Interrelationships Between Human Apolipoprotein A-I and Apolipoproteins B-48 and B-100 Kinetics Using Stable Isotopes

Francine K. Welty, Alice H. Lichtenstein, P. Hugh R. Barrett, Gregory G. Dolnikowski, Ernst J. Schaefer

Objective—Our purpose was to determine the relationship between apolipoprotein (apo) A-I and apoB-48 and apoB-100 metabolism in moderately hypercholesterolemic humans.

Methods and Results—The kinetics of apoA-I within high-density lipoprotein (HDL), apoB-48 and apoB-100 within triglyceride-rich lipoproteins, and apoB-100 within intermediate-density lipoprotein and low density-lipoprotein (LDL) were examined with a primed constant infusion of [5,5,5-²H₃] leucine in the fed state (hourly feeding) in 23 subjects after consumption of a 36% total fat diet. Lipoproteins were isolated by ultracentrifugation; apolipoproteins by SDS-PAGE gels; and isotope enrichment assessed by gas chromatograph/mass spectrometry. Kinetic parameters were calculated by multicompartamental modeling of the data with SAAM II. ApoA-I production rate (PR) was correlated with LDL apoB-100 pool size (PS; \( r = 0.49; P = 0.017 \)) and LDL cholesterol \( (r = 0.61; P = 0.002) \), whereas apoA-I fractional catabolic rate (FCR) was inversely correlated with apoB-48 FCR \( (r = -0.40; P = 0.05) \) but not with very low-density lipoprotein apoB-100 FCR.

Conclusions—Two links exist between apoA-I and apoB kinetics: 1) when LDL apoB-100 PS is high, there is increased apoA-I PR; and 2) delayed chylomicron remnant clearance (represented by apoB-48 FCR) is associated with enhanced apoA-I FCR, a finding indicating that alterations in intestinal lipoproteins may be more important in determining HDL cholesterol levels than changes in liver lipoproteins. (Arterioscler Thromb Vasc Biol. 2004;24:1-6.)

Key Words: apolipoprotein A-I ■ apolipoprotein B ■ lipoprotein metabolism ■ stable isotopes ■ HDL cholesterol

High-density lipoprotein cholesterol (HDL-C) levels are inversely correlated with the incidence of coronary heart disease (CHD) in epidemiological and observational population studies.¹⁻⁵ A combined analysis of 4 of the largest US epidemiological studies (Framingham Heart Study, Lipid Research Clinics Prevalence Mortality Follow-up Study, the Lipid Research Clinics Primary Prevention Trial, and the Multiple Risk Factor Intervention Trial) indicated that each 1 mg/dL (0.03 mmol/L) increase in HDL-C confers a 2% decrease in CHD risk in men and a 3% decrease in women.⁶ Therefore, elucidation of mechanisms controlling HDL-C levels is of interest.

Cholesteryl ester (CE) can be delivered from peripheral cells to the liver via 2 pathways, a process known as reverse cholesterol transport. The scavenger receptor class B type I mediates hepatic uptake of cholesterol from HDL particles without uptake of apoA-I in the particle.⁷ In the second pathway, CE transfer protein (CETP) transfers CE from HDL to apoB-containing lipoproteins as very low-density lipoprotein (VLDL) and LDL.⁸⁻⁹ Cholesterol is then taken up by hepatic LDL receptors.¹⁰ The cardioprotective effect of HDL-C has been attributed largely to its role in reverse cholesterol transport.

Elevated levels of apoB, the main protein in LDL, are associated with an increased risk of CHD.¹¹ ApoB exists in 2 forms in plasma, apoB-100 and apoB-48,¹² both of which are products of the same structural gene on chromosome 2.¹³ ApoB-100 is synthesized by the liver and secreted within VLDL, which is metabolized in plasma to form LDL. ApoB-100 contains the LDL receptor–binding domain; therefore, VLDL remnants (intermediate density lipoprotein [IDL]) and LDL are removed from circulation by binding to hepatic LDL receptors.¹² Synthesized in the intestine in response to dietary fat, apoB-48 is produced as a result of a premature stop codon at the apoB-100 codon 2153 by tissue-specific mRNA processing and secreted within chylomicrons.¹⁴ Chylomicrons and VLDL are the major triglyceride (TG) carriers in plasma, and
the TGs therein are hydrolyzed by lipoprotein lipase to form chylomicron remnants and VLDL remnants, respectively. ApoB-48 does not contain an LDL receptor–binding domain; therefore, the chylomicron remnants are most likely taken up by the liver by receptors that recognize apoE.15,16

An inverse relationship exists between plasma HDL-C levels and TG levels such that high plasma TG levels are associated with low plasma HDL-C levels and vice versa.17–21 One potential explanation for this is that in individuals with high TGs, the TG content of HDL increases as a result of the consequence of the action of CETP, which transfers TG from the apoB-containing lipoproteins (as chylomicrons or VLDL apoB-100) to HDL in exchange for CE, which is transferred from HDL to the apoB-containing lipoproteins. TG-enriched HDL has been associated with an increased fractional catabolic rate (FCR) of HDL-associated apoA-I,22 a finding that can explain the low plasma level of HDL-C in individuals with high TG levels.

To date, no studies have reported on the relationship between the kinetics of apoB-100 or of apoB-48 in the TG-rich lipoprotein (TRL) fraction and apoA-I. The purpose of the present study was to determine whether a correlation exists between the kinetic parameters for apoB and those for apoA-I. To examine this, we measured the kinetic behavior of both forms of apoB as well as that of apoA-I in the same individual in studies with stable isotopes.

Methods

Subjects

Twenty-three subjects, 6 females and 17 males, underwent a medical history and physical examination. They had no evidence of any chronic illness including endocrine, hepatic, renal, thyroid, or cardiac dysfunction. They did not smoke and were not taking any medications known to affect lipid levels. All female subjects were postmenopausal. The experimental protocol was approved by the Human Investigation Review Committee of the New England Medical Center and Tufts University. The subjects gave informed consent.

Experimental Protocol for In Vivo Stable Isotope Kinetics

To determine the kinetics of TRL apoB-48, VLDL, IDL, LDL, and apoB-100 and apoA-I, the subjects underwent a primed-constant infusion of deuterated leucine while they were in the fed state as described previously.23–28 Starting at 6 AM, subjects received 20 identical small hourly meals, each equivalent to one twentieth of their daily food intake with 15% of calories as protein, 49% carbohydrates, 36% fat (15% saturated, 15% monounsaturated, 6% polyunsaturated), and 180 mg cholesterol per 1000 kcal. At 11 AM, with 2 intravenous lines in place, 1 for the infusion and 1 for blood sampling, [5,5,5-2H3]-leucine (10 μmol/kg body weight) was injected as a bolus intravenously over 1 minute and then by continuous infusion (10 μmol/kg body weight per hour) over a 15-hour period. Blood samples (20 mL) were collected at hours 0, 1, 2, 3, 4, 6, 8, 10, 12, and 15.

Plasma Lipid and Lipoprotein Characterization

Blood was collected in sterile tubes containing EDTA (0.1% final concentration). Plasma was separated from red cells in a refrigerated centrifuge at 3000 rpm for 30 minutes at 4°C. Plasma and lipoprotein fractions were assayed for total cholesterol and TG with an Abbott Spectrum analyzer using enzymatic reagents.29,30 HDL-C was measured as described previously.31 Lipid assays were standardized through the Centers for Disease Control Lipid Standardization Program.

VLDL (d<1.006g/mL), IDL (d=1.006 to 1.019g/mL), LDL (d=1.019 to 1.063g/mL), and HDL (d=1.063 to 1.21g/mL) fractions were isolated from fresh plasma by ultracentrifugation.32 ApoB was assayed in plasma and lipoprotein fractions with a noncompetitive ELISA as described previously.33 ApoA-I was measured in plasma by an immunoturbidimetric assay as described previously.34

Apolipoprotein Quantitation and Isolation

ApoB-48, apoB-100, and apoA-I were isolated from lipoproteins by preparative SDS-PAGE using a Tris-glycine buffer system as described previously.28,34–36 On the basis of the assumption that apoB-100 and apoB-48 have the same chromogenicity, apoB concentrations within individual apoB species were assessed by scanning each gel with laser densitometry as described previously.73–28,37 We scanned VLDL fractions from each time point and averaged all 10 to calculate ratios and to estimate concentrations of apoB-48 and apoB-100 using the total apoB concentration as determined by ELISA.

Isotopic Enrichment Determinations

Isotopic enrichment of leucine in the apolipoproteins was expressed as tracer/tracer ratio (percentage).38

Kinetic Analysis

Kinetics of apoB-100 in VLDL, IDL, and LDL fractions and apoB-48 were reported for 17 of these subjects previously by multicompartamental models as described previously.72 Six additional subjects were studied and added to the present analysis for apoB and apoA-I.

Kinetics of apoA-I were described by the multicompartamental model shown in Figure 1. The model consists of a precursor compartment (compartment 1), which is the plasma leucine pool. Compartment 2 is an intracellular delay compartment accounting for apoA-I synthesis and lipoprotein assembly. Compartment 3 accounts for kinetics of the apoA-I. The FCR of apoA-I corresponds to the rate of irreversible loss from compartment 3. The SAAM II program was used to fit the model to the observed tracer data using a weighted least squares approach to find the best fit.39

It is assumed that plasma leucine (compartment 1) is the source of leucine that is incorporated into apoB and apoA-I and that all apolipoprotein enters the plasma via compartment 3 in each model. Therefore, transport rates into compartment 3 correspond to total apoB-100, apoB-48, and apoA-I production in all models. As described previously,40 we assumed a constant enrichment of the precursor pool and used the VLDL apoB-100 plateau for liver-derived VLDL apoB-100, and the TRL apoB-48 plateau for intestinally derived apoB-48.28 The VLDL apoB-100 plateau was used as the forcing function for apoA-I as described previously.41 Calculation of the plateau values was based on the solution of an exponential model assuming a single compartment.

It was assumed that each subject remains in steady state with respect to apoB-48, apoB-100, and apoA-I metabolism during the study as shown previously.23–27 Under this condition, the FCR is equivalent to the fractional synthetic rate. ApoB and apoA-I production rates (PRs) were determined by the following formula: PR (mg/kg per day) = [FCR (pools per day) × apoB (or apoA-I) concentration (mg/dL) × plasma volume/body weight (kilograms)].35,36 Plasma volume was estimated as 4.5% of body weight.
Statistical Analysis
Data were analyzed using the SysStat program and presented as mean ± SD. Unpaired t tests were performed. Spearman correlation coefficients were determined. P values ≤ 0.05 were considered significant.

Results

Subject Characteristics
Table 1 shows characteristics of the subjects for the total group and by gender. Women were slightly older than men (65 ± 8 versus 56 ± 12 years, respectively; P = 0.07) and had significantly higher levels of HDL-C compared with men (54 ± 7 mg/dL versus 42 ± 9 mg/dL; P = 0.01).

In Vivo ApoB Kinetics
During the kinetic studies, plasma apoB and lipid concentrations did not change significantly throughout the infusion period, indicating steady-state conditions as shown previously.23–27 Representative apoA-I leucine tracer/tracee ratios and model-predicted values are shown in Figure 2. Representative VLDL, IDL, and LDL apoB-100 and apoB-48 leucine tracer/tracee ratios and model-predicted values have been shown previously.28

Table 2 shows pool sizes (PSs), FCRs, and PRs for apoB-48, apoB-100, and apoA-I. There were no significant differences in kinetic parameters between men and women. For this reason, subsequent data analyses were done for the group as a whole. PSs of TRL apoB-48, VLDL apoB-100, LDL apoB-100, and apoA-I were 15 ± 9 mg, 237 ± 146 mg, 3607 ± 1020 mg, and 4278 ± 808 mg, respectively. Mean FCRs for TRL apoB-48, VLDL apoB-100, LDL apoB-100, and apoA-I were 4.3 ± 2.0, 6.1 ± 3.4, 0.24 ± 0.08, and 0.15 ± 0.04, respectively.

Table 1. Baseline Subject Characteristics

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (years)</th>
<th>Weight (kg)</th>
<th>Body Mass Index (kg/m²)</th>
<th>Total Cholesterol (mg/dL)</th>
<th>LDL-C mmol/L (mg/dL)</th>
<th>HDL-C (mg/dL)</th>
<th>TGs (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All, Mean ± SD</td>
<td>58 ± 11</td>
<td>77.5 ± 12.2</td>
<td>26.7 ± 3.2</td>
<td>5.93 ± 1.17 (229 ± 45)</td>
<td>4.07 ± 0.96 (157 ± 37)</td>
<td>1.17 ± 0.26 (45 ± 10)</td>
<td>1.32 ± 0.35 (117 ± 31)</td>
</tr>
<tr>
<td>Male</td>
<td>56 ± 12</td>
<td>79.4 ± 11.0</td>
<td>26.3 ± 2.4</td>
<td>5.88 ± 1.19 (227 ± 46)</td>
<td>3.99 ± 0.96 (154 ± 37)</td>
<td>1.09 ± 0.23 (42 ± 9)</td>
<td>1.34 ± 0.38 (119 ± 34)</td>
</tr>
<tr>
<td>Female</td>
<td>65 ± 8</td>
<td>72.2 ± 15.2</td>
<td>27.9 ± 4.8</td>
<td>6.11 ± 1.09 (236 ± 42)</td>
<td>4.25 ± 0.96 (164 ± 37)</td>
<td>1.40 ± 0.18 (54 ± 7)</td>
<td>1.24 ± 0.25 (110 ± 22)</td>
</tr>
<tr>
<td>P value</td>
<td>0.07</td>
<td>0.32</td>
<td>0.47</td>
<td>0.66</td>
<td>0.59</td>
<td>0.01</td>
<td>0.44</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SD.
Table 3 and Figure 3 show correlations. ApoA-I FCR was inversely correlated with apoB-48 FCR ($r = -0.40; P = 0.05$; Figure 3) but not with VLDL apoB-100 FCR. FCR of apoA-I was significantly and positively correlated with the plasma level of TRL cholesterol ($r = 0.48; P = 0.02$) more than the absolute TG level ($r = 0.36; P = 0.09$). ApoA-I PR was correlated with LDL apoB-100 PS ($r = 0.49; P = 0.017$; Figure 3) and specifically with concentration of LDL cholesterol (LDL-C; $r = 0.61; P = 0.002$). ApoA-I PS was positively correlated with apoA-I PR ($r = 0.65; P = 0.0009$) and not with apoA-I FCR ($r = 0.24; P = 0.272$). HDL-C was inversely correlated with apoB-48 FCR ($r = -0.405; P = 0.05$) and plasma TG ($r = -0.391; P = 0.06$).

### Discussion

The present study provides information on the interrelationships between kinetics of apoA-I, TRL apoB-48, and VLDL, IDL, and LDL apoB-100 in 23 human subjects on an average American diet in the fed state using stable isotopes and multicompartamental modeling. Our overall data support the concept of 2 significant links between metabolism of apoB and apoA-I-containing lipoproteins. First, we documented that LDL apoB-100 PS and LDL-C levels were positively correlated with apoA-I PR. Although correlations do not prove cause and effect, this finding suggests that when LDL-C levels are increased, apoA-I PR is increased possibly because of a greater need for reverse cholesterol transport in the setting of enhanced delivery of cholesterol to tissues. This relationship may be particularly apparent under the experimental protocol used because of the relatively high saturated fatty acid content of the diet. Under these circumstances, apoA-I production is increased, and there is presumably greater ABCA1-mediated cellular cholesterol efflux, more cholesterol esterification, and more transfer of CE to apoB-containing lipoproteins.

Second, apoA-I FCR was inversely correlated with apoB-48 FCR but not with VLDL apoB-100 FCR. These data indicate that when chylomicron apoB-48 clearance is delayed, apoA-I clearance is enhanced. This finding is consistent with the inverse correlation between plasma TG and HDL-C concentrations.17–21 The flux of TG from the intestine (≈80 g per day on the basis of a 36% fat diet in a person ingesting 2000 calories per day assuming 95% intestinal fat absorption) is 2× greater than the flux from the liver (≈35 g per day on the basis of a VLDL apoB-100 PR of 20 mg/kg per day28 in a 70-kg person and assuming that 2% of VLDL is apoB-100 and 50% is TG [weight percentage]). Therefore,
it would not be surprising that intestinal apoB-containing lipoproteins would be a stronger determinant of HDL-C levels than hepatic apoB-containing lipoproteins.

A likely explanation for the inverse relationship between apoA-I FCR and apoB-48 FCR is that when chylomicron clearance is delayed, there is enhanced CE uptake by TRL and apoA-I production is enhanced, probably because of a greater need for reverse cholesterol transport. Moreover, when chylo-

lomicon apoB-48 clearance is delayed, HDL apoA-I clearance is enhanced, presumably because of HDL TG enrichment.

Acknowledgments

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References


TABLE 3. Correlation Coefficient Analysis

<table>
<thead>
<tr>
<th></th>
<th>apoA-I PS</th>
<th>apoA-I PR</th>
<th>apoA-I FCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>apoA-I PS</td>
<td>0</td>
<td>0.646‡</td>
<td>0.239‡</td>
</tr>
<tr>
<td>apoA-I PR</td>
<td>0.646‡</td>
<td>0</td>
<td>0.808‡</td>
</tr>
<tr>
<td>apoA-I FCR</td>
<td>0.239</td>
<td>0.808‡</td>
<td>0</td>
</tr>
<tr>
<td>apoB-48 PS</td>
<td>0.029</td>
<td>0.059</td>
<td>0.011</td>
</tr>
<tr>
<td>apoB-48 PR</td>
<td>-0.359</td>
<td>-0.381</td>
<td>-0.261</td>
</tr>
<tr>
<td>apoB-48 FCR</td>
<td>-0.147</td>
<td>-0.334</td>
<td>-0.40‡</td>
</tr>
<tr>
<td>VLDL apoB-100 PS</td>
<td>0.026</td>
<td>-0.070</td>
<td>-0.057</td>
</tr>
<tr>
<td>VLDL apoB-100 PR</td>
<td>0.077</td>
<td>-0.157</td>
<td>-0.261</td>
</tr>
<tr>
<td>VLDL apoB-100 FCR</td>
<td>0.049</td>
<td>0.061</td>
<td>-0.006</td>
</tr>
<tr>
<td>LDL apoB-100 PS</td>
<td>0.518‡</td>
<td>0.492*</td>
<td>0.221</td>
</tr>
<tr>
<td>LDL apoB-100 PR</td>
<td>0.296</td>
<td>0.113</td>
<td>-0.104</td>
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<tr>
<td>LDL apoB-100 FCR</td>
<td>0.138</td>
<td>-0.075</td>
<td>-0.038</td>
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<tr>
<td>LDL apoB-100</td>
<td>0.195</td>
<td>0.199</td>
<td>-0.072</td>
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<tr>
<td>LDL apoB-100</td>
<td>0.370</td>
<td>0.608‡</td>
<td>0.310</td>
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<tr>
<td>TRL-C</td>
<td>-0.233</td>
<td>0.206</td>
<td>0.484*</td>
</tr>
<tr>
<td>TGs</td>
<td>0.017</td>
<td>0.175</td>
<td>0.363</td>
</tr>
</tbody>
</table>

*P<0.05; †P<0.01; ‡P<0.001.


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