Impact of hydrogenated fat on high density lipoprotein subfractions and metabolism

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Abstract **Relative to saturated fatty acids, *trans*-fatty acids/hydrogenated fat-enriched diets have been reported to increase low density lipoprotein (LDL) cholesterol levels and either decrease or have no effect on high density lipoprotein (HDL) cholesterol levels. To better understand the effect of *trans*-fatty acids/hydrogenated fat on HDL cholesterol levels and metabolism, 36 subjects (female, n = 18; male, n = 18) were provided with each of three diets containing, as the major sources of fat, vegetable oil-based semiliquid margarine, traditional stick margarine, or butter for 35-day periods. LDL cholesterol levels were 155 ± 27, 168 ± 30, and 177 ± 32 mg/dl after subjects followed the semiliquid margarine, stick margarine, and butter-enriched diets, respectively. HDL cholesterol levels were 43 ± 10, 42 ± 9, and 45 ± 10 mg/dl, respectively. Dietary response in apolipoprotein (apo) A-I levels was similar to that in HDL cholesterol levels. HDL_2 cholesterol levels were 12 ± 7, 11 ± 6, and 14 ± 7 mg/dl, respectively. There was virtually no effect of dietary fat on HDL_3 cholesterol levels. The dietary perturbations had a larger effect on particles containing apoA-I only (Lp A-I) than apoA-I and A-II (Lp A-I/A-II). Cholesterol ester transfer protein (CETP) activity was 13.28 ± 5.76, 15.74 ± 5.41, and 14.35 ± 4.77 mmol × h^{-1} × ml^{-1}, respectively. Differences in CETP, phospholipid transfer protein activity, or the fractional esterification rate of cholesterol in HDL did not account for the differences observed in HDL cholesterol levels. These data suggest that the saturated fatty acid component, rather than the *trans*- or polyunsaturated fatty acid component, of the diets was the putative factor in modulating HDL cholesterol response.**—Lichtenstein, A. H., M. Jauhiainen, S. McGladdery, L. M. Ausman, S. M. Jalbert, M. Vilella-Bach, C. Ehnholm, J. Frohlich, and E. J. Schaefer. **Impact of hydrogenated fat on high density lipoprotein subfractions and metabolism. *J. Lipid Res.* 2001.** 42: **597–604.**

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Trans-fatty acids are geometric isomers of unsaturated fatty acids that contain at least one double bond in the *trans* configuration. The presence of a *trans* double bond is in contrast to the presence of a more commonly occurring *cis* form. *Trans*-fatty acids are found naturally at low levels in meat and dairy products as a result of bacterial fermentation in ruminant animals. *Trans*-fatty acids are rarely found naturally in plants. During the partial hydrogenation of vegetable oils, frequently done to increase stability and decrease viscosity for subsequent use in food products (i.e., commercially baked and fried foods, traditional stick margarine), some of the *cis* double bonds are converted to *trans* double bonds. Other changes that occur during the hydrogenation process include hydration of double bonds and the migration of some double bonds along the acyl chain, forming multiple positional isomers (1).

Dietary *trans*-fatty acids/hydrogenated fat have consistently been reported to raise low density lipoprotein (LDL) cholesterol levels (2–4). This effect appears to be somewhat proportional to intake (4) and is not related to increased rates of endogenous cholesterol synthesis (5, 6). In contrast, the reports of the effects of dietary *trans*-fatty acids on high density lipoprotein (HDL) cholesterol levels have been less consistent. In the early 1990s it was reported that a diet enriched in elaidic acid (18:1t), relative to oleic acid (18:1c), not only resulted in higher LDL cholesterol levels but also lowered HDL cholesterol levels (1). Subsequent findings with regard to the effect of *trans*-fatty acids on HDL cholesterol levels have been inconsistent, with some workers reporting a decrease in levels (2–4, 7, 8) and others reporting no effect (9–17). Taking into consideration the potentially adverse effects of lower HDL cholesterol levels on the LDL/HDL cholesterol ratio, Ascherio and colleagues (18) have recently suggested that

Abbreviations: FER_{HDL}, fraction esterification rate of HDL; PLTP, phospholipid transfer protein activity; CETP, cholesterol ester transfer protein activity; HDL, high density lipoprotein.

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the adverse effects of *trans*-fatty acids might be stronger than those of saturated fatty acids.

On the basis of this and prior concerns, we have now characterized the effect of two commercially available forms of vegetable oil based spreads, on HDL cholesterol levels and HDL subfractions in moderately hypercholesterolemic individuals: one minimally hydrogenated, semiliquid margarine, and one heavily hydrogenated, traditional stick margarine, relative to the animal fat, butter. We have also investigated possible changes in factors associated with the metabolism of HDL: cholesterol fractional esterification rate of HDL (FER_{HDL}), cholesterol ester transfer protein (CETP) activity, and phospholipid transfer protein (PLTP) activity.

METHODS

Subjects

Thirty-six subjects, 18 female (age range, 57–73 years) and 18 male (age range, 52–73 years), with LDL cholesterol levels greater than 130 mg/dl (3.36 mmol/l) were recruited from the greater Boston area for this study. All the subjects fulfilled the following criteria: having normal kidney, liver, thyroid, and cardiac function; having normal fasting glucose levels; taking no medications known to affect blood lipid levels; and being a nonsmoker. All females were postmenopausal and were not taking hormone replacement therapy. Subjects treated for hypertension were excluded only if they were using β-blocker therapy. This protocol was approved by the Human Investigation Review Committee of New England Medical Center and Tufts University, and informed consent was obtained from all subjects. An initial report of total, LDL, and HDL cholesterol levels has appeared (4). The current data focus on the HDL subfractions and associated metabolic factors.

Experimental design

The study subjects were provided with six experimental diets containing varying amounts of soybean oil-derived spread or butter, for periods of 35 days each, according to a Latin square design. Only the results of three of the phases, the two margarines with the greatest differentials in *trans*- and polyunsaturated fatty acid levels and butter, are reported here. Both the subjects and investigators were blinded as to the diet phase. Details of this protocol have been published previously (4). The mean caloric intake (mean + SD) was 2,114 ± 320 kcal in women and 2,792 ± 518 kcal in men. Fasting blood samples were obtained for lipid and apolipoprotein (apo) determinations three times after day 28 of each diet phase. The mean value of measurement at the three time points is reported and was used for statistical analysis.

Diets

All diets were designed to contain 15% of energy as protein, 55% as carbohydrate, and 30% energy as fat. Two-thirds of the total fat (20% of calories) was provided as semiliquid margarine sold in squeeze bottles (semiliquid), traditional margarine sold in sticks, or butter. The fat, protein, carbohydrate, and cholesterol content of diet homogenates made from each complete meal cycle (three days) for all diet phases was analyzed by Hazleton Laboratories (Covance; Laboratories America, Inc., Madison, WI). The fatty acid profile of the diets was determined by Lipton using capillary gas chromatography (Baltimore, MD).

Biochemical analysis

Fasting (14-h) blood samples were collected in tubes containing 0.15% EDTA. Plasma was separated by centrifugation at 1,100 *g* at 4°C. A modification of the dextran sulfate-magnesium chloride method was used to determine the concentration of HDL_2 and HDL_3. This allowed for the sequential precipitation of apoB containing lipoproteins (very low density lipoprotein [VLDL], intermediate density lipoprotein [IDL], and LDL), and then in a separate step, $HDL\text{-}_2$ (20, 21). Thus the level of HDL cholesterol was determined directly after the precipitation of VLDL, IDL, and LDL, and the level of HDL_3 cholesterol was determined after the precipitation of HDL_2 cholesterol, whereas the level of HDL_2 cholesterol was calculated as the difference between total HDL cholesterol and HDL_3 cholesterol. VLDL was isolated from plasma by ultracentrifugation at 109,000 *g*, 4°C, according to Lipid Research Clinics methodology (22).

Plasma, the 1.006 g/ml infranatant, HDL, and HDL_3 were assayed for total cholesterol and/or triglycerides with a Spectrum CCX bichromatic analyzer (Abbott Diagnostics, North Chicago, IL) using enzymatic reagents (23). Lipid assays were standardized through the Lipid Standardization Program of the Centers for Disease Control, Atlanta, GA.

Plasma apoA-I levels were measured by an immunoturbidimetric assay using a Spectrum CCX analyzer with reagents and calibrators from INCSTAR (Stillwater, MN) (24). Levels of apoA-II and apoAI in particles without apoA-II were measured by an electroimmunodiffusion technique using commercially available agarose gels with polyclonal anti-apoA-II incorporated into the gels (Laboratoires Sebia, France) (25). Levels of apoA-I in particles with apoA-I and A-II were calculated by difference. The coefficients of variation between runs for both measurements were 4% and 10%, respectively. Within each run, the coefficient of variation was approximately 4% for apoA-I and 7% for apoA-II.

Enzyme assays

Cholesterol ester transfer protein. The activity of CETP was measured in plasma after removal of endogenous VLDL and LDL by phosphotungstate and magnesium chloride precipitation, as described previously (26).

Phospholipid transfer protein. PLTP activity in plasma was quantified by assessing the transfer of radioactively labeled phosphatidylcholine (PC) in PC-liposomes to HDL_3 according to the method of Damen, Regts, and Scherphof (27), with minor modifications (28, 29).

Cholesterol esterification rate (FER_{HDL}). Fractional esterification rate was determined by an isotopic assay method (30, 31). ApoB-containing lipoproteins were precipitated from the serum with phosphotungstic acid and magnesium chloride. A trace amount of tritiated cholesterol was applied to a paper disk and incubated with the sample to allow for spontaneous transfer, and after incubation the radioactivity in the free and esterified cholesterol fractions was quantified. The FER_{HDL} was calculated as the percentage of label found in cholesteryl ester relative to the total radioactivity in the sample.

Statistical analysis

Prior to the analysis, descriptive statistics and graphs (PROC UNIVARIATE and PROC MEANS) (SAS, Cary, NC) were used to summarize the overall effects of diets and distributions of the outcome measures. When violations of the basic testing assumptions were noted, appropriate transformations of the data were made. An analysis of variance (PROC GLM) with main effect of diet and subject as repeated measures was carried out for each outcome measure, followed by a Tukey's honestly significant dif-

ference (HSD) type of adjustment for the pairwise comparisons between each of the three treatment regimens.

RESULTS

The characteristics of the study subjects at the time of recruitment are shown in **Table 1**. As dictated by the recruitment criteria, this group of individuals represents a subset of moderately hypercholesterolemic subjects for whom dietary modification would be the first intervention to normalize their blood lipid levels. The female subjects recruited for the study tended to have higher mean HDL cholesterol and lower mean total cholesterol/HDL cholesterol ratios than the male subjects.

The composition of the experimental diets, as determined by chemical analysis, is shown in **Table 2**. As the degree of hydrogenation increased from semiliquid to stick margarine, the relative proportion of *trans*-fatty acids increased, while the proportion of polyunsaturated fatty acids decreased. Relative to the other two diets, the butter-enriched diet had about double the proportion of saturated fatty acids and a lower proportion of polyunsaturated fatty acids. Likewise, the cholesterol content of the butter-enriched diet, characteristic of fats of animal origin, was approximately twice that of the other two diets.

The highest total and LDL cholesterol levels were observed after the subjects consumed the butter-enriched diet, lowest after they consumed the semiliquid margarine-enriched diet, and intermediate after they consumed the stick margarine-enriched diet (**Table 3**). In contrast, HDL cholesterol levels followed a distinctly different pattern: HDL cholesterol levels were 7% lower after the subjects consumed the stick margarine- than after the butter-enriched diet, whereas consumption of semiliquid margarine resulted in HDL cholesterol levels similar to those measured after consumption of the stick margarine. The difference between the stick margarine and butter diets was 11% in the female subjects and virtually nil in the male subjects (**Table 4**). The intake of the stick margarine and butter resulted in the highest, least favorable total cholesterol/HDL cholesterol ratio, whereas intake of the semiliquid margarine resulted in the lowest, most favorable total cholesterol/HDL cholesterol ratio. Not only were HDL cholesterol levels lowest, but triglyceride levels were highest, after subjects consumed the stick margarine-enriched diet.

The differences in HDL cholesterol levels were attributable mainly to changes in HDL_2 cholesterol rather than HDL_3 cholesterol (Table 4). As with total HDL cholesterol, HDL_2 cholesterol levels were highest after the subjects consumed the butter-enriched diet, lowest after subjects consumed the stick margarine-enriched diet, and similar to the stick margarine after they consumed

Table 1. Characteristics of the subjects at the time of recruitment[a]

Variable	Females (n = 18)	Males (n = 18)	All (n = 36)
Age, years	67 ± 4	60 ± 7	63 ± 6
Body mass index, weight (kg)/height^2 (m^2)	26.6 ± 2.4	28.1 ± 3.4	27.4 ± 3.0
		mg/dl	
Totalcholesterol[b]	253 ± 32	237 ± 33	245 ± 33
VLDL cholesterol	31 ± 13	28 ± 11	29 ± 12
LDL cholesterol	167 ± 30	167 ± 26	167 ± 28
HDLcholesterol	53 ± 11	42 ± 9	48 ± 11
Triglyceride	158 ± 71	138 ± 55	148 ± 64
TC/HDL cholesterol	4.96 ± 1.22	5.75 ± 1.07	5.36 ± 3.41

Abbreviations: VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; TC, total cholesterol

[a] Plus-minus values are means ± SD.

[b] To convert total, VLDL, LDL, and HDL cholesterol values to mmol/l, divide by 38.67. To convert triglyceride values to mmol/l liter, divide by 88.75.

Table 2. Composition of experimental diets as determined by chemical analysis[a]

	Semiliquid	Stick	Butter
		% energy	
Protein	17.07	16.73	16.94
Carbohydrate	51.73	53.54	53.97
Fat	31.20	29.72	29.08
SFA	8.59	8.47	16.70
12:0	0.96	0.82	2.50
14:0	0.74	0.60	0.14
16:0	4.26	4.03	7.47
18:0	1.85	2.22	3.57
MUFA	8.08	8.46	8.07
18:1	7.11	6.53	6.97
PUFA	13.54	6.34	2.43
18:2	12.10	5.60	2.07
18:3	1.39	0.70	0.29
trans	0.91	6.72	1.25
Cholesterol (mg/1,000 kcal)[b]	68	67	121

Abbreviations: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

[a] Unless otherwise indicated, values are expressed as percentages of total daily energy intake. Values are based on chemical analysis of duplicate diets.

[b] To convert values to amounts per mj, divide by 239.

Table 3. Serum lipid, lipoprotein, FL and apolipoprotein levels after 5 weeks of diets containing different forms of hydrogenated fat*

Variable	Semiliquid	Stick	Butter
Total cholesterol†	226 ± 32^c	243 ± 37^b	251 ± 36^a
VLDL cholesterol	28 ± 9^b	33 ± 15^a	29 ± 13^b
LDL cholesterol	155 ± 27^c	168 ± 30^b	177 ± 32^a
HDL cholesterol	43 ± 10^{a,b}	42 ± 9^b	45 ± 10^a
Triglyceride	133 ± 54^b	156 ± 68^a	146 ± 57^{a,b}
TC/HDL	5.47 ± 1.2^b	6.03 ± 1.27^a	5.85 ± 1.40^a

Superscript letters indicate statistically significantly differences from the numbers without common letters; $P < 0.05$. VLDL, very low density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; TC, total cholesterol.

* Plus-minus values are means ± SD. The 18 female and 18 male subjects consumed each diet in random order for 33 days. With the exception of the ratios, values are expressed as mg/dl.

† To convert values for total, VLDL, LDL, and HDL cholesterol to mmol/l, divide by 38.67. To convert values for triglyceride to mmol/l, divide by 88.54. Triglyceride data log transformed prior to statistical analysis.

Table 4. Serum HDL cholesterol, apoA-I and apoA-II levels and subfraction levels after 5 weeks of diets containing different forms of hydrogenated fat*

Variable	Semiliquid	Stick	Butter
HDL cholesterol†	43 ± 10^{a,b}	42 ± 9^{b}	45 ± 10^{a}
Females	47 ± 10^{b}	45 ± 9^{b}	50 ± 10^{a}
Males	40 ± 8	39 ± 8	40 ± 9
HDL_2 cholesterol†	12 ± 7^{a,b}	11 ± 6^{b}	14 ± 7^{a}
Females	14 ± 8^{a,b}	13 ± 6^{b}	17 ± 7^{a}
Males	10 ± 5	9 ± 4	10 ± 4
HDL_3 cholesterol†	31 ± 5	31 ± 5	31 ± 6
Females	32 ± 4	32 ± 4	33 ± 5
Males	30 ± 5	30 ± 5	30 ± 6
ApoA-I	145 ± 23^{b}	141 ± 21^{b}	151 ± 25^{a}
Females	152 ± 22^{b}	145 ± 21^{c}	159 ± 24^{a}
Males	138 ± 22	137 ± 20	142 ± 24
ApoA-I in LpA-I	42 ± 11^{a,b}	39 ± 11^{b}	43 ± 11^{a}
Females	45 ± 11^{a,b}	42 ± 13^{a,b}	45 ± 12^{a,b}
Males	38 ± 9	36 ± 7	40 ± 9
ApoA-I in LpA-I/A-II	102 ± 16	103 ± 18	107 ± 22
Females	105 ± 16^{a,b}	104 ± 17^{b}	113 ± 22^{a}
Males	99 ± 15	102 ± 20	102 ± 20
ApoA-II	30 ± 6	30 ± 6	31 ± 7
Females	31 ± 4	30 ± 5	31 ± 6
Males	30 ± 7	30 ± 6	32 ± 8
HDL-C/apoA-I	0.296 ± 0.041	0.295 ± 0.044	0.298 ± 0.043
Females	0.306 ± 0.042	0.307 ± 0.036	0.312 ± 0.039
Males	0.287 ± 0.040	0.283 ± 0.048	0.283 ± 0.043

Superscript letters indicate statistically significantly differences from the numbers without common letters; $P < 0.05$. HDL, high density lipoprotein; HDL-C, high density lipoprotein cholesterol.

* Plus-minus values are means ± SD. The 18 female and 18 male subjects consumed each diet in random order for 33 days. With the exception of the ratios, values are expressed as mg/dl.

† To convert values for HDL, HDL_2 and HDL_3 cholesterol to mmol/l, divide by 38.67.

the semiliquid margarine-enriched diet. The difference in HDL_2 cholesterol levels between the stick margarine and butter was 27% in the group as a whole, 31% in the female subjects, and not significant in the male subjects.

As with HDL cholesterol levels, apoA-I levels were lowest after the subjects consumed the stick margarine-enriched diet and highest after they consumed the butter-enriched diet (7%, stick margarine vs. butter), with the semiliquid margarine-enriched diet resulting in apoA-I levels intermediate between the two (Table 4). Although a trend was apparent in male subjects, these differences were statistically significant in women only (10%, stick margarine vs. butter).

We also quantified the HDL fraction on the basis of its apolipoprotein composition, separating the HDL particles with apoA-I only (LpA-I) from those with apoA-I and A-II (LpA-I/A-II). ApoA-I in LpA-I were lower after the subjects consumed the diet enriched in stick margarine compared with the diet enriched in butter, with levels intermediate after the subjects consumed the semiliquid margarine-enriched diet. Differences observed for the whole group were reflected in the data for the female and male subjects, yet only the former reached statistical significance. Differences in apoA-I in LpA-I/A-II attributable to diet were also observed, although the pattern was not as distinct as that for apoA-I in LpA-I, either for the group as a whole or when the data for women and men were analyzed separately. The differences reached statistical significance only for women. Variations in apoA-II levels were low and did not appear to be affected by diet in any consistent manner.

Owing to the parallel changes in HDL cholesterol and apoA-I levels, the ratios of HDL cholesterol/apoA-I were similar, regardless of dietary treatment. These data suggest that despite differences in the levels of HDL cholesterol and/or apoA-I induced by diet, it is unlikely that the composition of the particles was changed to a significant extent.

In an attempt to determine the mechanism by which the experimental diets altered HDL cholesterol levels, we measured the activity of proteins associated with HDL metabolism. No significant difference among the three diet phases was observed for FER_{HDL} (**Table 5**). PLTP activity was somewhat elevated in male subjects after they consumed the stick margarine-enriched diet and lowest after they consumed the butter-enriched diet, and was negatively correlated with HDL_2 levels (partial correlation = −0.356; $P = 0.0304$). CETP activity was highest after subjects consumed the stick margarine-enriched diet and lowest after the semiliquid margarine-enriched diet. CETP activities measured after the subjects consumed the butter-enriched diet were statistically indistinguishable from those measured after consumption of the semiliquid margarine-enriched diets.

Table 5. FER_{HDL}, PLTP activity, and CETP activity after 5 weeks of diets containing different forms of hydrogenated fat*

Variable	Semiliquid	Stick	Butter
FER_{HDL} (%/hour)	21.2 ± 5.7	21.6 ± 5.2	21.4 ± 5.2
Females	20.3 ± 6.1	20.8 ± 5.7	20.0 ± 5.2
Males	22.1 ± 5.3	22.4 ± 4.5	22.1 ± 5.0
PLTP (nmol × h^{-1} × L^{-1})	5,197 ± 1,617	5,648 ± 1,542	5,320 ± 1,611
Females	5,483 ± 1,844	5,772 ± 1,468	5,753 ± 1,779
Males	4,910 ± 1,345^{a,b}	5,523 ± 1,646^{a}	4,888 ± 1,334^{b}
CETP(nmol × h^{-1} × ml^{-1})	13.28 ± 5.76^{b}	15.74 ± 5.41^{a}	14.35 ± 4.77^{b}
Females	13.41 ± 7.17	15.21 ± 6.25	14.85 ± 5.29
Males	13.15 ± 4.09^{b}	16.27 ± 4.53^{a}	13.84 ± 4.29^{b}

Superscript letters indicate statistically significantly differences from the numbers without common letters; $P < 0.05$. FER_{HDL}, fraction esterification rate of HDL; PLTP, phospholipid transfer protein activity; CETP, cholesterol ester transfer protein activity.

* Plus-minus values are means ± SD. The 18 female and 18 male subjects consumed each diet in random order for 33 days.

DISCUSSION

The intake of hydrogenated fat and/or *trans*-fatty acids and saturated fatty acids has been associated with changes in LDL and HDL cholesterol levels consistent with increased risk of developing cardiovascular disease (19). In the present study we have attempted to characterize the changes in HDL cholesterol levels resulting from the substitution for butter of two commonly available forms of vegetable oil-based spreads, one lightly and one heavily hydrogenated, in moderately hypercholesterolemic individuals, and relate these changes to factors that alter HDL metabolism in circulation.

The magnitude of the difference in HDL cholesterol levels after the subjects ate the lightly and heavily hydrogenated margarine-enriched diets (degree of hydrogenation as estimated from *trans*- and polyunsaturated fatty acid levels) was not significant, as was predicted by some studies (2–4, 7, 8) but not others (9–17). Despite the small effect on HDL cholesterol and HDL subfractions observed with the two very different types of margarines, of note is the large effect on total and LDL cholesterol levels and, consequently, the total cholesterol to HDL cholesterol ratio. In contrast, the HDL levels were significantly higher after the subjects consumed the butter-enriched diet than they were after the two margarine-enriched diets, with the largest difference evident after the subjects consumed the stick margarine-enriched diet. From these data we can suggest that the major determinant of HDL cholesterol levels in the present study is the saturated fatty acid content of the diet, rather than either the *trans*- or polyunsaturated fatty acid content of the diet. Consistent with this finding, Judd et al. (3) have compared the effect of diets containing two different levels of *trans*-fatty acids (3.9% and 6.8% of energy) to diets high in either oleic acid or saturated fatty acids. They found no significant difference in HDL cholesterol levels between the two *trans*-fatty acid diets but lower levels than when people ate the high saturated fat diet.

Reduced levels of HDL_2 cholesterol have been associated with increased risk of developing cardiovascular disease (32). HDL_2, the larger cholesteryl ester rich fraction, rather than HDL_3, the smaller cholesteryl ester poor fraction, is the most responsive to alterations in environmental factors (34–37). This pattern was observed in the present study. HDL_2 cholesterol levels were lowest after the subjects consumed the margarine-enriched diets compared with the butter-enriched diet. There was little effect of diet on HDL_3 levels; hence the differences observed in HDL_2 cholesterol levels were the major determinant of HDL cholesterol levels. The pattern of the changes again suggests that the saturated fat component of the diets, rather than either the *trans*- or polyunsaturated fatty acid components of the diet, accounted for the differences in HDL_2 levels. These data are consistent with those reported by Judd et al. (3) and Aro et al. (38).

The distributions of LpA-I and LpA-I/A-II particles were also assessed on the basis of dietary fat modification. LpA-I particles are thought to be more effective than LpA-I/A-II particles in promoting cellular cholesterol efflux (39, 40). LpA-I levels followed a pattern similar to that of total apoA-I, that is, the lowest levels were displayed after the subjects consumed the stick margarine-enriched diet and highest after they consumed the butter-enriched diet. This pattern was less pronounced for apoA-I in LpA-I/A-II. As for both cholesterol in HDL_2 and HDL_3, and apoA-I in LpA-I and LpA-I/A-II, the response was more distinct in the female subjects than in the males.

CETP activity has a direct effect on the level of cholesterol in HDL and apoB containing lipoproteins by facilitating the transfer of cholesteryl esters out of HDL in exchange for triglycerides (41, 42). Hence, the activity of CETP could be responsible for the changes in HDL or LDL cholesterol levels observed. CETP activity was highest after subjects consumed stick margarine, the diet that resulted in the lowest HDL and intermediate LDL cholesterol levels, as compared with both the diet enriched in semiliquid margarine or the butter diet. This trend was observed in both the female and male subjects, although the difference in CETP activity, reached statistical significance only in the males. Notable is the greater variability in response of the female subjects, which may have confounded the analysis. Nevertheless, the data suggest that the differences in HDL cholesterol levels between the diets enriched in the two margarines and butter are not likely due solely to CETP activity.

Our observation with respect to the effect of hydrogenated fat/*trans*-fatty acids on CETP activity, is consistent with that in model systems. Enrichment of HDL with *cis*-fatty acids has been reported to inhibit CETP activity, whereas enrichment with *trans*-fatty acids has been reported to increase CETP activity (43). The results of two experiments involving humans suggested that CETP activity was higher after subjects consumed a diet rich in *trans*-fatty acid (18:1t) relative to one rich in a monounsaturated fatty acid (18:0c) (44), or a diet rich in polyunsaturated (18:2) or saturated (18:0) fatty acids (45). In contrast, Aro et al. (38) reported no significant effect of *trans*-fatty acids on CETP activity relative to diets high in saturated fatty acids, despite a significant effect on HDL cholesterol levels.

PLTP plays an important role in remodeling HDL, thereby facilitating the interconversion of HDL subpopulations (28, 29). Studies in PLTP transgenic mice, as well as studies in which the human PLTP gene has been transferred to mice by adenovirus technique, have demonstrated the role of PLTP in the generation of pre-beta HDL (46–49). Additionally, recent data from PLTP knock-out mice suggest that transfer of lipolytic surface remnants, mainly phospholipids from triglyceride-rich lipoproteins, into HDL is facilitated by PLTP and that this process is a major determinant of HDL levels (50). PLTP activity was highest after the subjects consumed the stick margarine-enriched diet. This was the experimental fat that resulted in the lowest levels of HDL cholesterol, although, paradoxically, in contrast to that observed for HDL cholesterol levels, the relationship was significant only in male subjects. However, a negative relationship of HDL_2 cholesterol levels with PLTP activity was observed.

Aro et al. (38) reported higher PLTP activities and lower HDL cholesterol levels after their subjects consumed a diet high in *trans*- relative to stearic acid. In a recent report concerning subjects from the Finnish cross-sectional study, we demonstrated a negative correlation between PLTP activity and HDL cholesterol levels (51). This potential factor awaits further investigation.

The FER_{HDL} is also a determinant of HDL cholesterol levels (52). The ability of lecithin:cholesterol acyltransferase (LCAT) to increase the content of HDL cholesteryl ester, specifically in HDL_2, facilitates the efflux of cholesteryl ester to catabolic sites, contributing to its antiatherogenic potential. In our study, no significant effect of diet on mean FER_{HDL} was observed. Previous work has resulted in contradictory findings with regard to the effect of *trans*-fatty acids on cholesterol esterification. Early work in rats suggested that *trans*-fatty acids either increased (53) or decreased (54) LCAT activity. In a cell-free system composed of phosphatidylcholine vesicles and LCAT purified from both rats and humans, *trans*-fatty acids were reported to inhibit LCAT, and this inhibition appeared to be due to the position that the *trans*-fatty acid occupied in the phosphatidylcholine vesicle, rather than changes in the fluidity of the substrate particle (55). Although there were no significant differences in this parameter in the present study, the possibility cannot be ruled out that the enzyme was affected by the concentration of *trans*-fatty acids in the sample. Such an inhibition may explain why the diet rich in *trans*-fatty acid did not result in higher FER_{HDL}, as would have been expected from the HDL_2 and HDL_3 ratios (52).

A limitation of this study is that we did not formulate diets to vary in a single fatty acid, but substituted commercially available forms of margarine and butter. This allowed us, albeit in an extreme situation, to mimic the actual effect of products currently available to individuals, especially those instructed to modify their diet to reduce their risk of developing cardiovascular disease (CVD), but did not allow us to attribute the changes observed to individual fatty acids. Another limitation of this study is the relatively narrow range of HDL cholesterol levels observed as a result of the dietary manipulations that hampered our attempts to precisely elucidate the mechanism by which dietary fatty acids, in this case saturated, polyunsaturated, and *trans*, alter HDL cholesterol levels. However, similar to the limitations stated above, we were able to determine the magnitude of the effect of substituting different forms of commonly available fats on lipoprotein levels, with specific emphasis on the HDL subfractions. The data generated from this study should be useful as a guide when making recommendations about choices of dietary fats specific to individual products or a mixture of different products.

In conclusion, our findings suggest that consumption of diets low in saturated and minimally hydrogenated fat results in the most favorable lipoprotein profile with respect to risk of developing CVD. The range of *trans*-fatty acids/hydrogenated fat, polyunsaturated fatty acids, and saturated fatty acids in the experimental diets, although broad with respect to that attainable even with extreme dietary patterns in the United States, may not have been sufficiently extreme to precisely define the mechanisms. However, the data suggest that the difference in the saturated fatty acid, rather than the *trans*- or polyunsaturated fatty acid, content of the diets was the major determinant of HDL cholesterol levels. The findings are consistent with the recommendation to restrict both saturated and hydrogenated fats.

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