Hydrogenated fat consumption affects cholesterol synthesis in moderately hypercholesterolemic women

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Abstract To determine mechanisms by which hydrogenated fat influences plasma lipid levels, 14 women (65–71 yrs with LDL-C $\geq$ 130 mg·dl^{-1}) consumed, for 5-week periods each, a baseline (BL) diet (39% kcal fat, 164 mg chol·1000 kcal^{-1}) and reduced fat diets (30% kcal) where two-thirds of the fat was either soybean oil (SO), low *trans* squeeze (SQM), medium *trans* tub (TM), or high *trans* stick (SM) margarines, or butter (BT). Plasma lipid levels were analyzed at the end of each phase. Fractional synthesis rates (FSR) in pools/day (p·d^{-1}) and absolute synthesis rates (ASR) in grams/day (g·d^{-1}) of free cholesterol (FC) were measured using the deuterium incorporation methodology. Plasma total ($P < 0.01$) and low density lipoprotein ($P < 0.05$) cholesterol levels increased with increasing degree of hydrogenation or saturated fat intake. High density lipoprotein cholesterol levels ($P < 0.05$) were lowest on the SM diet when compared to the BT diet. Low *trans* SQM (0.081 ± 0.019 p·d^{-1}) and medium *trans* TM (0.086 ± 0.029 p·d^{-1}) diets elicited responses similar to the SO (0.078 ± 0.024 p·d^{-1}) diet, whereas high *trans* SM (0.053 ± 0.029 p·d^{-1}) diet mimicked the BT (0.062 ± 0.017 p·d^{-1}) and high fat BL (0.053 ± 0.023 p·d^{-1}) diet in its suppression ($P < 0.05$) of FSR-FC. ASR-FC, which is an approximation of the daily production of newly synthesized cholesterol, showed a trend similar to the FSR-FC data. These results indicate that reduced synthesis is not responsible for the higher plasma TC levels seen with consumption of the SM, BT, and BL diets, and suggest that another mechanism, possibly impairment of the catabolic pathway of cholesterol, is involved.—Matthan, N. R., L. M. Ausman, A. H. Lichtenstein, and P. J. H. Jones. **Hydrogenated fat consumption affects cholesterol synthesis in moderately hypercholesterolemic women. *J. Lipid Res.* 2000.** 41: **834–839.**

Supplementary key words reduced fat diets • margarines • hydrogenation • *trans* fatty acids • cholesterol synthesis

Trans fatty acids are geometrical isomers of the naturally occurring *cis* fatty acids. They are produced as a result of biohydrogenation in the ruminant fat of animals or by commercial hydrogenation of vegetable oils (1). The majority of dietary *trans* fatty acids in Western diets is contributed from commercial hydrogenation. Hence it is not surprising that *trans* fatty acid intake mirrors consumption of margarines, baked products, and commercially prepared foods, which has steadily increased since the beginning of this century (2). Their consumption has come under scrutiny in view of recent studies indicating adverse health effects with *trans* fatty acids relative to their *cis* counterparts (3–5) or unhydrogenated oils (6–10). These effects include elevated total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C) and triglycerides (TG), as well as decreased high density lipoprotein cholesterol (HDL-C) levels (3–10). This is of concern because dietary counseling aimed at lowering plasma lipid levels usually encourages substitution of vegetable oils and its products such as margarines and shortenings for those of animal origin (11). In addition, over the past few years there has been a proliferation of new products on the market that vary in their degree of hydrogenation. These include softer margarines in semi-liquid (squeeze) or soft (tub) forms intended to substitute for hard margarines in stick form. Whether the consumption of these products has a similar or less dramatic effect relative to butter on measures of cardiovascular disease (CVD) risk was recently evaluated (12). Findings revealed that consumption of products low in *trans* fatty acids like soybean oil, squeeze and tub margarines had a beneficial effect on the serum lipid profile compared to diets enriched in stick margarine, shortening (hydrogenated soybean oil) or butter. However, the mechanisms responsible for the adverse effects on circulating lipid levels seen after consumption of the latter diets remain to be elucidated.

One possible mechanism involves alterations in en-

Abbreviations: FC, free cholesterol; FSR, fractional synthesis rate; ASR, absolute synthesis rate; DI, deuterium incorporation; D_2O, deuterium oxide; ACAT, acyl-CoA:cholesterol acyltransferase; MIDA, mass isotopomer distribution analysis.

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dogenous cholesterol synthesis. In humans biosynthesis of cholesterol accounts for 60 to 80% of whole body cholesterol input (13, 14), and has been shown to be influenced by dietary fatty acid composition. Saturated fats tend to suppress whereas polyunsaturated fats tend to increase cholesterol synthesis rates (15–17). Cuchel and colleagues (18) have shown that feeding subjects corn oil margarine in stick form as compared to a corn oil diet increased plasma lipid and lipoprotein levels in spite of a trend toward decreased de novo cholesterol synthesis rates. However, the issue remained unresolved as this declining trend in fractional synthetic rate of cholesterol (FSR-FC) did not reach statistical significance, possibly due to sample size limitations and high variability in synthesis among the male and female subjects. With the exception of the above study, the effect of hydrogenation, specifically degree of hydrogenation on cholesterogenesis, remains unknown. Thus, the present study was designed to evaluate the impact of consumption of commonly available sources of dietary fats subjected to different degrees of hydrogenation on endogenous synthesis rate of free cholesterol (FC) using the deuterium incorporation (DI) methodology.

METHODS

Subjects

Fourteen postmenopausal, middle-aged to elderly women (65–71 yrs) were selected to participate in this study from an original study population of 18 female subjects as previously described (12). Subjects had LDL-C levels $\geqslant$130 mg·dl^{-1}, 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 $\geqslant$2 alcoholic drinks per day were also excluded from the study. The protocol was reviewed and approved by the human investigation review committee of New England Medical Center and Tufts University. All potential subjects were given a verbal and written description of the study prior to obtaining consent. However, during the study period, subjects were blinded to the dietary phase assignments. A portion of the data focusing on plasma lipid and lipoprotein parameters has been published previously (12).

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 and was separated by a washout period ranging from 2 to 4 weeks, during which subjects consumed their habitual diets. All diets were isocaloric. The soybean oil diet was formulated to meet NCEP 2 dietary guidelines, providing 15% energy as protein, 55% as carbohydrate, and 30% as fat ($\leqslant$7% SFA, 10–15% MUFA, and $\leqslant$10% PUFA) and less than 85 mg cholesterol per 1000 kcal. The soybean oil component comprised 20% of energy. In subsequent diets, the soybean oil was replaced by the experimental fat, so that the effect of consuming diets enriched in hydrogenated fats could be assessed within the general context of current recommendations for individuals with elevated plasma lipid levels. The specific fats investigated were soybean oil (SO) and soybean oil-based margarines in the squeeze (SQM), tub (TM), and stick (SM) form as well as butter (BT). These fats were especially chosen because as a group they represent a broad range of *trans* and fatty acid profiles. The baseline diet was included in order to access the biochemical parameters proposed for each individual on a defined diet with a fatty acid composition similar to that currently consumed in North America to best characterize the study population. 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 take-out. Energy intakes of the subjects were tailored to individual requirements, as verified by the ability to maintain stable body weight. Analyses of the macronutrient, fatty acid, and cholesterol contents of the diets were carried out by Covance Laboratories (Madison, WI) and Best Foods Research and Engineering Center (Union, NJ).

Protocol and analyses

During week 5 of each dietary phase a fasting blood sample was drawn followed by administration of a bolus oral dose of deuterium oxide (1.2 g D_2O per kg total body water). Another fasting blood sample was taken 24 h after dosing. Blood samples were centrifuged at 3000 rpm at 4°C and plasma was separated, aliquoted, and frozen at −80°C until further analysis. VLDL was isolated from plasma by ultracentrifugation at 39,000 rpm for 18 h at 4°C (19). Serum and the 1.006 g/ml 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 (20). HDL-C levels were measured after precipitation of the apolipoprotein B containing lipoproteins by a dextran–magnesium sulfate procedure (21). Lipid assays were standardized through the Lipid Standardization Program at the Center for Disease Control (Atlanta, GA).

Additional plasma aliquots were used to determine deuterium enrichment in body water and FC as previously described (13, 22). Briefly, lipids were extracted (23) and the FC band was separated by thin-layer chromatography using petroleum ether–ethyl ether–acetic acid 135:15:1.5 (v/v/v). FC was eluted with hexane–chloroform–ethyl ether 5:2:1 (v/v/v) and dried under a stream of nitrogen. The purified cholesterol was converted into water and carbon dioxide by combustion over cupric oxide and silver wire at 520°C for 2 h. In addition, 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. This enabled measurement of deuterium enrichment of plasma water. The combustion water from FC 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 deuterium, Cheshire, England). The instrument was calibrated daily using water standards of known isotopic composition. Values were expressed relative to the enrichment of standard mean ocean water (SMOW) in parts per mil. The per mil designation was used because of the relatively small enrichments encountered. Duplicate samples for each subject were analyzed concurrently against a single set of standards.

Calculation of cholesterol synthesis rates

FSR-FC, defined as the proportion of the central or M_1 pool replaced daily by newly synthesized cholesterol, was calculated as the change in product enrichment over time divided by the maximum possible enrichment, based on a linear rate of uptake of label into cholesterol over time (12). The equation used was:

$$\text{FSR-FC (pools/day)} = \frac{\delta \text{ cholesterol (\%)}}{\delta \text{ plasma water (\%)} \times 0.478}$$

where δ is the difference in deuterium enrichment over 24 h. Model parameters and assumptions underlying use of D_2O as tracer for FSR measurements have been described previously (13,

TABLE 1. Baseline characteristics of study subjects (n = 14)

Characteristic	Mean ± SD
Age (yrs)	68.1 ± 2.5
Body mass index ($kg \cdot m^{-2}$)	26.5 ± 2.4
Serum cholesterol ($mg \cdot dl^{-1}$)[a]	
Total	258.3 ± 30.8
LDL-C	171.5 ± 29.8
HDL-C	53.9 ± 12.2
VLDL-C	30.9 ± 13.2
Serum triglyceride ($mg \cdot dl^{-1}$)[b]	158.1 ± 75.2

[a] To convert values for cholesterol to millimoles per liter, divide by 38.67.
[b] To convert values for triglycerides to millimoles per liter, divide by 88.54.

22). ASR-FC, which is an approximation of the daily production of newly synthesized cholesterol expressed in g/day ($g \cdot d^{-1}$), was derived by multiplying the FSR-FC by M_1 pool size and a factor of 0.33. The M_1 pool size was calculated using Goodman's equation (24) which takes into account the subjects body weight, plasma TC, and TG concentrations. The factor of 0.33 was included to account for the proportion of FC in the overall plasma TC pool.

Statistical analysis

One-way analyses of variance with the main effect of diet and subject as the repeated measure was used on each outcome variable using an SAS general linear model program (SAS version 6, SAS Institute Inc, Cary, NC). Group means were separated by Tukey's test at a significance level of $P < 0.05$. Because the TG, VLDL-C, FSR-FC, and ASR-FC data were not normally distributed, logarithmic transformations were performed prior to statistical testing. Untransformed data are presented in text and tables as means ± standard deviation (SD).

RESULTS

Baseline characteristics at the time of screening are shown in **Table 1**. By design, all subjects had LDL-C concentrations ⩾130 $mg \cdot dl^{-1}$ indicating that they were in the borderline high risk or high risk categories for CVD as defined by the Adult Treatment Panel (25). Mean (± SD) TC, LDL-C, HDL-C, VLDL-C, and TG concentrations were 258 ± 31, 172 ± 30, 54 ± 12, 31 ± 13, and 158 ± 75 $mg \cdot dl^{-1}$, respectively. The average body mass index (BMI) was 26.5 ± 2.4 $kg \cdot m^{-2}$, which is characteristic of a group of moderately hypercholesterolemic middle-aged and elderly women.

The composition of the experimental diets based on chemical analyses is shown in **Table 2.** Excluding the BL diet, all other reduced fat diets had similar protein, fat, and carbohydrate contents. Dietary *trans* fatty acid levels ranged from 0.6 to 6.7% of total energy. This was accompanied by lower PUFA levels, which are consistent with effects of the hydrogenation process. The cholesterol content of the butter diet was approximately twice that of the other diets. The option to compensate for this endogenous cholesterol content was not proposed because cholesterol is an inseparable component of butter and it would be artificial to assess the impact of the fatty acid composition of the product on various physiological parameters without including the cholesterol.

Mean (± SD) plasma lipid and lipoprotein concentrations during the six dietary phases are shown in **Table 3.** Plasma TC levels were elevated with consumption of the BL, BT, and SM diets when compared to the SO, SQM, and TM diets ($P < 0.05$ for BT vs. SO, SQM, TM; BL vs. SO, SQM; and SM vs. SO). The LDL-C levels followed a similar pattern, with consumption of the BT, BL, and SM diets resulting in higher ($P < 0.05$) levels relative to the SO diet. Switching from the BL to all five reduced fat diets resulted in a significant ($P < 0.05$) lowering of HDL-C. Among the reduced fat diets, plasma HDL-C concentrations were lowest on the SM diet (45 ± 9 $mg \cdot dl^{-1}$) and highest on the BT diet (50 ± 10 $mg \cdot dl^{-1}$). A trend toward higher plasma VLDL-C and TG levels was observed with SM feeding, but values did not reach statistical significance.

TABLE 2. Composition of test diets as determined by chemical analysis[a]

Constituent	Baseline (BL)	Soybean Oil (SO)	Squeeze Margarine (SQM)	Tub Margarine (TM)	Stick Margarine (SM)	Butter (BT)
	percentage of total daily energy intake					
Protein	16.8	15.7	17.1	16.3	16.7	16.9
Carbohydrate	44.6	55.8	51.7	52.9	53.5	53.9
Fat	38.6	28.5	31.2	30.8	29.7	29.1
Saturated fatty acids	15.45	7.30	8.59	8.40	8.47	16.70
12:0	1.77	0.83	0.96	0.67	0.82	1.35
14:0	0.12	0.63	0.74	0.55	0.60	2.50
16:0	7.77	3.65	4.26	4.18	4.03	7.47
18:0	3.61	1.45	1.85	2.28	2.22	3.57
Monounsaturated fatty acids[b]	15.05	8.14	8.08	8.04	8.46	8.07
18:1	12.15	7.20	7.11	6.65	6.53	6.97
Polyunsaturated fatty acids[b]	6.97	12.48	13.54	11.14	6.34	2.43
18:2	5.86	10.74	12.10	9.99	5.60	2.07
18:3	1.00	1.67	1.39	1.10	0.70	0.29
Trans fatty acids	1.69	0.55	0.91	3.30	6.72	1.25
Cholesterol ($mg \cdot 1000\ kcal^{-1}$)	163.8	65.9	68.0	70.3	66.5	121.0

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

TABLE 3. Serum lipid and lipoprotein profile at the end of each phase

Variable	Baseline (BL)	Soybean Oil (SO)	Squeeze Margarine (SQM)	Tub Margarine (TM)	Stick Margarine (SM)	Butter (BT)
			mg·dl^{-1}			
TC	252.2 ± 31.9^{ab}	229.5 ± 28.3^{d}	232.8 ± 28.1^{cd}	239.9 ± 29.9^{bcd}	247.3 ± 33.5^{abc}	255.9 ± 33.3^{a}
LDL-C	175.5 ± 30.8^{a}	156.5 ± 32.4^{b}	157.5 ± 22.4^{bc}	165.2 ± 29.3^{ab}	171.9 ± 30.3^{ac}	176.8 ± 28.7^{a}
HDL-C	51.7 ± 12.0^{a}	46.5 ± 9.2^{bc}	46.5 ± 10.8^{bc}	47.2 ± 9.7^{bc}	45.3 ± 9.4^{c}	50.2 ± 9.6^{ab}
VLDL-C	24.1 ± 7.2	27.1 ± 11.2	26.8 ± 7.9	26.6 ± 7.5	29.6 ± 13.5	28.8 ± 11.1
TG	131.9 ± 44.6	136.9 ± 52.8	131.2 ± 53.5	140.5 ± 55.8	154.5 ± 64.2	139.1 ± 45.6

Within a row, values (mean ± SD) with different superscripts are significantly different ($P < 0.05$). To convert values for cholesterol to millimoles per liter, divide by 38.67. To convert values for triglycerides to millimoles per liter, divide by 88.54.

With respect to the cholesterol kinetic data, FSR-FC rates (**Fig. 1**) were higher ($P < 0.05$) on the SO (0.078 ± 0.024 p·d^{-1}), SQM (0.081 ± 0.019 p·d^{-1}), and TM (0.086 ± 0.029 p·d^{-1}) diets when compared to the SM (0.053 ± 0.029 p·d^{-1}) and BL (0.053 ± 0.023 p·d^{-1}) diets. These rates represent the proportion of the rapidly turning over pool of cholesterol synthesized per day. In order to determine the absolute amount of de novo cholesterol synthesized per day, ASR-FC (**Fig. 2**) rates were calculated. The ASR-FC data mimicked the FSR-FC data with rates being lower ($P < 0.05$) on the low fat high *trans* SM (0.450 ± 0.258 g·d^{-1}) and high fat BL (0.458 ± 0.195 g·d^{-1}) diets when compared to the SO and other two margarine diets (0.661 ± 0.234, 0.689 ± 0.192, and 0.739 ± 0.273 g·d^{-1}, respectively). The BT diet also resulted in lower FSR-FC (0.062 ± 0.017 p·d^{-1}) and ASR-FC (0.557 ± 0.193 g·d^{-1}) rates, but differences were significant only when compared to the TM (0.086 ± 0.029 p·d^{-1} and 0.739 ± 0.273 g·d^{-1}, respectively) diet.

DISCUSSION

Considerable interest has been focussed on the mechanism by which dietary fatty acids influence plasma TC and LDL-C concentrations because elevated levels are associated with a greater risk of developing coronary heart disease (26, 27). One specific class of dietary fatty acids that has received growing attention is *trans* fatty acids. These fatty acids are known to cause changes in plasma lipid and lipoprotein cholesterol profiles (3–10, 12), but the mechanisms involved are unclear. Thus the present study was conducted to determine whether endogenous cholesterogenesis changes in response to shifts in dietary *trans* and fatty acid profile, and consequently influences circulating lipid levels.

Using the DI method to measure de novo cholesterol synthesis, we have demonstrated for the first time that FSR-FC and ASR-FC rates were lower with stick margarine and high fat BL feeding when compared to the oil and softer margarine diets. The BT diet was intermediate in its effects on cholesterol synthesis relative to the other diets. However, the reverse was seen with regard to the plasma lipid and lipoprotein data, with consumption of the former diets producing the most favorable profile and the latter diets producing the least desirable profile. As circulating cholesterol levels reflect changes in synthesis and clearance, it can be speculated that the enhanced biosynthesis seen with SO, SQM, and TM feeding was compensated by an even greater rate of clearance, which could, in part, explain the lower lipid levels seen after consumption

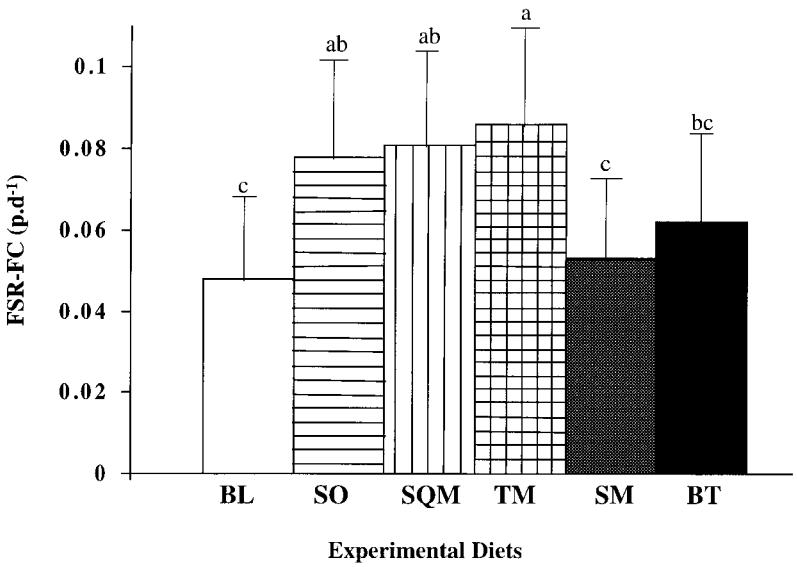

Fig. 1. Fractional synthesis rates of free cholesterol (FSR-FC) at the end of each dietary phase. Data are presented as means ± SD, n = 14. Diet abbreviations are as follows: BL, baseline; SO, soybean oil; SQM, squeeze margarine; TM, tub margarine; SM, stick margarine; BT, butter. Columns with different letters are significantly different ($P < 0.05$).

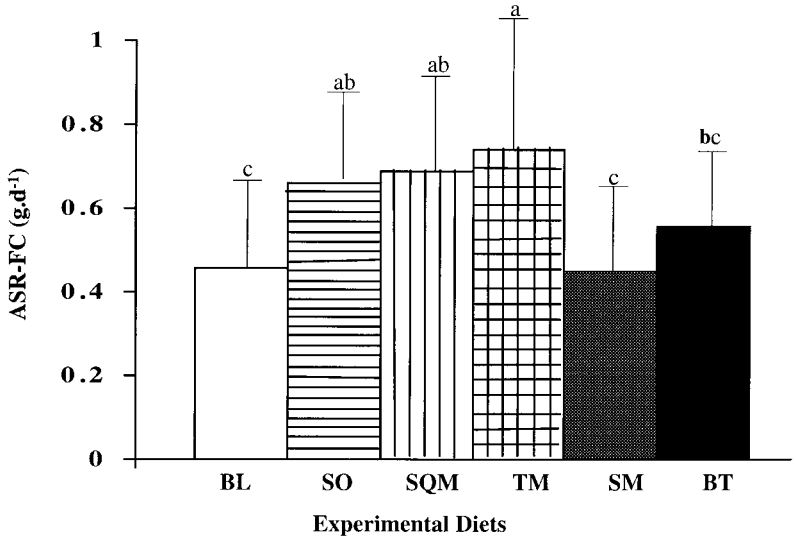

Fig. 2. Absolute synthesis rates of free cholesterol (ASR-FC) at the end of each dietary phase. Data are presented as means ± SD, n = 14. Diet abbreviations are as follows: BL, baseline; SO, soybean oil; SQM, squeeze margarine; TM, tub margarine; SM, stick margarine; BT, butter. Columns with different letters are significantly different ($P < 0.05$).

of these diets. This hypothesis is consistent with the findings of increased net fecal neutral and acidic sterol excretion seen with corn oil feeding compared to stick margarine or beef tallow (28). Conversely, despite marked suppression of synthesis, the higher plasma TC and LDL-C levels seen on the SM, BT, and BL diets suggest some impairment in the catabolic pathway of cholesterol. One mechanism (29) proposed involves interference with LDL receptor-mediated clearance of cholesterol-rich lipoproteins. Spady and Dietschy (30), using a hamster model, have demonstrated that dietary saturated fatty acids (SFA) affect LDL cholesterol levels through changes in the activity of the hepatic LDL receptor. The liver enzyme acyl-CoA:cholesterol acyltransferase (ACAT) that converts free cholesterol to its esterified form has a lower affinity for saturated than unsaturated fatty acids. In fact, Woollett, Daumerie, and Dietschy (31) have demonstrated in animals that the selectivity of ACAT for unsaturated fatty acids is lost when the double bond is in the *trans* configuration (specifically *t*-C18:1n–9). The resulting accumulation of hepatic free cholesterol could lead to down-regulation of the LDL receptor, causing accumulation of LDL particles in plasma and increased formation of LDL from its precursor, VLDL. An alternate mechanism, supported by in vitro studies (32–34) involves enrichment of cell membrane phospholipids with *trans* and saturated fatty acids. The resultant alterations in membrane fluidity could reduce binding or internalization of circulating LDL, thus accounting for the higher plasma cholesterol levels despite lowered synthesis.

These findings suggest that *trans* and SFA-rich diets elevate TC and LDL-C levels by similar mechanisms. However, among the reduced fat diets, the BT diet, despite its higher SFA and cholesterol content, produced a similar reduction in endogenous cholesterol synthesis when compared to the SM diet. This finding is not readily explainable but could relate to the fatty acid profile of the BT diet. It has been reported (35) that consumption of diets high in 16:0 but low in linoleic acid (18:2 n–6), which is similar to the fatty acid profile of the BT diet, does not alter endogenous cholesterol synthesis rates relative to either a low 16:0/high 18:2 n–6 diet or a high 16:0/high 18:2 n–6 diet. Thus, the high 16:0 content of the BT diet could attenuate the 12:0 + 14:0, and exogenous cholesterol-induced reduction in synthesis, and account for the observed modest but not marked suppression of FSR-FC and ASR-FC rates.

A limitation of the present study is the difficulty in partitioning out effects of *trans* from other fatty acids in the experimental diets. However, our study does allow assessment of the altered fatty acid profile resulting from hydrogenation, and consequently the impact of consuming various hydrogenated products. Secondly, the validity of the DI method has been questioned because there is no physiological basis for a model based on linear regression (36). While this may be true, the initial, short term deuterium oxide incorporation rate is linear (13, 22), unaffected by flux rates of other unlabeled substances into the system and thus can be taken to represent a direct measure of synthesis independent of total whole body production rate. In addition, the DI method has been compared and validated against the classic sterol balance technique (28). More recently, Di Buono et al (37) measured fractional and absolute synthesis rates of cholesterol simultaneously by the DI and mass isotopomer distribution analysis (MIDA) methods. The authors concluded that both techniques yield comparable rates of cholesterogenesis in humans when measurements are made over 24 h. Consequently, we feel that this method offers a safe, reliable, and non-invasive tool for accurate assessment of human cholesterol biosynthesis.

In summary, cholesterol synthesis rates were lower with consumption of the high *trans* SM and high fat BL diets, when compared to the SO, SQM, and TM diets, with rates after subjects consumed the BT diet being intermediate. However, plasma lipid and lipoprotein concentrations were higher on the former than on the latter diets. This clearly demonstrates that the elevations in circulating levels of cholesterol are not due to a rise in cholesterol synthesis, thereby supporting the hypothesis of an impairment in the

catabolic pathway of cholesterol-rich lipoproteins. While these results support present dietary guidelines to reduce the SFA content of diets, they also suggest the need to specify optimal ratios of individual fatty acids classes including *trans* fatty acid contributions from hydrogenated fats.

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