Impact of Hydrogenated Fat Consumption on Endogenous Cholesterol Synthesis and Susceptibility of Low-Density Lipoprotein to Oxidation in Moderately Hypercholesterolemic Individuals

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The effects of replacing corn oil with corn oil margarine in stick form on endogenous cholesterol synthesis and susceptibility of low-density lipoprotein (LDL) to oxidation were assessed in 14 middle-aged and elderly men and women aged 63 ± 12 years (mean ± SD) with moderate hypercholesterolemia (mean LDL-cholesterol [LDL-C], 4.24 ± 0.59 mmol/L at the time of recruitment). Subjects consumed each of two diets for 32-day periods, one enriched in corn oil, which contained 30% of energy as fat (7% saturated fatty acid [SFA], 9% monounsaturated fatty acid [MUFA] [0.4% 18:1n9 *trans*], and 11% polyunsaturated fatty acid [PUFA]) and 85 mg cholesterol/4.2 MJ, and one enriched in stick corn oil margarine, which contained 30% fat (8% SFA, 12% MUFA [4.2% 18:1n9 *trans*], and 8% PUFA) and 77 mg cholesterol/4.2 MJ. Both diets were isocaloric and supplied by a metabolic research kitchen. Mean total cholesterol levels were lowest ($P = .039$) when subjects consumed the corn oil–enriched diet (5.01 ± 0.51 mmol/L) as compared with the margarine-enriched diet (5.30 ± 0.58 mmol/L). LDL-C levels were 3.24 ± 0.51 and 3.50 ± 0.54 mmol/L when subjects consumed corn oil– and margarine-enriched diets, respectively ($P = .058$). There were no significant differences in high-density lipoprotein cholesterol (HDL-C) or triglyceride concentrations between the two experimental periods. Consumption of the margarine-enriched diet versus the corn oil–enriched diet tended to result in lower cholesterol fractional synthetic rates ([C-FSRs] 0.0466 ± 0.0175 and 0.0668 ± 0.0298, respectively, $P = .080$) and cholesterol absolute synthetic rates ([C-ASRs] 1.1761 ± 0.5375 and 1.6954 ± 0.8685, respectively, $P = .092$); however, differences did not reach statistical significance. Consumption of the margarine-enriched diet versus the corn oil–enriched diet resulted in a significantly higher concentration of α-tocopherol in both plasma and LDL ($P = .004$ and $P = .011$, respectively). LDL particle size tended to be smaller after subjects consumed the margarine-enriched diet versus the corn oil–enriched diet ($P = .103$). Susceptibility of LDL to oxidation was similar after consumption of the corn oil– and margarine-enriched diets. These data suggest that an increased rate of endogenous cholesterol synthesis did not contribute to the higher plasma cholesterol concentrations during the period when subjects consumed the margarine-enriched diet. Therefore, the increase in cholesterol concentration resulting from margarine consumption was likely attributable, at least in part, to a decreased catabolic rate of cholesterol. Additionally, susceptibility of LDL to in vitro oxidation was not altered by consumption of hydrogenated fat.

IN RESPONSE TO longstanding recommendations to reduce the saturated fat content of the diet and to comply with current National Cholesterol Education Program guidelines,[1] there has been a shift in Western countries away from the use of animal fats toward the use of vegetable oils and products derived thereof such as margarines and partially hydrogenated commercial cooking fats.[2] While this trend has resulted in a decrease in consumption of saturated fat, especially from animal sources, there has been a reciprocal increase in hydrogenated fat intake and consequently *trans* fatty acid consumption.[2]

Hydrogenation transforms vegetable oils from a liquid to a semisolid state, resulting in products that are less susceptible to oxidation and have a higher viscosity, thus allowing them to be tailored to specific uses.[3,4] Early studies on the effects of *trans* fatty acid consumption in humans on plasma lipid concentrations were inconclusive.[4-8] Their consumption has recently come under scrutiny in light of newer reports suggesting that intake of *trans* double-bond–containing fatty acids or hydrogenated fat results in elevated plasma cholesterol concentrations relative to *cis* double-bond–containing fatty acids or unhydrogenated oils.[9-15] Recent epidemiological studies using food-frequency questionnaires to estimate *trans* fatty acid intake have suggested an association between consumption of *trans* double-bond–containing fatty acids and plasma cholesterol concentrations or risk of cardiovascular disease.[16-18] In contrast, studies using adipose tissue fatty acid patterns as a long-term indicator of *trans* fatty acid intake did not support an association with the risk of death from cardiovascular disease.[19-20]

The hydrogenation process results in multiple changes in the composition of fat. The proportion of saturated (SFA) and monounsaturated (MUFA) fatty acids increases with a concomitant decrease in the proportion of polyunsaturated

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fatty acids (PUFAs).[14] Additionally, some of the naturally occurring *cis* double bonds are isomerized to *trans* double bonds, and the position of some double bonds is shifted. These factors contribute to a change in physical characteristics of the acyl chain. Consumption of diets high in *trans* double-bond–containing fatty acids or hydrogenated fat clearly results in higher plasma cholesterol concentrations than diets high in *cis* double-bond–containing fatty acids or the naturally occurring oil. Limited evidence suggests that subgroups of people consuming diets high in *trans* fatty acids are at higher risk of developing cardiovascular heart disease than those consuming low levels.[16,18] Unclear to date are the actual mechanism(s) responsible for these changes; two possibilities are alterations in the rate of endogenous cholesterol synthesis and susceptibility of low-density lipoprotein (LDL) to become oxidized.

In humans, de novo cholesterogenesis accounts for up to 80% of whole-body cholesterol input and has been shown to be sensitive to dietary fatty acid composition.[21-24] We have previously reported that the cholesterol fractional synthetic rate (C-FSR) was lower after subjects consumed a diet enriched in SFA (beef tallow) versus PUFA (corn oil).[24] The effect of *trans* fatty acid intake on de novo cholesterogenesis is unknown.

There is evidence that oxidative modification of LDL enhances its atherogenic properties[25,26] and that this process occurs in vivo.[27,28] Oxidatively modified LDL is recognized by specific receptors on macrophages that do not recognize unmodified LDL.[29,30] This leads to development of foam cells, since activity of these receptors is not downregulated by the cholesterol content in macrophages.[25,31] Factors that affect the susceptibility of LDL to oxidation include the content of endogenous antioxidants,[32] particle size,[33-36] and fatty acid composition, the latter of which reflects the fatty acid composition of the diet.[36-38]

Given the current interest in the effects of *trans* fatty acid and hydrogenated fat intake on plasma cholesterol concentrations and incidence of atherosclerosis, it was the objective of the present investigation to determine whether consumption of stick corn oil margarine, a common hydrogenated fat product, impacts endogenous cholesterol synthesis rates and the susceptibility of LDL to oxidation in humans. The population studied was middle-aged and elderly women and men with moderately elevated LDL-cholesterol (LDL-C) concentrations, the group for whom dietary modification is frequently recommended. For this reason, the effect of hydrogenated fat consumption on plasma lipid concentrations and susceptibility of LDL to oxidation was studied within the context of a reduced-fat diet (30% of energy) consistent with the recommendations given to this study population.[1]

SUBJECTS AND METHODS

Subjects

Fourteen subjects (eight women and six men) with a mean age of 63 years (range, 44 to 78) were recruited to participate in this study as previously described.[14] The initial entrance criterion for this study was an LDL-C concentration greater than 3.36 mmol/L. Subjects reported no evidence of chronic illness, did not smoke, and were not taking medications known to affect plasma lipid concentrations (lipid-lowering drugs, β-adrenergic–blocking agents, diuretics, or hormones). All women were postmenopausal. Data sets for cholesterol synthesis rate measurements were incomplete in three subjects (two women and one man).

Experimental Design

The study consisted of three dietary phases of 32 days, separated by periods during which subjects consumed their habitual diets. Subjects were first provided with the baseline diet designed to approximate that currently consumed in the United States,[39] containing 15% of energy as protein, 49% as carbohydrate, and 36% as fat (15% SFA, 15% MUFA, and 6% PUFA) and approximately 128 mg cholesterol/4.2 MJ, which was used to stabilize study subjects at a defined level of fat and cholesterol intake. Subjects then ate each of two diets devised to conform to Step 2 guidelines, which were designed to provide 15% as protein, 55% as carbohydrate, and 30% as fat ($\leq$ 7% SFA, 10% to 15% MUFA, and $\leq$ 10% PUFA) and less than 85 mg cholesterol/4.2 MJ. In the latter two diets, one fifth or two thirds of total fat energy was provided as corn oil or corn oil margarine in stick form, so that the effect of consuming diets enriched in hydrogenated fat could be assessed within the context of current recommendations for individuals with elevated plasma lipid concentrations. However, the results of chemical analysis indicated that the margarine-enriched diet exceeded Step 2 guidelines for saturated fat by 0.73% (Table 1). All food and drink were provided by the Metabolic Research Unit of the Jean Mayer US Department of Agriculture Human Nutrition Research Center on Aging at Tufts University to be consumed on-site or packaged for takeout. Energy intakes of the subjects were tailored to individual requirements, as verified by the ability to maintain body weight. A portion of these data focusing on plasma lipid responses to experimental diets have been previously published.[14,40-42] We have previously reported that diet order had no effect on the outcome.[40]

During the final week of each dietary phase, four fasting blood samples were obtained for lipid, lipoprotein, and apolipoprotein determinations. Fractional (C-FSR) and absolute (C-ASR) synthetic rates of endogenous cholesterol were assessed 24 hours following oral administration of deuterium oxide (1.2 g/kg esti-

Table 1. Composition of the Study Diets (% of energy) as Determined by Chemical Analysis

Variable	Corn Oil	Corn Oil Margarine
Protein	17.4 ± 0.9	16.4 ± 1.6
Carbohydrate	53.3 ± 2.4	53.8 ± 2.5
Fat	29.4 ± 1.5	29.7 ± 1.13
SFA		
Total	6.90 ± 0.60	7.73 ± 0.13
12:0	0.09 ± 0.01	0.07 ± 0.01
14:0	0.40 ± 0.04	0.32 ± 0.01
16:0	4.33 ± 0.30	4.18 ± 0.09
18:0	1.60 ± 0.21	2.00 ± 0.02
MUFA		
Total	8.98 ± 0.64	12.16 ± 0.25
16:1n7	0.30 ± 0.08	0.23 ± 0.01
18:1n9	8.40 ± 0.55	11.65 ± 0.24
18:1 *cis*	9.62	7.49
18:1 *trans*	0.44	4.16
PUFA		
Total	11.21 ± 0.52	8.30 ± 0.16
18:2n6	10.67 ± 0.53	7.85 ± 0.18
18:3n3	0.40 ± 0.06	0.33 ± 0.01
20:4n6	0.05 ± 0.01	0.04 ± 0.01
Cholesterol (mg/4.2 MJ)	85 ± 4	77 ± 9

NOTE. Values are the mean ± SD; n = 3.

mated body water).[21-24] Both susceptibility of LDL to oxidation and endogenous cholesterol synthesis were assessed during the final week of the dietary periods.

Analytical Procedures

Fasting (14-hour) blood was collected in tubes containing EDTA (0.1%), and plasma was separated by centrifugation at 3,000 rpm at 4°C. Very-low-density lipoprotein ([VLDL] d < 1.006 g/mL) was isolated from plasma by ultracentrifugation at 39,000 rpm for 18 hours at 4°C. Plasma and the 1.006-g/mL infranatant fraction were assayed for total cholesterol and triglyceride with an Abbott Diagnostics (North Chicago, IL) Spectrum CCX biochromatic analyzer using enzymatic reagents as previously described.[43] High-density lipoprotein cholesterol (HDL-C) level was measured in the supernatant fraction after precipitation of apolipoprotein B–containing lipoproteins by a dextran sulfate–magnesium procedure.[44] Lipid assays were standardized through the Lipid Standardization Program of the Centers for Disease Control (Atlanta, GA). Within-run and between-run coefficients of variation for these assays were less than 2% for cholesterol and 2.5% for triglyceride.

α-Tocopherol concentration in plasma and LDL was analyzed by the reverse-phase high-performance liquid chromatography method.[45] Retinyl hexanoate in methanol was used as an internal standard. The mobile phase consisted of acetonitrile:dichloromethane:methanol:*n*-butanol (90:10:5:0.1 vol/vol/vol/vol) with 0.1% ammonium acetate. The high-performance liquid chromatography system was equipped with a Waters (Milford, MA) pump (flow rate, 1.5 mL/min), a 5-μm stainless steel Waters resolve column, an autosampler (Millipore, Bedford, MA), two absorbance detectors connected in series, and a dual-channel integrator. Analyte peaks were identified by retention times and quantified using the standard curve of the external standard.

Cholesterol Synthesis Determinations

Deuterium enrichments in body water and in combined free and esterified plasma cholesterol were determined as previously described.[21-24] Briefly, after lipid extraction and saponification, free cholesterol was quantitatively separated by thin-layer chromatography. Cholesterol was eluted from the silica and combusted at 520°C in Pyrex tubing in the presence of cupric oxide and sterling silver. Combustion-product carbon dioxide was removed while water was transferred by vacuum distillation into tubes containing 60 mg zinc reagent (Biogeochemical Laboratories, Indiana University, Bloomington, IN). In addition, plasma samples from the 0- and 24-hour time points were distilled into zinc-containing tubes to measure deuterium enrichment of plasma water. Water from cholesterol and plasma samples was combined with zinc shot and reduced to hydrogen gas at 520°C under vacuum in Pyrex tubing. Hydrogen gas deuterium enrichment was measured by differential isotope ratio mass spectrometry (903D; VG Isomass, Cheshire, England). The instrument was calibrated daily using water standards of known isotopic composition. Samples of each subject were analyzed using a single set of standards.

Calculation of Cholesterol Synthesis Rates

C-FSRs (pools per day) were determined from incorporation of precursor deuterium into plasma total cholesterol, relative to the maximum theoretical enrichment using the linear regression model. The following equation was used:

$$\text{C-FSR (pools per day)} = \frac{\delta \text{ cholesterol } (‰)}{\delta \text{ plasma water } (‰) \times 0.478},$$

where δ is the difference in deuterium enrichment over 24 hours. Assumptions underlying the use of labeled water as tracer for measurements of C-FSR and model consideration have been extensively described elsewhere.[22-25] C-ASRs (grams per day) were derived by multiplying C-FSR by cholesterol central pool size as calculated from the equation reported by Goodman et al.[46] Central pool size, ie, the rapid-turnover M_1 cholesterol pool, was derived as a function of body weight and plasma concentration of both cholesterol and triglyceride. C-ASR represents an approximation of the daily production of newly synthesized cholesterol.

LDL Isolation

LDL was isolated from plasma by modified single discontinuous density-gradient ultracentrifugation in a near-vertical rotor (NVT90; Beckman Instruments, Palo Alto, CA).[47] Plasma density was adjusted to 1.35 g/mL with KBr by layering it under 0.9% NaCl (pH 7.2) with phenylmethylsulfonyl fluoride (0.5 mmol/L) in Beckman Optiseal polyallomer tubes. Samples were centrifuged immediately in a Beckman L8-80 ultracentrifuge at 70,000 rpm at 4°C for 90 minutes. The LDL fraction was separated using a gradient fractionator (Hoefer Scientific Instruments, San Francisco, CA). Cholesterol concentration in samples was analyzed immediately after isolation using a modified enzymatic method previously described.[48]

Susceptibility of LDL to Oxidation

LDL was oxidized with hemin and H_2O_2 within 2 hours of isolation, as previously described.[49] Final assay concentrations were 0.56 mmol/L LDL-C, 5 μmol/L hemin, and 50 μmol/L H_2O_2 in 0.9% NaCl, in a final assay volume of 0.15 mL. Each sample was analyzed in quadruplicate. Oxidation of LDL was monitored by measuring the decreasing absorbance of hemin at 405 nm for 150 minutes at 90-second intervals using an MR600 Microplate reader (Dynatech Laboratories, Chantilly, VA). Resistance of LDL to oxidation is expressed as lag time to oxidation, which was assessed by calculating the time required for the reaction to reach maximum velocity.[50]

LDL Particle Size Determination

LDL particle size was calculated as previously described.[51] Briefly, LDL subclasses were separated using 2% to 16% polyacrylamide agarose gel electrophoresis (PAA 2-16%; Pharmacia, Piscataway, NJ). An LKB Ultrascan XL laser densitometer (LKB Instruments, Paramus, NJ) was used to scan the gels, and LKB GSXL software was used for peak integration. The LDL score represents the relative area under each LDL peak.[51] A smaller particle score corresponds to larger LDL particle size.

Statistical Analysis

Data were analyzed using a paired *t* test to assess statistical significance of dietary effects on the biochemical parameters measured. Because the data were not normally distributed, C-FSR and C-ASR values were logarithmically transformed before statistical analysis. Untransformed data are presented in the text and tables. Since responses in men and women were similar, data from both groups were pooled into a common group. Pearson's correlation coefficient was used to explore the relationship between variables. Results are expressed as the mean ± SD.

RESULTS

At the time of recruitment for this study, the mean age of the subjects was 63 ± 12 years. All subjects had total cholesterol and LDL-C concentrations that were in the borderline-high–risk or high-risk categories as defined by the Adult Treatment Panel (LDL > 3.36 mmol/L).[1] Mean total cholesterol, LDL-C, and HDL-C concentrations were 6.15 ± 0.62, 4.24 ± 0.59, and 1.24 ± 0.28 mmol/L,

respectively. As a group, subjects had an elevated body mass index (27.2 ± 4.3 kg/m^2), characteristic of a group of middle-aged and older subjects with elevated plasma cholesterol concentrations.

Composition of the study diets based on food analysis is shown in Table 1. There was generally good agreement between nutrient content as estimated from food tables and chemical analysis. Substitution of hydrogenated fat (stick corn oil margarine) for vegetable oil (corn oil) resulted in an approximately 10-fold increase in the concentration of *trans* double-bond–containing fatty acids in the diet. This was accompanied by higher concentrations of SFA and MUFA and lower concentrations of PUFA. All differences observed are consistent with the known effects of the hydrogenation process. α-Tocopherol content of the corn oil and margarine was 14.80 and 14.26 mg/100 g, respectively (University of Minnesota School of Public Health, Nutrition Coordinating Center). All other food components of the diets were identical. Therefore, it is assumed that the α-tocopherol intake of the subjects per 4.2 MJ was equivalent.

After consumption of the baseline diet before the two experimental diets, plasma lipid concentrations were similar to those observed during the recruitment period. Total cholesterol, VLDL-C, LDL-C, HDL-C, and triglyceride levels were 5.77 ± 0.83, 0.56 ± 0.19, 3.96 ± 0.76, 1.25 ± 0.27, and 1.22 ± 0.34 mmol/L, respectively.

During the period when subjects consumed the margarine-enriched diet, total cholesterol concentrations were 5.5% higher (P = .039) than during the period when subjects consumed the corn oil–enriched diet (Table 2). Similarly, during the period when subjects consumed the margarine-enriched diet, LDL-C concentrations were 9% higher (P = .058) than during the period when subjects consumed the corn oil–enriched diet. Concentrations of HDL-C were unaffected by the source of dietary fat within the context of the reduced-fat diets (P = .373). Together, these findings resulted in a total cholesterol to HDL-C ratio that was significantly higher after consumption of the margarine-enriched diet versus the corn oil–enriched diet (P = .037). Plasma triglyceride concentrations were not affected by consumption of the different diets.

With respect to cholesterol kinetics, C-FSR, ie, the proportion of the rapidly cycling pool of cholesterol synthesized per day, was higher after subjects consumed the corn oil–enriched diet versus the margarine-enriched diet (P = .080; Table 3). These data suggest that consumption of the corn oil–enriched diet resulted in a more rapid rate of cholesterol synthesis relative to the margarine-enriched diet, although the difference did not quite reach statistical significance by conventional standards.

Table 2. Plasma Lipid Concentrations (mmol/L) at the End of Each Study Phase

Variable	Corn Oil	Margarine	P
Total cholesterol	5.01 ± 0.51	5.30 ± 0.58 (6 ± 11)	.039
Triglyceride	1.25 ± 0.34	1.29 ± 0.34 (7 ± 31)	.588
VLDL-C	0.63 ± 0.11	0.69 ± 0.15 (12 ± 23)	.096
LDL-C	3.24 ± 0.51	3.50 ± 0.54 (9 ± 16)	.058
HDL-C	1.14 ± 0.24	1.11 ± 0.24 (−2 ± 8)	.373
Total cholesterol/HDL-C	4.59 ± 1.04	4.97 ± 1.17 (9 ± 14)	.037

NOTE. Values are the mean ± SD; n = 14. Mean percent change is shown in parentheses.

Table 3. C-FSR at the End of Each Dietary Period (pools per day)

Subject No.	Corn Oil	Margarine
1	0.0345	0.0268
2	0.0870	0.0548
3	0.0517	0.0277
4	0.0278	0.0345
5	0.1657	0.0319
6	0.0808	0.0636
7	0.0474	0.0515
8	0.0721	0.0670
9	0.0338	0.0217
10	0.0570	0.0820
11	0.0766	0.0515
Mean ± SD	0.0668 ± 0.0298	0.0466 ± 0.0175
P		.080

NOTE. All values were log-transformed before statistical analysis; arithmetic means are reported.

Mean central pool sizes of study subjects during the periods when they consumed the two diets were 24.24 ± 4.08 and 24.83 ± 4.48 g for the corn oil– and margarine-enriched diets, respectively. No differences in central cholesterol pool size were detected resulting from the experimental conditions as described.

When C-ASR was calculated, ie, the absolute amount of de novo cholesterol synthesized per day, a pattern similar to that seen for C-FSR was observed (Table 4). C-ASR was higher when subjects consumed the corn oil–enriched diet versus the margarine-enriched diet (P = .092), although differences approached but did not reach statistical significance.

α-Tocopherol concentration in plasma and LDL was higher after consumption of the margarine-enriched diet relative to the corn oil–enriched diet (Table 5). LDL particle score appeared to be slightly smaller after subjects consumed the margarine-enriched diet versus the corn oil–enriched diet, although differences did not reach statistical significance (P = .103). Despite differences in α-tocoph-

Table 4. C-ASR at the End of Each Dietary Period (g/d)

Subject No.	Corn Oil	Margarine
1	0.6572	0.5342
2	2.3803	1.4549
3	1.4991	0.8273
4	0.5610	0.6908
5	4.4933	0.9266
6	2.1960	1.5958
7	0.9197	1.0449
8	2.2017	2.2036
9	0.7719	0.5238
10	1.2456	1.8413
11	1.8855	1.2944
Mean ± SD	1.7101 ± 1.1357	1.1761 ± 0.5375
P		.0923

NOTE. All values were log-transformed before statistical analysis; arithmetic means are reported.

Table 5. α-Tocopherol Concentration in Plasma and LDL, LDL Particle Score, and Lag Time to Oxidation

	Corn Oil	Corn Oil Margarine	*P*
α-Tocopherol concentration			
Plasma (μmol/L)	20.90 ± 6.99	28.09 ± 4.74	.004
LDL (μg/mg LDL-C)	4.78 ± 1.37	5.94 ± 1.11	.011
LDL particle score	3.48 ± 0.66	3.75 ± 0.62	.103
Lag time (min)	93 ± 26	98 ± 23	.461

NOTE. Values are the mean ± SD; n = 14.

erol concentration between the two diet periods, susceptibility of LDL to oxidation did not differ between corn oil– and margarine-enriched diets. Plasma or LDL α-tocopherol concentrations were not correlated with LDL concentration in either of the diet phases. LDL particle score or α-tocopherol concentration in plasma or LDL did not correlate significantly with the lag time to oxidation in this study group.

DISCUSSION

Consumption of *trans* double-bond–containing fatty acids or hydrogenated fat has been reported to result in higher plasma cholesterol concentrations as compared with the corresponding *cis* double-bond–containing fatty acids or unhydrogenated fat.[9-15] The first intent of the present study was to determine whether, within the context of a diet meeting Step 2 criteria and in hypercholesterolemic individuals, hydrogenated fat influences circulating plasma cholesterol concentrations by perturbing rates of endogenous cholesterol synthesis. The second intent of the present study was to assess the impact of hydrogenated fat intake on the susceptibility of LDL to in vitro oxidation given the purported relationship between *trans* fatty acid intake and the incidence of cardiovascular heart disease and the atherogenic nature of oxidized LDL. The study design allowed for a direct comparison of hydrogenated stick margarine derived from corn oil to corn oil itself.

Increased plasma cholesterol concentrations can be the result of either an increase in the production rate or a decrease in the catabolic rate. C-ASR tended to be lower when study subjects consumed the margarine-enriched diet versus the corn oil–enriched diet. These data suggest that an increased rate of endogenous cholesterol synthesis did not contribute to the higher plasma cholesterol concentrations during the period when subjects consumed the margarine-enriched diet. In contrast, although not quite significant ($P = .080$), C-FSR was lower when study subjects consumed the margarine-enriched diet versus the corn oil–enriched diet. In steady-state conditions, which the subjects appeared to be in on the basis of stable plasma cholesterol concentrations during the period when cholesterol kinetic measurements were made, cholesterol synthetic rate can be assumed to equal the catabolic rate. Therefore, the increase in cholesterol concentration resulting from margarine consumption was likely attributable, at least in part, to a decreased catabolic rate of cholesterol. We did not specifically address the issue of in which lipoprotein fraction were endogenous cholesterol kinetics most affected. However, since the majority of change in circulating cholesterol concentration occurred in the LDL fraction, it is likely that this was the lipoprotein fraction most impacted by increased hydrogenated-fat consumption.

Previous study has suggested that the cholesterol-increasing properties of dietary hydrogenated fat may be due to a combination of factors, which include replacement of PUFA with SFA and MUFA, as well as to more specific effects of the *trans* bonds within acyl chains. Evidence for the replacement mechanism and a similar behavior of *trans* fatty acids to SFAs is seen in the findings of the present study. Both total cholesterol and LDL-C concentrations observed during the period when subjects consumed the margarine-enriched diet were higher than those observed during periods when subjects consumed corn oil–enriched diets. The predicted change in plasma total cholesterol concentrations when margarine was substituted for corn oil in the diet, estimated using the Keys[52] or Hegsted[53] equations, was similar to what was observed only when *trans* fatty acids were treated as SFAs.[14]

Independent direct actions of *trans* fatty acids on circulating cholesterol concentrations can be speculated to occur secondarily to conformational changes of the fatty acids. Displacement of *cis* double-bond– by *trans* double-bond–containing fatty acids may lead to changes in either the physicochemical properties of the lipoprotein particle or the membrane, such as fluidity, which would result in a decrease in the ability of the lipoprotein particle to bind to cell surface receptors, resulting in higher plasma cholesterol levels. This hypothesis is supported by the lower C-FSR, or fraction of endogenous cholesterol cycled, during the period when subjects consumed the margarine-enriched diet.

No significant difference in susceptibility of LDL to oxidation was observed after periods when study subjects consumed corn oil– and margarine-enriched diets. It is unclear whether the magnitude of the differences in fatty acid composition of the diet due to the hydrogenation process itself would manifest as differences in the susceptibility of LDL to oxidation. However, there were lower concentrations of α-tocopherol in both plasma and LDL after consumption of the corn oil–enriched diet versus the margarine-enriched diet, which may have been predicted to increase susceptibility of LDL to oxidation. The effect of α-tocopherol concentration in LDL on the susceptibility of LDL to oxidation is far more complex than initially thought. Although α-tocopherol has been reported to be the primary antioxidant in LDL[32] and intervention studies have demonstrated that α-tocopherol supplementation increases the resistance of LDL to oxidation,[54-57] it has also been reported that α-tocopherol in LDL acts as a pro-oxidant and increases the susceptibility of LDL to oxidation.[58-59] Furthermore, the susceptibility of LDL to oxidation does not appear to be related to α-tocopherol concentration in LDL in people who have not been taking α-tocopherol supplements.[60-62]

There is some evidence that small dense LDL particles are more susceptible to oxidation than large buoyant

particles.[33-36] In the present study, LDL particle score tended to be lower, indicating larger particle size after consumption of the corn oil–enriched diet versus the margarine-enriched diet. However, no correlation was observed between susceptibility of LDL to oxidation and LDL particle size.

In summary, our data suggest that replacing corn oil with corn oil margarine in stick form impaired the clearance of endogenously synthesized cholesterol. Additionally, within the context of a low-fat diet, the one-to-one substitution of hydrogenated fat for the unhydrogenated oil did not significantly impact the susceptibility of LDL to oxidation. Whether the former observation is attributable to the increase in *trans* fatty acid intake or a shift in the fatty acid composition of the diet as a result of hydrogenation to an increase in SFA and MUFA and a decrease in PUFA remains to be determined.

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