,[22] failed to show any measurable quantities of *trans* fatty acids. In a similar study to the one described here, *trans* fatty acids represented 5.5% of the total energy of the diet, but only traces of these acids could be found in the serum lipids.[6] The absence of *trans* fatty acids, little or no change in saturated and C-20 polyunsaturated fatty acids, and only marginal changes in mono and diunsaturated fatty acids in the serum lipids after 6 weeks on a diet containing a high energy content of a specific type of fat illustrates the tight control of the serum lipid fatty acid composition.

Effects of dietary fats on serum lipids

The butter diet did not elevate total serum cholesterol or LDL cholesterol significantly relative to either baseline *(Tables 6 and 7)* in this group of middle-aged men. The degree of elevation of cholesterol in this study was surprisingly low compared with results with institutionalized subjects or patients on liquid formula diets[30,32,34–36] and approximately one-half the rise above pre-study values for a group of hypercholesterolemic men that consumed 34% of their energy as butter for 2 weeks.[37] In a similar study to the one described here that contained 38 participants, butter produced a small (5%), but significant rise in the total serum cholesterol and LDL-cholesterol relative to baseline and four other dietary fats.[6] However, not all studies have shown butter to elevate serum cholesterol. In a recent study conducted under free-living conditions in which subjects consumed two eggs per day and 5–6% of their energy as butter, normocholesterolemic and hypercholesterolemic males did not show a statistically significant rise in serum cholesterol after the first 12 weeks relative to entry values, and those that consumed butter the second 12 weeks after consuming margarine first either showed no change in serum cholesterol or a decreased level.[29] Both the butter-and-egg diet and the margarine-and-egg diet produced a significant rise in serum cholesterol in normocholesterolemic females. Serum apolipoprotein B, the major protein of LDL,[38] was reduced significantly on the butter diet relative to the two-point baseline, whereas LDL cholesterol was elevated but not significantly. This observation agrees with the results from our study involving 38 participants in which LDL

cholesterol was elevated significantly relative to a 10-point baseline, while apolipoprotein B values on the butter diet were reduced but not significantly.[6] Small decreases in apolipoprotein B values are possible when LDL cholesterol values increase because HDL-cholesterol and triglyceride values influence the calculated LDL cholesterol values.[15]

Total serum cholesterol in the participants on the palm oil diets, CPO and RPO, was unchanged from habitual or baseline values in this population of middle-aged men. These results are in agreement with all the results from studies in which palm oil was used in human dietary fat studies,[1] which showed that palm oil did not elevate cholesterol above pre-study habitual diet values. Consistent with palm oils not elevating total serum cholesterol levels, LDL-cholesterol was not elevated, instead the CPO diet produced a significant decrease in LDL cholesterol relative to the two-point baseline. Additionally, apolipoprotein B levels were reduced significantly in the subjects on both CPO and RPO diets relative to the two-point baseline. This observation is in contrast to the results of a recent study that showed palm olein increased apolipoprotein B levels by 9%.[39] The RPO diet apolipoprotein A1 levels were the highest of any diet, but were only significantly different from SUN and butter diets *(Table 7)*. These results are in agreement with an earlier study that showed palm olein increased apolipoprotein A1 values 11%.[39] Overall, if one considers the LDL:HDL cholesterol ratio as an indicator of the desirability of a dietary fat,[40,41] the ranking from the most desirable to the least desirable are: RPO, 3.22; SUN, 3.23; CPO, 3.26; B-line, 3.29; SPO, 3.31; PAR, 3.36; and BU, 3.42.

The addition of 20% sunflower oil to refined palm oil to give SPO did not improve the serum lipid profile of the RPO diet. Actually the resulting profile was more undesirable: the LDL:HDL cholesterol ratio above put SPO on the undesirable end of the ranking, while RPO was the most desirable. This observation provokes three comments. First, the results from the SPO diet illustrate the fallacy of evaluating dietary fat according to the level of saturated fatty acids they contain. *Table 4* shows that the SPO diet provided approximately 10 grams/day more polyunsaturated fatty acids and 10 grams/day less saturated fatty acids than the RPO diet, yet a more desirable serum lipid profile was observed on the RPO diet. Secondly, the results from the SPO diet illustrate that the lipid profile resulting from a mixture of dietary fats is not the sum of the individual parts. There appears to be interactions that have not been identified. Thirdly, the results of the lipid profiles resulting from these diets will hopefully prompt the expert panel[12] and the Council on Scientific Affairs[42] to reconsider their recommendations on reducing and eliminating certain foods and fats from the diet of the public.

The margarine, PAR, derived from partially hydrogenated vegetable oil, did not result in a significantly different serum lipid profile from the habitual diet with one exception. The PAR diet participants had a significantly lower apolipoprotein B level than the 12-point baseline value, whereas LDL cholesterol values were not changed significantly *(Table 7)*. There are several explanations for why apolipoprotein B and LDL cholesterol values do not show the same degree of change as expected and exemplified by the SUN diet values. First, LDL cholesterol is a calculated value that includes triglyceride values, which can be affected by dietary fat independently of cholesterol. Secondly, a type of dietary fat could change the ratio of cholesterol to apolipoprotein. Thirdly, dietary fat could change the level of very low density lipoproteins that also contain apolipoprotein B. PAR diet HDL-cholesterol values were significantly lower than RPO values. Early studies[34,43] questioned the use of partially hydrogenated fats, while other studies[44,45] indicated that the effect of partially hydrogenated fats on serum cholesterol differed little from the natural fat. More recent studies,[4,5] in which lipoprotein fractions were measured, showed HDL cholesterol was decreased and LDL cholesterol was increased relative to high *cis* mono- and poly-unsaturated diets. We did not observe the increase in LDL cholesterol, perhaps because of the lower percentages of *trans* fatty acids in the PAR diet. Another recent study in which cholesterol was included in the form of two eggs per day showed that margarine containing partially hydrogenated fat produced a significant rise in serum cholesterol of normocholesterolemic subjects relative to entry levels.[29]

The SUN diet, containing a high level of polyunsaturated fatty acids, caused a significant decrease in total serum cholesterol and apolipoprotein B levels relative to both baselines, but unfortunately it lowered HDL-cholesterol and apolipoprotein A1 significantly relative to the two-point baseline. HDL cholesterol and apolipoprotein A1 values on the SUN diet were significantly lower than RPO diet values. Several earlier studies[46–51] have reported that high levels of dietary polyunsaturated fatty acids produce a significant decrease in HDL-cholesterol. Serum SUN LDL-cholesterol levels were decreased significantly relative to butter and SPO diets, but not relative to baseline values. The SUN diet also lowered total serum cholesterol more than the other diets, as expected. The effects of the SUN diet on serum lipids and lipoproteins were very similar to the effects we obtained in a similar study using a soft margarine that did not contain partially hydrogenated fats.[6]

Effect of dietary fatty acid on serum lipids

This study was not designed to measure the effect of individual fatty acids on serum lipids, but some useful information has emerged. *Table 4* gives the minimal quantities of the major fatty acids that were consumed for each of the diets. Except for the butter and baseline diets, the greater than 75% accountability of the fat in the test fats by the major fatty acids shown in *Table 4* provides a reliable base for comparison (it is unlikely the unaccounted fat would be of such extreme composition as to affect the results significantly). Excluding stearate, which has no effect on serum lipids,[34,52] the quantities of saturates (14:0 + 16:0) consumed on SUN and PAR diets were similar. Likewise, the sum of the

18:1 and 18:2 consumed on the two diets was nearly equal. Any difference in the serum lipid profiles between the two diets can be attributed to the different proportions of mono- and poly-unsaturated fatty acids. However, because it has been shown that natural *cis* 18:1 and 18:2 fatty acids affect total serum cholesterol equally,[40,46,53] any difference in response must be attributed to the presence of the 26% *trans* fatty acids in the margarine. Although not quite statistically significant at the 95% level, SUN diet total cholesterol values were 0.15 mmol/L lower than the PAR diet. In a similar study with 38 participants, the difference between a hard margarine diet containing 29% *trans* fatty acids and the total serum cholesterol of a polyunsaturated diet was statistically significant.[6] Our data are also in agreement with two recent studies that showed diets containing *trans* monounsaturated fatty acids elevated total serum cholesterol values relative to natural *cis* 18:1 and 18:2 diets.[4,5]

HDL-cholesterol, reported to be decreased by high levels of poly-unsaturated fatty acids in the diet,[46–51] was significantly lower on both the SUN and PAR diets relative to the RPO diet *(Table 7)*. Because the PAR diet contained only one-third the level of polyunsaturated fatty acids as the SUN diet *(Table 4)* and natural *cis* monounsaturated fatty acids do not reduce HDL cholesterol levels,[52] the decreased level of HDL cholesterol in the PAR diet can be attributed to the *trans* fatty acids. This observation is in agreement with two earlier reports[4,5] that showed diets containing *trans* fatty acids reduced HDL cholesterol relative to both natural *cis* 18:1 and 18:2 diets. They also reported an increase in LDL cholesterol on the diets containing the *trans* fatty acids.

The similar effect on HDL cholesterol by two distinctly different types of fatty acids is most interesting. The poly-unsaturated fatty acids, predominantly linoleate, is a single component, whereas the *trans* fatty acid fraction is composed primarily of a mixture of positional octadecenoate isomers that have dramatically different physical properties from linoleate. Because many dietary fats contain the same natural fatty acids as found in the oils containing high levels of linoleate but do not reduce HDL-cholesterol, we can be reasonably confident that linoleate is responsible for lowering HDL-cholesterol. However, because partially hydrogenated fats contain 8–10 *trans* monounsaturated positional isomers, 6–7 unnatural *cis* monounsaturated positional isomers, and numerous geometrical and positional octadecadienoate isomers in small amounts, we are less confident that the *trans* fatty acids are responsible for lowering HDL-cholesterol. Because *trans* fatty acids represent the major products of partial hydrogenation, they are assumed to be the culprits. Even if the assumption is correct, we should remember that it is very unlikely that all the *trans* positional isomers reduce HDL-cholesterol equally. Given that both the *trans* fatty acids and linoleic acid reduce HDL-cholesterol when consumed at high levels, we are faced with explaining how compounds with dramatically different physical properties can have a similar effect on HDL-cholesterol. HDL is a macromolecule composed of 50% protein of multiple types and 50% lipid, of which cholesterol is one of many classes. Because it is likely that an altered metabolism of any of the components would affect HDL biosynthesis and composition, it is very conceivable that the two different types of dietary fatty acids could act independently to produce the same net effect.

Effect on dietary choices

This study indicates that the extreme measure of replacing half the fat in the diet with various types of fat, ranging from saturated to highly poly-unsaturated, has only marginal effects on the serum lipid profile of healthy middle-aged men when compared with their normal or habitual diet. Surprisingly, butter did not elevate total serum cholesterol, suggesting that the replacement of butter in the diet of healthy individuals is not likely to have a significant effect. Palm oil, often proclaimed to be absent from many manufactured foods, did not elevate serum cholesterol, even at these high levels. Actually palm oil-diet lipoprotein and apolipoprotein profiles were equal to or more desirable than the other test fats. The sunflower oil diet, high in poly-unsaturated fatty acids, reduced total serum cholesterol the most of any diet, along with reduced LDL-cholesterol and apolipoprotein B. Unfortunately, the desirable HDL-cholesterol and apolipoproteins A1 were also reduced. Despite the latter negative effects, individuals that must lower total serum cholesterol could expect the greatest effect from poly-unsaturated fatty acids. This recommendation is based on average values, but as we will show in a separate publication, this is not the best regime for all individuals. The addition of sunflower oil to palm oil did not result in an improved serum lipid profile, but gave a more undesirable LDL:HDL-cholesterol ratio. The hard margarine, produced by the partial hydrogenation of oils, did not improve the normal diet serum lipid profile, but an undesirable LDL:HDL-cholesterol ratio attributable to approximately 13% *trans* fatty acids in the diet was observed. These data, along with other recent studies, suggest that the *trans* fatty acids are not equal to the natural *cis* isomers regarding their effect on serum lipids. This should be taken into account in the consideration of food labeling in the future and in the interpretation of existing food labels. Presently the quantity of monounsaturated fatty acids listed for a food does not distinguish between *cis* and *trans* isomers. Food labels should clearly indicate the quantities of natural and unnatural monounsaturated fatty acids present for individuals who may wish to restrict their consumption of *trans* fatty acids. Although these data do not indicate that partially hydrogenated fats and oils represent a major health risk, they do raise the question of their continued use to replace fats containing saturated fats in processed foods and point to the need for additional research.

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