Dietary Hydrogenated Fat Increases High-Density Lipoprotein apoA-I Catabolism and Decreases Low-Density Lipoprotein apoB-100 Catabolism in Hypercholesterolemic Women


Objective—To determine mechanisms contributing to decreased high-density lipoprotein cholesterol (HDL-C) and increased low-density lipoprotein cholesterol (LDL-C) concentrations associated with hydrogenated fat intake, kinetic studies of apoA-I, apoB-100, and apoB-48 were conducted using stable isotopes.

Methods and Results—Eight postmenopausal hypercholesterolemic women were provided in random order with 3 diets for 5-week periods. Two-thirds of the fat was soybean oil (unsaturated fat), stick margarine (hydrogenated fat), or butter (saturated fat). Total and LDL-C levels were highest after the saturated diet (P<0.05; saturated versus unsaturated) whereas HDL-C levels were lowest after the hydrogenated diet (P<0.05; hydrogenated versus saturated). Plasma apoA-I levels and pool size (PS) were lower, whereas apoA-I fractional catabolic rate (FCR) was higher after the hydrogenated relative to the saturated diet (P<0.05). LDL apoB-100 levels and PS were significantly higher, whereas LDL apoB-100 FCR was lower with the saturated and hydrogenated relative to the unsaturated diet. There was no significant difference among diets in apoA-I or B-100 production rates or apoB-48 kinetic parameters. HDL-C concentrations were negatively associated with apoA-I FCR (r = –0.56, P = 0.03) and LDL-C concentrations were negatively correlated with LDL apoB-100 FCR (r = –0.48, P = 0.05).

Conclusions—The mechanism for the adverse lipoprotein profile observed with hydrogenated fat intake is determined in part by increased apoA-I and decreased LDL apoB-100 catabolism. (Arterioscler Thromb Vasc Biol. 2004; 24:1092-1097.)

Key Words: trans fatty acids ■ hydrogenated fat ■ apolipoprotein kinetics ■ stable isotopes ■ soybean oil ■ margarine ■ butter

Considerable interest has been focused on the mechanism by which dietary fatty acids influence plasma lipoprotein and lipoprotein profiles and subsequently cardiovascular disease (CVD) risk.1–3 One specific class of dietary fatty acids that has received increased attention since the early 1990s is trans fatty acids. These fatty acids are produced as a result of biohydrogenation in the ruminant fat of animals or by commercial hydrogenation of vegetable oils.4 The majority of trans fatty acids in the US diet is contributed by products made with hydrogenated fat such as commercially baked goods, fried foods, and margarines.5,6 Relative to unsaturated fatty acids, trans fatty acid/hydrogenated fat consumption results in higher low-density lipoprotein cholesterol (LDL-C) concentrations and relative to saturated fat lower high-density lipoprotein cholesterol (HDL-C) concentrations.7–13 Several investigators have also reported modest but significant elevations in plasma triglyceride and Lp(a) concentrations.14–16

There are limited data on the mechanism(s) by which trans fatty acid/hydrogenated fat intake alters plasma lipoprotein patterns. Areas investigated thus far include endogenous cholesterol synthesis and esterification17–19 and the activities of cholesterol ester transfer protein (CETP)20–23 and lecithin cholesterol acyltransferase (LCAT).24–26 An alternate approach to defining a mechanism by which trans fatty acids/
TABLE 1. Composition of the Experimental Diets as Determined by Chemical Analysis

<table>
<thead>
<tr>
<th>Dietary Constituent</th>
<th>Unsaturated Fat*</th>
<th>Hydrogenated Fat*</th>
<th>Saturated Fat*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate</td>
<td>55.8</td>
<td>53.5</td>
<td>54.0</td>
</tr>
<tr>
<td>Protein</td>
<td>15.7</td>
<td>16.7</td>
<td>16.9</td>
</tr>
<tr>
<td>Fat</td>
<td>28.5</td>
<td>29.7</td>
<td>29.1</td>
</tr>
<tr>
<td>SFA</td>
<td>7.3</td>
<td>8.5</td>
<td>16.7</td>
</tr>
<tr>
<td>MUFA†</td>
<td>8.1</td>
<td>8.5</td>
<td>8.1</td>
</tr>
<tr>
<td>PUFAt</td>
<td>12.5</td>
<td>6.3</td>
<td>2.4</td>
</tr>
<tr>
<td>Trans</td>
<td>0.6</td>
<td>6.7</td>
<td>1.3</td>
</tr>
<tr>
<td>Cholesterol (mg/1000 kcal)</td>
<td>66</td>
<td>67</td>
<td>121</td>
</tr>
</tbody>
</table>

* Percentage of total daily energy intake. Percentages may not total 100 because of rounding.
†Only cis isomers are included.

hydrogenated fat alters lipoprotein concentrations relative to other fats is to directly assess the kinetic behavior of major apoproteins associated with HDL and LDL particles. This area remains largely unexplored in humans.

The aim of the present study was to determine the effects of a diet consistent with current dietary recommendations1,27 enriched in soybean oil (unsaturated fat), soybean oil-based traditional stick margarine (hydrogenated fat), or butter (saturated fat) on lipoprotein metabolism, with the intent of elucidating mechanisms contributing to the adverse lipoprotein profile reported with hydrogenated fat intake.

Methods

Subjects

Eight postmenopausal women (older than 50 years) with LDL-C concentrations ≥3.36 mmol/L at the time of screening were recruited for this study. Subjects were free from chronic illness and were not using medications known to affect lipid metabolism (lipid-lowering drugs, fish-oil capsules, beta-blockers, diuretics, or hormones). Subjects who smoked or reported consuming ≥2 alcoholic drinks per day were excluded from the study. The protocol was approved by the Human Investigation Review Committee of the New England Medical Center and Tufts University. All subjects gave written informed consents.

Experimental Design and Diets

Subjects were provided with each of 3 diets for 5-week periods according to a randomized, blinded, crossover design as previously described for an earlier study.7 The experimental fats comprised 20% of energy in each diet. All food and drink were provided to the subjects. Initial energy intakes were calculated using the Harris-Benedict equation and adjustments were made when necessary to maintain body weight. Analysis of the macronutrient and cholesterol contents of the diets were performed by Covance Laboratories (Madison, Wis) and fatty acid profiles by Best Foods Research and Engineering Center (Union, NJ) (Table 1).

Measurement of Lipoprotein Kinetics

At the end of each experimental diet, a primed-constant infusion of deuterated leucine was performed in the fed state to determine the kinetics of HDL apoA-I, triglyceride-rich lipoprotein (TRL–apoB-100, intermediate-density lipoprotein (IDL–apoB-100, and LDL–apoB-100, and TRL apoB-48. After fasting for 12 hours, subjects were fed hourly for 20 hours starting at 6:00 AM. Each identical meal consisted of one-twentieth their daily caloric intake specific for each dietary phase as previously described.28 Five hours after their first meal, subjects received an intravenous bolus dose (10 μmol/kg) followed by a constant infusion (10 μmol/kg per hour) of [5,5,5-H2]-l-leucine over a 15-hour period. Blood samples (20 mL) were collected via a second intravenous line at 0, 0.5, 0.75, 1, 2, 3, 4, 6, 8, 10, 12, and 15 hours.

The protocol for plasma lipid and lipoprotein characterization, quantification, and isolation of the apolipoproteins, isotopic enrichment determinations, and kinetic analysis were performed as previously described.29–37 For HDL compositional analysis, total and free cholesterol, triglyceride, and phospholipid concentrations were determined using commercially available enzymatic assays (Roche Diagnostics, Indianapolis, Ind and Wako Diagnostics, Richmond, Va). Total protein was determined using the Biuret method. The cholesteryl ester and phospholipid fatty acid composition of the HDL fraction was determined using gas chromatography.28 At the end of each dietary infusion protocol, plasma samples were collected 10 minutes before and after the administration of heparin (100 U heparin sodium/kg body weight) for the measurement of lipoprotein lipase (LPL) and hepatic lipolytic activity (HTGL).35,36

Kinetic Analysis

The kinetics of HDL apoA-I and TRL apoB-48 (Figure I, available online at http://atvb.ahajournals.org) and apoB-100 (Figure II, available online at http://atvb.ahajournals.org) in TRL, IDL, and LDL fractions were determined by fitting the multicompartamental model to the tracer/tracer (T/T) ratio data using the SAAM II program (SAAM Institute, Seattle, Wash) as previously described.35–37 After fitting the observed data to the respective models, fractional catabolic rates (FCR) (in pools/d) and production rates (PR) (in mg/kg per day) of apoA-I, apoB-100, and apoB-48 were calculated as described.35–37

Statistical Analyses

One-way analyses of variance with diet as the study factor and subject as the repeated measure was used on each outcome variable using a SAS general linear model program (SAS version 6; SAS Institute Inc, Cary, NC). Differences in group means were identified using Tukey honestly significant differences at a significance level of P<0.05. Within subject partial correlation coefficients adjusted for diet were calculated between variables of interest. If data were not normally distributed, appropriate transformations were performed before statistical testing. Untransformed data are presented in text and tables as means±standard deviation (SD). The lipid and lipoprotein values are averages of 3 measurements taken at the end of each dietary phase.

Results

Lipids and Lipoproteins

Total and LDL-C concentrations were 11% and 15% higher after subjects consumed the saturated fat relative to the unsaturated fat diet (P<0.05, Table 2), with levels being intermediate between the saturated and unsaturated diets after subjects consumed the hydrogenated fat diet (7% and 12%, respectively). In contrast, HDL-C concentrations were 7% lower after subjects consumed the hydrogenated relative to saturated fat diet (P<0.05). Triglyceride concentrations were highest after subjects ate the hydrogenated and lowest after the unsaturated fat diets, although these differences did not reach statistical significance. These data suggest that the subjects participating in this kinetic study responded to the dietary fat similarly to that as would be predicted on the basis of other human metabolic studies.7,10,12,14
ApoA-I concentrations and PS followed a pattern similar to that of HDL-C, lowest after the hydrogenated and highest after the saturated fat diet (Table 3). These differences were accounted for by an 11% higher apoA-I FCR after subjects consumed the hydrogenated relative to the saturated fat diet ($P<0.05$). The FCR after subjects ate the unsaturated diet was intermediate, with the differential between the hydroge-

### TABLE 2. Lipid and Lipoprotein Concentrations at the End of Each ExperimentalPhase

<table>
<thead>
<tr>
<th>Variable</th>
<th>Experimental Diets</th>
<th>Experimental Diets</th>
<th>Experimental Diets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unsaturated Fat</td>
<td>Hydrogenated Fat</td>
<td>Saturated Fat</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>4.85±0.73*</td>
<td>5.16±0.57†</td>
<td>5.39±0.76†</td>
</tr>
<tr>
<td>LDL-C</td>
<td>2.99±0.57*</td>
<td>3.27±0.39†</td>
<td>3.43±0.72†</td>
</tr>
<tr>
<td>HDL-C</td>
<td>1.32±0.39†</td>
<td>1.30±0.35*</td>
<td>1.39±0.33†</td>
</tr>
<tr>
<td>HDL2</td>
<td>0.27±0.16</td>
<td>0.28±0.18</td>
<td>0.29±0.18</td>
</tr>
<tr>
<td>HDL3</td>
<td>1.05±0.23</td>
<td>1.02±0.24</td>
<td>1.10±0.19</td>
</tr>
<tr>
<td>VLDL-C</td>
<td>0.55±0.24</td>
<td>0.59±0.29</td>
<td>0.57±0.29</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.21±0.52</td>
<td>1.29±0.64</td>
<td>1.25±0.63</td>
</tr>
</tbody>
</table>

Values are mean±SD.

*†For each variable, values with different superscripts are significantly different at $P<0.05$. To convert values for cholesterol to milligrams per deciliter, multiply by 38.67. To convert values for triglycerides to milligrams per deciliter, multiply by 88.54.

### ApoA-I Kinetics

ApoA-I concentrations and PS followed a pattern similar to that of HDL-C, lowest after the hydrogenated and highest after the saturated fat diet (Table 3). These differences were accounted for by an 11% higher apoA-I FCR after subjects consumed the hydrogenated relative to the saturated fat diet ($P<0.05$). The FCR after subjects ate the unsaturated diet was intermediate, with the differential between the hydroge-

### TABLE 3. Kinetic Parameters of ApoA-I, HDL Fatty Acid Profile, and HDL Composition at the End of Each Experimental Phase

<table>
<thead>
<tr>
<th>Variable</th>
<th>Experimental Diets</th>
<th>Experimental Diets</th>
<th>Experimental Diets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unsaturated Fat</td>
<td>Hydrogenated Fat</td>
<td>Saturated Fat</td>
</tr>
<tr>
<td>ApoA-I (mg/dL)</td>
<td>117±13†</td>
<td>114±14*</td>
<td>118±12†</td>
</tr>
<tr>
<td>Pool Size (mg)</td>
<td>3326±287†</td>
<td>3211±358*</td>
<td>3353±198†</td>
</tr>
<tr>
<td>FCR (pools/d)</td>
<td>0.19±0.04†</td>
<td>0.20±0.04‡</td>
<td>0.18±0.03*</td>
</tr>
<tr>
<td>PR (mg/kg per day)</td>
<td>9.79±0.89</td>
<td>10.14±1.94</td>
<td>9.53±1.12</td>
</tr>
<tr>
<td>HDL Fatty Acid Profile (molar percent)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesteryl Ester</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SFA</td>
<td>7.7±2.1</td>
<td>6.4±1.5</td>
<td>8.1±3.1</td>
</tr>
<tr>
<td>MUFA‡</td>
<td>18.1±2.2*</td>
<td>18.9±2.3*</td>
<td>25.9±3.5†</td>
</tr>
<tr>
<td>PUFA</td>
<td>73.8±3.0†</td>
<td>74.1±2.9†</td>
<td>65.3±7.0*</td>
</tr>
<tr>
<td>Trans (18:1)</td>
<td>0.1±0.2†</td>
<td>0.4±0.2†</td>
<td>0.1±0.1*</td>
</tr>
<tr>
<td>Phospholipid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SFA</td>
<td>39.5±3.3</td>
<td>37.5±2.6</td>
<td>40.2±2.5</td>
</tr>
<tr>
<td>MUFA‡</td>
<td>7.7±0.9*</td>
<td>8.5±1.5*</td>
<td>9.9±2.1†</td>
</tr>
<tr>
<td>PUFA</td>
<td>50.0±3.3</td>
<td>49.2±2.7</td>
<td>46.8±3.7</td>
</tr>
<tr>
<td>Trans (18:1)</td>
<td>0.8±0.5*</td>
<td>3.0±0.9†</td>
<td>1.2±0.8*</td>
</tr>
<tr>
<td>HDL Composition (% wt/wt)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>43.6±2.8</td>
<td>44.9±3.0</td>
<td>42.4±7.3</td>
</tr>
<tr>
<td>Free cholesterol</td>
<td>3.1±1.0</td>
<td>2.6±0.5</td>
<td>3.3±1.2</td>
</tr>
<tr>
<td>Cholesteryl ester</td>
<td>20.3±2.1</td>
<td>19.3±2.2</td>
<td>20.6±4.0</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>6.3±2.0</td>
<td>6.6±2.1</td>
<td>5.9±1.3</td>
</tr>
<tr>
<td>Phospholipid</td>
<td>26.7±1.4</td>
<td>26.0±2.1</td>
<td>28.4±4.0</td>
</tr>
</tbody>
</table>

‡Only cis isomers are included.

Values are mean±SD.

FCR indicates fractional catabolic rate; PR, production rate.

*†For each variable, values with different superscripts are significantly different at $P<0.05$. 

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The relative proportion of trans fatty acids was significantly higher at the expense of monounsaturated fatty acids. These differences in the fatty acid profile of the HDL subfraction are consistent with an earlier study that had a larger cohort. There were no significant differences in HDL composition as a result of altering dietary fat.

**ApoB-100 Kinetics**

Plasma TRL and LDL apoB-100 values, PS, FCR, and PR were not significantly different among diet phases (Table 4). Plasma LDL apoB-100 concentrations and PS were 12% and 14% higher after subjects consumed the hydrogenated relative to the unsaturated fat diet (P<0.05), with concentrations being intermediate after subjects consumed the hydrogenated fat diet. LDL apoB-100 FCR were 20% and 13% lower after subjects consumed the hydrogenated relative to the saturated fat diet (P<0.05). There was no significant effect of dietary fat type on LDL apoB-100 PR. LDL apoB-100 FCR was negatively correlated with plasma LDL-C (r=−0.48; P<0.05) (Figure B) and LDL apoB-100 concentrations (r=−0.35; P=0.05). Plasma LDL-C concentrations were not significantly related to differences in LDL-C apoB-100 PR. These data suggest the mechanism for the LDL-C elevating effect of hydrogenated fat is similar to that of saturated fat, primarily determined by decreased catabolism, resulting in a shorter residence time.

**ApoB-48 Kinetics**

ApoB-48 concentrations and PS values tended to be 78% (P=0.19) and 70% (P=0.21) higher after subjects consumed...
the hydrogenated diet relative to the unsaturated fat diet, which was accompanied by a 19% lower FCR (P = 0.15) and 44% higher PR (P = 0.19) (Table 4). Because of the high degree of variability in response among subjects, these differences did not attain statistical significance.

**Lipoprotein Lipase Activity**

There was no significant effect of dietary fat type on lipase activities. Total lipoprotein lipase activity was 3105 ± 2998, 3562 ± 948, and 4471 ± 1116 nEq of free fatty acids (FFA)/mL per hour, and HTGL activity 12 168 ± 1665, 11 654 ± 1279, and 11 901 ± 1398 nEq FFA/mL per hour, after subjects consumed the unsaturated, hydrogenated, and saturated fat diets, respectively.

**Discussion**

Dietary trans fatty acid/hydrogenated fat intake has been shown to increase plasma LDL-C concentrations relative to unhydrogenated oil and decrease HDL-C concentrations relative to saturated fat.7–9 The results of this study are consistent with previous observations. The unique finding of this study is that the metabolic basis for these effects is predominantly caused by changes in FCR rather than PR for HDL apoA-I and LDL apoB-100.

Data identifying the metabolic basis for differences in HDL-C concentrations induced by trans fatty acid/hydrogenated fat intake are limited. In the only report currently available, Khosla et al 26 assessed the effect of diets high in elaidic acid (18:1 t) or palmitic acid (C 16:0) in normocholesterolemic cubus monkeys. They reported that elaidic acid lowered HDL-C concentrations relative to palmitic acid and that this difference was attributable to an increase in apoA-I FCR. These findings in nonhuman primates are consistent with the results of the current study.

Differences observed in LDL-C concentrations after shifting the fatty acid pattern of the diet from unsaturated to trans fatty or saturated fatty acids were caused by changes in FCR rather than PR. No significant difference in TRL and IDL apoB-100 PS or kinetic behavior was observed attributable to the different experimental fats. These data suggest that differences in LDL-C concentrations were unlikely caused by changes in the conversion rate of TRL to IDL or IDL to LDL, but solely to the clearance of LDL. Similar to these observations, Khosla et al 26 reported no significant effect of elaidic relative to palmitic acid on apoB-100 metabolism in normocholesterolemic cubus monkeys.

In addition to elevated LDL-C concentrations, trans fatty acid intake has been associated with small but at times significant increases in triglyceride concentrations.14–16 Whether the higher triglycerides concentrations are attributable to an increase in the chylomicron remnants or the VLDL fraction is unclear. Our previous work measuring remnant-like particle cholesterol or triglyceride concentrations after subjects consumed diets enriched in polyunsaturated or hydrogenated fat was not helpful in addressing this issue.41 In the present study, triglyceride concentrations and apoB-48 concentrations, PS and PR, tended to be higher, whereas apoB-48 FCR was lower, after subjects consumed the hydrogenated fat diet relative to the other diets. However, these differences did not reach statistical significance. It is important to note that triglyceride carrying lipoprotein particles tend to show a higher degree of lability than other lipoprotein particles. Likewise, differences in the activities of HTGL or LPL (involved in postprandial lipid metabolism by facilitating the clearance of chylomicrons derived from dietary fat) do not appear to explain potential differences in plasma triglyceride concentrations resulting from hydrogenated fat feeding.

In conclusion, the results of the present study demonstrate that an increase in apoA-I clearance rather than a decrease in production rates was the putative factor in modulating HDL-C concentrations after consumption of diets enriched in hydrogenated relative to saturated fat. The mechanism for the LDL-C elevating effect of hydrogenated fat appears to be similar to that of saturated fat, primarily determined by decreased catabolism. These findings support current recommendations to reduce saturated and hydrogenated fat consumption.

**Acknowledgments**

This work was supported by grant (HL 54727) from the National Institute of Health (A.H.L. and N.R.M.). P.H.R.B. and F.K.W. were partially supported by NIH grants P41 EB-001975 and HL 56895, respectively. We are indebted to the staff of the Metabolic Research Unit, including Helen Rasmussen, for their expert care of the study subjects, to Susan Jalbert for the apolipoprotein isotypic enrichment determinations, to Dalan Jensen for the lipase activity measurements, and to the study subjects, without whom this investigation would not be possible.

**References**


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Supplementary Figures for Online Version

ApoA-I or ApoB-48 Compartmental Model. Compartment 1 was the apolipoprotein precursor pool, defined by a forcing function representing the apoB-100 enrichment plateau. Compartment 2 was the intracellular delay compartment representing the time required for the synthesis and secretion of apoB-48 or apoA-I from the intestine and liver. Compartment 3 represented the apoB-48 or apoA-I T/T ratio with direct catabolism of apoB-48 or apoA-I from this compartment.

ApoB-100 Compartmental Model. Compartment 1 was the apolipoprotein precursor pool, defined by a forcing function representing the apoB-100 enrichment plateau. Compartment 2 was an intracellular delay compartment accounting for the synthesis of apoB and assembly of lipoproteins. Compartment 3 represented rapidly turning over TRL particles, while compartments 4, 5 and 6 represented a classical delipidation chain accounting for TRL particles that turned over more slowly. IDL apoB-100 (compartment 7) was derived from either TRL compartment 3 or the delipidation chain. LDL apoB-100 (compartment 8) was derived from the IDL fraction or directly from TRL compartment 3.
Supplementary Figure 1: ApoA-I or ApoB-48 Compartmental Model
Supplementary Figure 2: ApoB-100 Compartmental Model