Hydrogenated fat consumption affects acylation-stimulating protein levels and cholesterol esterification rates in moderately hypercholesterolemic women

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Abstract **To determine whether hydrogenated fat consumption alters triglyceride metabolism and cholesterol esterification rates, 14 women (65–71 years of age) were provided with each of four diets for 5-week periods according to a randomized cross-over design. The experimental diets contained either soybean oil (SO), low *trans* squeeze (SQM), medium *trans* tub (TM), or high *trans* stick (SM) margarines. Triglyceride uptake by adipose tissue was determined by measuring plasma acylation-stimulating protein (ASP), FFA, glucose, and insulin levels, while rates of transfer and esterification rate of newly synthesized cholesterol (ER) were derived by using plasma CETP levels and the deuterium incorporation methodology. Plasma ASP levels were lowest ($P < 0.05$) in subjects on the SM diet (33.4 ± 12.7 nM) compared with the SO (48.7 ± 17.0 nM) and SQM (50.7 ± 15.7 nM) diets. Conversely, FFA were highest ($P < 0.05$) on the SM diet (0.86 ± 0.45 mM) relative to all the other diets. No differences were observed in plasma glucose and insulin levels among diets. A trend toward higher CETP levels after consumption of the SM diet was observed. However, the ER was lowest ($P < 0.05$) after the SM (0.111 ± 0.062 g·day^{-1}) diet and highest after consumption of the SQM (0.216 ± 0.123 g·day^{-1}) diet. In addition, ASP levels were negatively correlated with FFA ($r = -0.63$, $P < 0.05$), LDL cholesterol ($r = -0.56$, $P < 0.05$), and TG ($r = -0.41$, $P < 0.05$), whereas FFA was positively correlated with apolipoprotein B-containing lipoproteins ($r = 0.58$ and 0.47, for VLDL and LDL cholesterol, $P < 0.05$), and negatively correlated with HDL cholesterol ($r = -0.51$, $P < 0.05$). The ER was found to positively correlate with HDL cholesterol and HDL_2 subfraction ($r = 0.53$ and 0.45, respectively, $P < 0.05$). Taken together, these data demonstrate that the alterations in circulating lipid levels, commonly observed with consumption of hydrogenated fat-rich diets, can be explained in part by changes in ASP activity as well as newly synthesized cholesterol ER.**—Matthan, N. R., K. Cianflone, A. H. Lichtenstein, L. M. Ausman, M. Jauhiainen, and P. J. H. Jones. **Hydrogenated fat consumption affects acylation-stimulating protein levels and cholesterol esterification rates in moderately hypercholesterolemic women.** ***J. Lipid Res.*** **2001.** 42: **1841–1848.**

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Available data from human studies (1–10) indicate that *trans* fatty acids and/or hydrogenated fat tend to increase total cholesterol (TC) and LDL cholesterol, and at relatively high concentrations decrease HDL cholesterol levels. Some investigators have also reported modest but significant elevations in plasma TG concentrations (1, 4, 5, 9). Given that the effect of hydrogenated fat consumption on plasma lipoprotein profiles appears to be as unfavorable as saturated fat, it is of considerable interest to determine precisely the mechanisms responsible for these changes.

Although several animal studies have attempted to evaluate the mechanism of action of *trans* fatty acids from hydrogenated fat, scant information is available from human studies to resolve this issue (11). A few authors have suggested that *trans* fatty acids behave like saturated fatty acids and increase plasma LDL cholesterol levels by down-regulating LDL receptors (12, 13). Others suggest that reduced cholesterol esterification by LCAT, accompanied by increased transfer of cholesteryl esters from HDL to LDL mediated by CETP, may account for the increase in LDL cholesterol and decrease in HDL cholesterol (14–17). Because CETP activity is influenced by the presence of TG-rich lipoproteins, it is possible that abnormalities in TG metabolism could be the underlying cause for the subsequent alterations in lipoprotein profile.

Abbreviations: ASP, acylation-stimulating protein; ER, esterification rate of newly synthesized cholesterol; SM, stick margarine; SO, soybean oil; SQM, squeeze margarine; TM, tub margarine.

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In this regard, in vivo (18, 19) and in vitro (20) studies have shown that acylation-stimulating protein (ASP) is an important determinant of the rate of adipocyte TG uptake and redistribution. ASP action is achieved through an interaction with the cell membrane, which results in stimulation of the second messenger diacylglycerol. Diacylglycerol then mediates stimulation and translocation of protein kinase C, producing a downstream stimulation of TG synthesis (21, 22). It has also been reported that ASP increases specific membrane transport of glucose via translocation of glucose transporters from intracellular storage sites to the plasma membrane. The effect on glucose uptake has been shown to be independent and additive to that of insulin (23, 24). In addition, Cianflone et al. (25) documented alterations in plasma ASP levels in a number of dyslipoproteinemic states that suggest a relationship between ASP and other determinants of lipoprotein metabolism. One such determinant is the rate of uptake and subsequent metabolism of TG-rich lipoproteins, which in turn depends, in part, on the amount and type of fatty acid consumed. Thus, the objective of the present study was to explore whether alterations in *i*) TG uptake by adipose tissue determined by measuring plasma ASP, FFA, glucose, and insulin levels, and *ii*) esterification rates of newly synthesized cholesterol (ER) and transfer by CETP could account for the changes in circulating lipid levels observed with consumption of different forms of hydrogenated fats.

MATERIALS AND METHODS

Subjects

Eighteen postmenopausal (age >50 years), moderately hypercholesterolemic (LDL cholesterol levels $\geq$130 mg·dl^{-1}) women were recruited to participate in this study as previously described (1). Sample losses during shipping and preparation precluded generation of complete data sets for four subjects, and hence they could not be included in the present study. Subjects were free from chronic illness and were not taking any medication known to affect lipid metabolism (lipid-lowering drugs, β-blockers, diuretics, or hormones). Subjects who smoked or consumed two or more alcoholic drinks per day were excluded from the study. The protocol was reviewed and approved by the Human Investigation Review Committee of the New England Medical Center and Tufts University. All potential subjects were given a verbal and written description of the study before obtaining consent. During the study period, subjects, investigators, and laboratory personnel were blinded to the dietary phase assignments. A portion of the data focusing on plasma lipid levels and endogenous cholesterol synthesis rates has been published previously (1, 13, 15).

Experimental design and diets

Subjects consumed each of six diets according to a randomized cross-over design. Each dietary phase had a duration of 5 weeks, separated by washout periods ranging from 2 to 4 weeks, during which subjects consumed their habitual diets. To focus on the effects of hydrogenation per se, only the results from four of the phases, the soybean oil (SO) and soybean oil-based margarines in the squeeze (SQM), tub (TM), and stick (SM) forms, are reported here. The soybean oil diet was formulated to meet National Cholesterol Education Panel 2 dietary guidelines, providing 15% energy as protein, 55% as carbohydrate, and 30% as fat [$\leq$7% saturated fatty acids (SFA), 10–15% monounsaturated fatty acids (MUFA), and $\leq$10% PUFA] and less than 85 mg of cholesterol per 1,000 kcal. The SO component comprised 20% of energy. In subsequent diets, the SO was replaced by the experimental fats, so that the effect of consuming diets enriched in hydrogenated fat could be assessed within the general context of current recommendations for individuals with elevated plasma lipid levels. All food and drink were provided by the Metabolic Research Unit of the Jean Mayer U.S. Department of Agriculture Human Nutrition Research Center on Aging at Tufts University to be consumed on site or packaged for take-out. Subjects were required to consume all food provided to them and not to supplement their diet with any other food or drink except water and noncaloric beverages. Initial energy intakes of the subjects were calculated according to the Harris-Benedict equation, and adjustments were made when necessary to maintain body weight. Analysis of the macronutrient, fatty acid, and cholesterol contents of the diets was carried out by Covance Laboratories (Madison, WI) and Best Foods Research and Engineering Center (Union, NJ).

PROTOCOL AND ANALYSES

Blood draws

Three times during week 5 of each dietary phase fasting blood samples were drawn for analyses of plasma lipid and lipoprotein concentrations. On two of the 3 days an additional blood sample was taken before and 24 h after the administration of an oral dose of deuterium oxide (1.2 g of D_2O per kg of total body water) for the determination of endogenous cholesterol synthesis and esterification. Blood samples were centrifuged at 3,000 rpm at 4°C and plasma was separated, aliquoted, and frozen at −80°C until further analysis.

Plasma lipid and lipoprotein analyses

VLDL was isolated from plasma by ultracentrifugation at 39,000 rpm for 18 h at 4°C (26). Serum and the 1.006 g·ml^{-1} infranatant fraction were assayed for TC and TG with an Abbott Diagnostic (North Chicago, IL) spectrum CCX biochromatic analyzer, using enzymatic reagents as previously described (27). HDL cholesterol levels were measured after precipitation of the apolipoprotein B (apoB)-containing lipoproteins by a dextran-magnesium sulfate procedure (28). Lipid assays were standardized through the Lipid Standardization Program at the Centers for Disease Control and Prevention (Atlanta, GA).

Plasma CETP, FFA, glucose, and insulin analyses

Plasma CETP activity was measured after removal of endogenous VLDL and LDL by phosphotungstate and magnesium chloride as described by Groener, Pelton, and Kostner (29). Plasma FFA levels were analyzed by an enzymatic method (Wako NEFA kit; Alpha Laboratories, Eastleigh, UK). Plasma glucose concentrations were determined by an enzymatic colorimetric assay (Roche Laboratories, Nutley, NJ), and plasma insulin levels were measured by a human insulin-specific competitive binding radioimmunoassay (Linco Research, St. Louis, MO).

Plasma ASP assay

Plasma ASP levels were determined by an ELISA sandwich immunoassay (30). The procedure is as follows: a murine (in-house) monoclonal antibody was used as the capture antibody. This antibody was coated at 7 μg·ml^{-1} in PBS (100 μg per well in a 96-well plate) overnight at 4°C and blocked with 1% ovalbumin for 2 h. The plate was washed three times with wash solution (0.5% Tween 20 in 0.9% NaCl) between every step. Standard so-

lutions of 0.78–27.46 ng·ml^{-1}ASP, purified as described previously (30), as well as the test and control plasma samples (precipitated and diluted appropriately), were added at 100 μl per well. The plate was incubated for 1 h at 37°C and washed, followed by an incubation for 1 h at 37°C with 100 μl of rabbit antiserum to human ASP (raised against holoprotein), diluted 1:2,000 in PBS with 0.05% Tween 20. The plate was then incubated for 30 min at 37°C with 100 μl of goat anti-rabbit IgG conjugated to horseradish peroxidase (1:5,000; Sigma, St. Louis, MO) diluted in PBS with 0.05% Tween 20. After the final wash, the color reaction was initiated with 100 μl of *o*-phenylenediamine (1 mg·ml^{-1}) in 100 mM sodium citrate and 0.05% Tween 20. After visual development the reaction was stopped with 50 μl of 4 N H_2SO_4, and the absorbance was read at 490 nm. ASP concentration versus absorbance was graphed and calculated by linear regression. The within- and between-assay coefficient of variation was less than 4%.

Determination of ER

Deuterium enrichment in body water, free cholesterol (FC), and cholesteryl ester (CE) fractions were measured as previously described (31, 32). Lipids were extracted from plasma (33) and the FC and CE bands were separated by thin-layer chromatography, using petroleum ether–ethyl ether–acetic acid 135:15:1.5 (v/v/v). The FC band was eluted from silica with hexane–chloroform–ethyl ether 5:2:1 (v/v/v) and dried under a stream of nitrogen. The CE band was saponified with 5 ml of methanolic potassium hydroxide, capped tightly, and boiled at 100°C for 55 min. After cooling, 2 ml of distilled water and 13 ml of petroleum ether were added per tube, which was shaken for 5 min, and then centrifuged at 1,500 *g* for 15 min. The upper ether phase was separated, and the extraction procedure was repeated two more times, omitting the addition of water. Combined ether phases were dried down under a stream of nitrogen and then plated to separate the cholesterol from free fatty acids. The resultant deesterified cholesteryl ester (d-CE) was purified from silica as described above. The FC and d-CE were then converted into water and carbon dioxide by combustion over cupric oxide and silver wire at 520°C for 2 h. In addition, to measure deuterium enrichment of plasma water, pre- and post-D_2O samples diluted 2-fold and 10-fold, respectively, to produce deuterium enrichments within detectable ranges on the mass spectrometer were distilled into zinc-containing tubes. The combustion water from FC, d-CE, and plasma were then vacuum distilled into Pyrex™ tubes containing zinc reagent and reduced to hydrogen-deuterium gas by heating at 520°C for 30 min. Deuterium enrichment of the gas was analyzed by isotope ratio mass spectrometry (VG Isomass 903 D; VG Gas Analysis Systems, Cheshire, England). The instrument was calibrated daily with water standards of known isotopic composition. Values were expressed relative to the enrichment of standard mean ocean water in parts per mil (‰). Samples for each subject were analyzed in duplicate against a single set of standards. Maximum acceptable precision for deuterium was 5‰ at enrichments over 500‰ and 2‰ at enrichments below 200‰.

Fractional synthesis rates (FSR) in pools per day (p·day^{-1}) were calculated as cholesterol deuterium enrichment in FC and CE in relation to that of the precursor body water pool adjusted for the fraction of hydrogens of cholesterol derived from labeled substrate (31). The equation used was

$$\text{FSR (p·day}^{-1}) = \frac{\delta \text{ cholesterol (\%)}}{\delta \text{ plasma water (\%)} \times 0.478}$$

where δ is the difference in deuterium enrichment over 24 h. Model parameters and assumptions underlying use of D_2O as tracer for endogenous cholesterol measurements have been described previously (31, 32). The absolute esterification rate (AER), expressed in grams per day (g·day^{-1}), was derived by multiplying the FSR-CE by M_1 pool size and a factor of 0.67. The M_1 pool size was calculated according to the Goodman, Noble, and Dell (34) equation, which takes into account the subject's body weight and plasma TC and TG concentrations. The factor of 0.67 accounts for the proportion of CE in the overall plasma TC pool. Given that newly synthesized cholesterol initially appears into the FC pool, the ER, expressed as grams per day (g·day^{-1}), was computed to reflect the appearance of label in cholesteryl ester from the FC pool after correcting for pool size differences. The equation used was as follows:

$$\text{ER (g·day}^{-1}) = \frac{\text{FSR-CE/CE-M}_1 \text{ pool}}{\text{FSR-FC/FC-M}_1 \text{ pool}}$$

The size of the FC-M_1 pool was calculated in a manner similar to that described for CE, with replacement of the factor of 0.67 by 0.33 to account for the contribution of FC to the TC pool.

Statistical analysis

One-way analyses of variance with the main effect of diet, and subject as the repeated measure, were applied to each outcome variable, using an SAS Institute (Cary, NC) general linear model program (SAS version 6). Differences in group means were identified by Tukey's test at a significance level of $P < 0.05$. However, before statistical analyses, descriptive statistics (Proc Univariate) were used to summarize the overall distributions of the outcome variables, and when appropriate logarithmic transformations were performed. Untransformed data are presented in text and tables as means ± standard deviation (SD). Partial correlations with "subject" as the repeated measure were performed between variables of interest, using the data derived after consumption of the SO, SQM, TM, and SM diets.

RESULTS

At the time of recruitment, all subjects had LDL cholesterol concentrations ⩾130 mg·dl^{-1}, indicating that they were in the borderline high risk to high risk categories for cardiovascular disease, a subset for whom dietary modification is the first intervention to normalize blood lipid levels. Mean (±SD) TC, LDL cholesterol, HDL cholesterol, VLDL cholesterol, and TG levels were 258 ± 31, 172 ± 30, 54 ± 12, 31 ± 13, and 158 ± 75 mg·dl^{-1}, respectively. The average body mass index was 26.5 ± 2.4 kg·m^{-2}, which remained constant over the duration of the study as the subjects' body weights were carefully monitored to prevent either a gain or loss.

The composition of the experimental diets based on chemical analyses is shown in **Table 1**. Details pertaining to individual fatty acid species have been previously reported (1). All diets had similar protein, carbohydrate, fat, and cholesterol contents. However, increasing the degree of hydrogenation resulted in higher *trans* fatty acid and lower PUFA levels on the margarine-based diets. However, the SFA and MUFA contents remained relatively similar.

Plasma ASP and FFA levels at the end of each dietary phase are depicted in **Fig. 1**. Plasma ASP levels were lower ($P < 0.05$) after consumption of the SM (33.4 ± 12.7 nM) diet relative to the SO (48.7 ± 17.0 nM) and SQM (50.7 ±

TABLE 1. Composition of test diets as determined by chemical analysis[a]

Constituent	Soybean Oil	Squeeze Margarine	Tub Margarine	Stick Margarine
		Percentage of total daily energy intake		
Protein	15.7	17.1	16.3	16.7
Carbohydrate	55.8	51.7	52.9	53.5
Fat	28.5	31.2	30.8	29.7
Saturated fatty acids	7.3	8.6	8.4	8.5
Monounsaturated fatty acids[b]	8.1	8.1	8.0	8.5
Polyunsaturated fatty acids[b]	12.5	13.5	11.1	6.3
Trans fatty acids	0.6	0.9	3.3	6.7
Cholesterol (mg·4.2 MJ^{-1})	65.9	68.0	70.3	66.5

[a] Because of rounding, percentages may not total 100.
[b] Only *cis* isomers are included.

15.7 nM) diets. Reciprocally, plasma FFA levels were significantly elevated with SM feeding when compared with SO and the other two margarine diets.

With respect to the cholesterol kinetic data (**Table 2**), FSR-FC rates ($p \cdot day^{-1}$) were higher ($P < 0.05$) on the SO, SQM, and TM diets when compared with the SM diet. FSR-CE ($p \cdot day^{-1}$) was higher ($P < 0.05$) on the SO and SQM diets and lower on the SM diet, with esterification rates on the TM diet being intermediate. AER ($g \cdot day^{-1}$) values were similar, with consumption of the SM diet resulting in a significant ($P < 0.05$) reduction of esterification relative to the SO and SQM diets. AER values did not differ among the other diets. The ER data ($g \cdot day^{-1}$), which corrects for input of deuterium label from the FC pool, was lower ($P < 0.05$) on the SM diet relative to the SQM diet, but differences among other diets were no longer observed.

As previously reported for a larger data set (1), in this subgroup of female subjects (**Table 3**), plasma TC levels were elevated ($P < 0.05$) with consumption of the SM diet when compared with the SO diet. A similar pattern of response was observed with LDL cholesterol levels, with consumption of the SM diet resulting in higher LDL cholesterol levels relative to the SO diet ($P < 0.05$). The HDL cholesterol and HDL_2 levels were lowest after consumption of the SM diet but differences between the diets did not attain statistical significance. The apoB levels were higher ($P < 0.02$) on the SM diet when compared with the other diets. No differences in plasma levels of glucose and insulin were observed among diets. A trend toward elevated plasma TG and CETP levels ($P = 0.06$ and $P = 0.07$) on the SM diet was observed; however, results did not reach statistical significance. This could be attributable to the smaller sample size in our study as these variables were significantly different in the overall study population (n = 36).

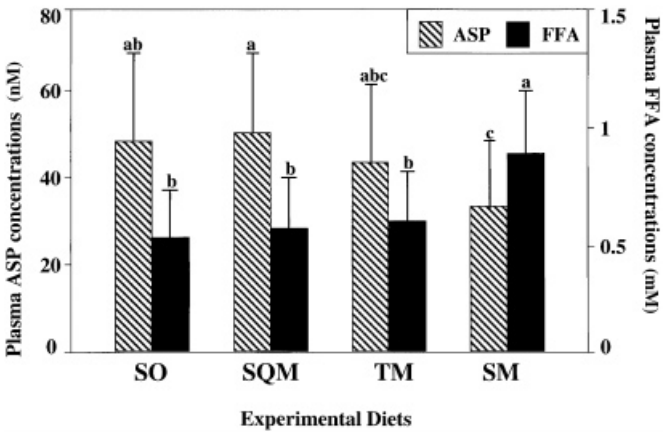

Fig. 1. Plasma acylation-stimulating protein (ASP) and FFA concentrations (nanomolar and millimolar, respectively) at the end of each dietary phase. Data are presented as means ± SD, n = 14. Diet abbreviations are as follows: SO, soybean oil; SQM, squeeze margarine; TM, tub margarine; SM, stick margarine. Columns with different letters are significantly different ($P < 0.05$).

To determine whether the changes observed in circulating LDL cholesterol and HDL cholesterol with hydrogenated fat consumption could be a consequence of alterations in components of the ASP pathway as well as ER, partial correlations between these variables were computed by pooling data from the oil and hydrogenated fat diets. Plasma ASP levels (**Fig. 2A** and **B**) were found to negatively correlate with FFA ($r = -0.63$, $P < 0.05$), LDL cholesterol ($r = -0.56$, $P < 0.05$), as well as TG concentrations ($r = -0.41$, $P < 0.05$). FFA levels in turn were positively correlated with apoB-containing lipoproteins VLDL cholesterol and LDL cholesterol (Fig. 2C), but negatively associated with HDL cholesterol (Fig. 2D) concentrations ($r = 0.58$, 0.47, and -0.51, respectively, $P < 0.05$). When the ER data (not shown) were correlated with HDL cholesterol and HDL_2 levels a positive association was obtained ($r = 0.53$ and 0.45, respectively, $P < 0.05$).

DISCUSSION

Considerable attention has been focused on the effect of dietary hydrogenated fat on blood lipid and lipoprotein profiles; however, the mechanisms responsible for these changes remain unclear. It has been proposed that the cholesterol-raising ability of hydrogenated fat is related to either delayed LDL clearance or increased LDL production (11). With regard to the former, Hayashi and colleagues (12) have demonstrated in hamsters that hy-

TABLE 2. Rates of newly synthesized and esterified cholesterol as a function of dietary fat consumed

Variable	Soybean Oil	Squeeze Margarine	Tub Margarine	Stick Margarine
		Mean ± SD		
Fractional synthesis rate of free cholesterol (FSR-FC, p·day^{-1})	0.078 ± 0.024^{a}	0.081 ± 0.019^{a}	0.086 ± 0.029^{a}	0.053 ± 0.029^{b}
Fractional synthesis rate of cholesterol ester (FSR-CE, p·day^{-1})	0.038 ± 0.029^{a}	0.032 ± 0.019^{a}	0.024 ± 0.012^{a,b}	0.017 ± 0.009^{b}
Absolute esterification rate (AER, g·day^{-1})	0.648 ± 0.506^{a}	0.537 ± 0.324^{a}	0.417 ± 0.390^{a,b}	0.292 ± 0.165^{b}
Esterification rate of newly synthesized cholesterol (ER, g·day^{-1})	0.193 ± 0.084^{a,b}	0.208 ± 0.137^{a}	0.133 ± 0.044^{a,b}	0.111 ± 0.065^{b}

Within a row, values with different superscripts are significantly different ($P < 0.05$), n = 14.

drogenated corn oil elevates plasma VLDL cholesterol and LDL cholesterol levels through the suppression of hepatic LDL receptor activity. We have also previously reported (13) that an impairment in the catabolic pathway of cholesterol rather than reduced synthesis is responsible for the higher plasma cholesterol levels seen with consumption of hydrogenated and saturated fat-enriched diets. In the present study, we provide new evidence that alterations in ASP production and ER could account for the changes in circulating lipoprotein levels after consumption of different forms of hydrogenated fats.

Our data suggest that hydrogenated fat intake interferes with ASP production, resulting in lower plasma ASP and higher plasma FFA levels. Consequently, it is hypothesized that less hydrolyzed FFA is taken up by the adipose tissue and more is diverted to the liver, where they are secreted as an increased number of hepatic B-100-containing particles (35, 36). This could explain the increase in circulating levels of the apoB-containing lipoproteins seen after consumption of the SM diet. Furthermore, lower ASP levels were associated with higher plasma FFA concentrations, which in turn were positively correlated with the apoB-containing lipoproteins and negatively associated with HDL cholesterol concentrations. Although these associations do not support causation, it is interesting that they are consistent with the above-described hypothesis. The above-described hypothesis is also supported by data from hyper-apoB subjects, in whom decreased peripheral cellular response to ASP leads to delayed postprandial TG and consequently increased postprandial FFA, which in turn is associated with increased apoB production (37). In addition, Murray, Sniderman, and Cianflone (20) have also demonstrated a delay in postprandial TG clearance in mice lacking ASP, reflected as an increased circulating FFA level, with corresponding increases in plasma VLDL cholesterol, LDL cholesterol, and TG.

Present results also provide evidence of a lower ER on the SM diet relative to the SQM diet. Given that HDL is the predominant site of cholesterol esterification in plasma, the reduction in the esterification of newly synthesized cholesterol seen with SM feeding could account for the trend toward lower HDL cholesterol concentrations. Other investigators (38–40) have determined cholesterol esterification rates by measuring the activity of LCAT with HDL-like analogs, or by measuring the heterogeneity of the HDL pool, using LCAT as a probe. However, results using these in vitro assays have been conflicting, and possibly relate to differences in methodology (41). Consequently we utilized the deuterium uptake methodology to obtain a direct in vivo measure of ER after hydrogenated fat consumption. Mazier and Jones (42), using the same

TABLE 3. Serum lipid, lipoprotein, insulin, glucose, and CETP levels as a function of dietary fat type consumed

Variable	Soybean Oil	Squeeze Margarine	Tub Margarine	Stick Margarine
		Mean ± SD		
TC (mg·dl^{-1})	229.5 ± 28.3^{c}	232.8 ± 28.1^{b,c}	239.9 ± 29.9^{b,c}	247.3 ± 33.5^{a,b}
LDL-cholesterol (mg·dl^{-1})	156.5 ± 32.4^{c}	157.5 ± 22.4^{b,c}	165.2 ± 29.3^{a,b,c}	171.9 ± 30.3^{a,b}
HDL-cholesterol (mg·dl^{-1})	46.5 ± 9.2	46.5 ± 10.8	47.2 ± 9.7	45.3 ± 9.4
HDL_2	14.6 ± 6.9	14.2 ± 8.3	13.9 ± 6.9	12.9 ± 6.6
HDL_3	31.9 ± 3.8	32.3 ± 3.9	33.1 ± 5.3	32.3 ± 3.5
VLDL-cholesterol (mg·dl^{-1})	27.1 ± 11.2	26.8 ± 7.9	26.6 ± 7.5	29.6 ± 13.5
TG (mg·dl^{-1})	136.9 ± 52.8	131.2 ± 53.5	140.5 ± 55.8	154.5 ± 64.2
Apo B (mg·dl^{-1})	136.2 ± 24.8^{b}	137.9 ± 24.2^{b}	141.2 ± 26.2^{a,b}	148.0 ± 27.2^{a}
CETP (nmol·ml^{-1}·h^{-1})	13.9 ± 5.7	13.4 ± 7.8	14.8 ± 5.4	15.9 ± 6.7
Insulin (μU·ml^{-1})	13.1 ± 6.6	13.2 ± 6.5	12.2 ± 5.2	13.3 ± 6.9
Glucose (mM)	5.1 ± 0.6	5.1 ± 0.6	5.1 ± 0.6	5.2 ± 0.6

Within a row, values with different superscripts are significantly different ($P < 0.05$), n = 14. To convert values for cholesterol and triglycerides to millimoles per liter, divide by 38.67 and 88.54, respectively.

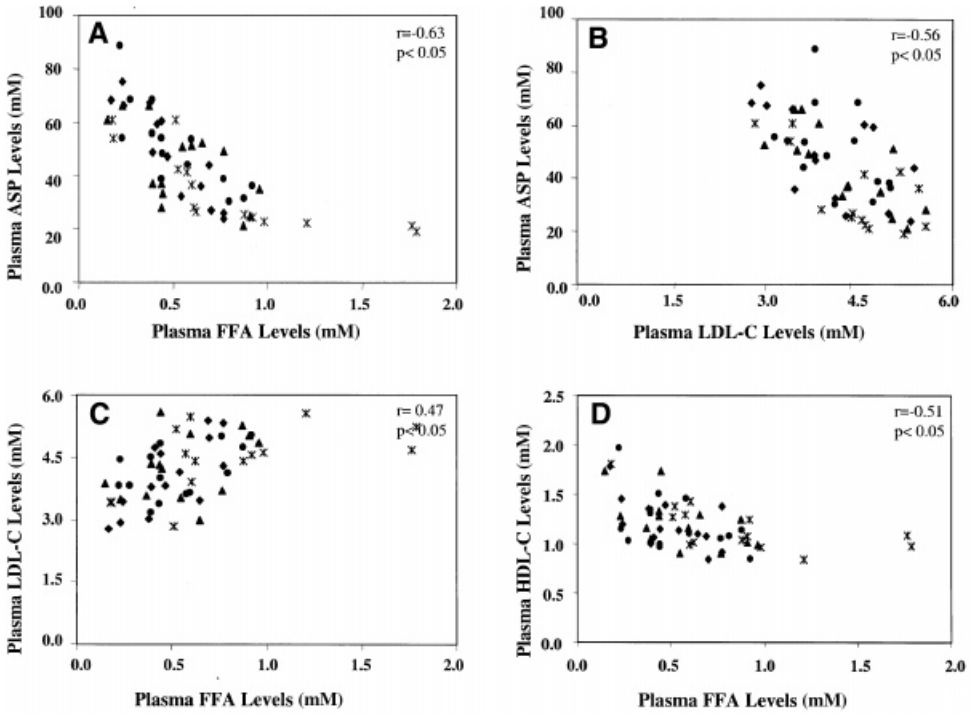

Fig. 2. Association between (A) plasma ASP (nanomolar) and plasma FFA concentrations (millimolar), (B) plasma ASP (nanomolar) and plasma LDL cholesterol (LDL-C, millimolar), (C) plasma FFA concentrations (millimolar) and plasma LDL-C (millimolar), and (D) plasma FFA concentrations (millimolar) and plasma HDL cholesterol (HDL-C, millimolar) concentrations. Data are presented for 14 subjects after consumption of the SO (solid diamonds), SQM (solid circles), TM (solid triangles), and SM (asterisks) diets. The *r* values refer to partial correlations.

method, calculated AER as the product of FSR-CE and M_1 pool size, the latter derived from a decay curve after injection of [4-^{14}C]cholesterol. The authors demonstrated that AER were higher after consumption of diets containing PUFA versus MUFA or SFA; however, this trend was not statistically significant, possibly because of the small sample size (n = 9) used in the study. Given the invasive nature of the above-described technique to estimate M_1 pool size, we opted to use the Goodman equation, which is considered to yield an accurate estimate of the overall M_1 pool size. We observed lower AER after consumption of the high *trans*-containing SM diet when compared with the SO and SQM diets. However, the AER calculation does not account for movement of FC into the ester pool. Consequently, the ER model was devised. Our revised model assumes that free cholesterol migrates rapidly between lipoproteins, erythrocytes, and other cellular structures (31, 43). Thus, deuterium enrichment of plasma FC represents that of free sterol within the rapidly exchanging M_1 pool found in the liver, intestine, and circulation. Turnover rates for CE are slower, thus, present data likely reflect events occurring within the plasma, rather than the overall CE pool within the M_1 compartment. To validate our model, we recalculated CE pool size values and derived ER according to the Nestel and Monger model (44) and determined the agreement (**Fig. 3**) between ER derived by both methods, using the Bland-Altman technique (45). As can be observed, both methods yield comparative results; however, the present model derived by stable isotope methodology has the advantage of being more easily applicable when compared with the greater labor intensiveness and invasiveness of radioactive isotope studies.

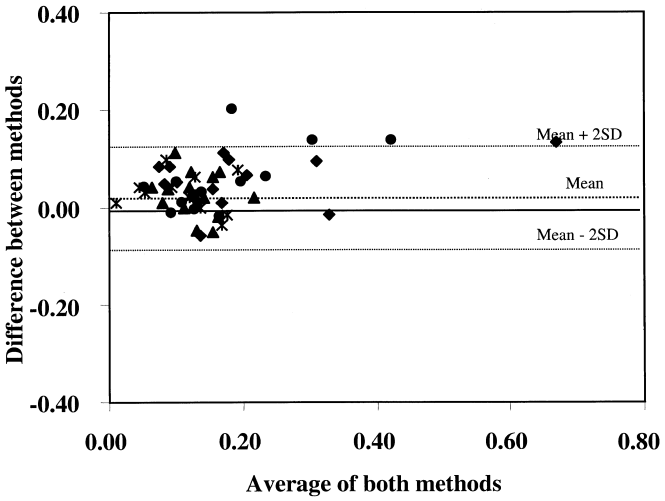

Fig. 3. The difference between the present model and the Nestel-Monger model, with upper and lower 95% limits of agreement (2 SD), plotted against means of the two measurements for each subject's cholesterol esterification data. The results are presented for 14 subjects after consumption of the SO (solid diamonds), SQM (solid circles), TM (solid triangles), and SM (asterisks) diets.

Another explanation for the increase in LDL cholesterol and decrease in HDL cholesterol levels after hydrogenated fat consumption could be alterations in CETP activity. Lagrost (17) suggested that high concentrations of *trans* fatty acids may increase the rate of transfer of cholesteryl esters from HDL to LDL when added to plasma in vitro, a hypothesis later confirmed by Abbey and Nestel (14). However, a significant increase was detected only with an assay that used endogenous lipoproteins, but no effect on CETP activity was observed when assayed in lipoprotein deficient plasma. Van Tol et al. (16) measured the serum activity levels of CETP by using excess exogenous substrate in sera of subjects who had consumed diets enriched in either *trans* fatty acids or stearic or linoleic acid. The *trans* fatty acid diet resulted in an 18% increase in CETP activity, which coincided with the expected lower ratio of cholesteryl esters to TG in HDL. In the present study, CETP activity appeared to be lowered after consumption of the SM diet, but results did not attain the level of statistical significance. However, in the original subset studied (15), CETP activity was found to be highest after subjects consumed the SM diet relative to the SQM diet. This trend was observed in both female and male subjects, although the difference in CETP activity reached statistical significance only in the males. The greater variability in response of the female subjects may have confounded the analysis.

In conclusion, our results suggest that the alterations in circulating lipoprotein levels, commonly observed with consumption of hydrogenated fat-rich diets, are at least in part due to lowered ASP levels as well as ER. Given these unfavorable effects, dietary guidelines should continue to focus on the substitution of unhydrogenated oil for hydrogenated or saturated fat in processed foods. In addition, the recommendation to substitute softer for harder margarines and cooking fats seems justified.

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