Effects of ApoE Genotype on ApoB-48 and ApoB-100 Kinetics With Stable Isotopes in Humans

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

Abstract—Subjects with the apolipoprotein (apo) E4 allele have been shown to have higher low density lipoprotein (LDL) cholesterol and apoB levels than do subjects with the other alleles. To elucidate the metabolic mechanisms responsible for this finding, we examined the kinetics of apoB-48 within triglyceride-rich lipoproteins (TRLs) and of apoB-100 within very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), and LDL by using a primed constant infusion of [5,5,5-2H3]leucine in the fed state (hourly feeding) during consumption of an average American diet in 18 normolipidemic subjects, 12 of whom had the apoE3/E3 genotype and 6, the apoE3/E4 genotype. Lipoproteins were isolated by ultracentrifugation and apolipoproteins, by sodium dodecyl sulfate gels; isotope enrichment was assessed by gas chromatography–mass spectrometry. Kinetic parameters were calculated by multicompartamental modeling of the data with SAAM II software. Compared with the apoE3/E3 subjects, the apoE3/E4 subjects had significantly higher levels of total apoB, 100.1 ± 17.8 versus 135.4 ± 34.0 mg/dL (P = 0.009), and significantly higher levels of LDL apoB-100, 88.1 ± 19.2 versus 127.5 ± 32.7 mg/dL (P = 0.005), respectively. The pool size of TRL apoB-48 was 17.4% lower for apoE3/E4 subjects compared with apoE3/E3 subjects due to a 33.3% lower production rate (P = 0.28). There was no significant difference in the TRL apoB-48 fractional catabolic rate (5.1 ± 2.2 versus 5.0 ± 2.1 pools per day). The pool size for VLDL apoB-100 was 36% lower for apoE3/E4 subjects compared with apoE3/E3 subjects due entirely to a 30% lower production rate (P = 0.04). The LDL apoB-100 pool size was 57.8% higher (P = 0.003) for apoE3/E4 subjects compared with apoE3/E3 subjects due to a 35.5% lower fractional catabolic rate of LDL apoB-100 (P = 0.003), with no significant difference in production rate. In addition, 77% of VLDL apoB-100 in apoE3/E4 subjects compared with 58% in apoE3/E3 subjects (P = 0.05). In conclusion, the presence of 1 E4 allele was associated with higher LDL apoB-100 levels owing to lower fractional catabolism of LDL apoB-100 and a 33% increase in the conversion of VLDL apoB-100 to LDL apoB-100. (Arterioscler Thromb Vasc Biol. 2000;20:1807-1810.)

Key Words: apolipoprotein B ■ apolipoprotein E ■ stable isotopes ■ LDL cholesterol ■ lipoprotein kinetics

The mechanisms regulating the synthesis and secretion of apoB-100 and apoB-48 are incompletely understood but important, since elevated levels of apoB, the main protein in LDL, are associated with an increased risk of developing coronary heart disease.1 ApoB exists in 2 forms in plasma, apoB-100 and apoB-48,2 both of which are products of the same structural gene on chromosome 2.3 ApoB-100 is synthesized by the liver and secreted within VLDLs, which are hydrolyzed by lipoprotein lipase to form VLDL remnants (IDLs). Approximately 50% of VLDL remnants are removed directly from plasma by an apoE-mediated process; the remainder are metabolized in plasma to form LDL. ApoB-100 contains the LDL receptor–binding domain; therefore, LDLs are removed from the circulation by binding to hepatic LDL receptors.2

Produced as a result of a premature stop codon at the apoB-100 codon 2153 by tissue-specific mRNA processing, apoB-48 is synthesized in the intestine and secreted within chylomicrons.4 Both chylomicrons and VLDL are the major triglyceride carriers in plasma, and the triglycerides 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, chylomicron remnants are most likely taken up by hepatic LDL receptors via apoE and by other hepatic receptors that recognize apoE.5,6 such as LDL receptor–related protein and heparan sulfate proteoglycans.7,8

ApoE is an important protein component of VLDL and functions as a ligand in the receptor-mediated clearance of cholesterol.9 ApoE exists in 3 forms in plasma, apoE2, apoE3, and apoE4,9,10 all of which are produced in the liver.11 ApoE4 is associated with higher cholesterol levels in VLDL, intermediate density lipoprotein (IDL), and LDL by using a primed constant infusion of [5,5,5-2H3]leucine in the fed state (hourly feeding) during consumption of an average American diet in 18 normolipidemic subjects, 12 of whom had the apoE3/E3 genotype and 6, the apoE3/E4 genotype. Lipoproteins were isolated by ultracentrifugation and apolipoproteins, by sodium dodecyl sulfate gels; isotope enrichment was assessed by gas chromatography–mass spectrometry. Kinetic parameters were calculated by multicompartamental modeling of the data with SAAM II software. Compared with the apoE3/E3 subjects, the apoE3/E4 subjects had significantly higher levels of total apoB, 100.1 ± 17.8 versus 135.4 ± 34.0 mg/dL (P = 0.009), and significantly higher levels of LDL apoB-100, 88.1 ± 19.2 versus 127.5 ± 32.7 mg/dL (P = 0.005), respectively. The pool size of TRL apoB-48 was 17.4% lower for apoE3/E4 subjects compared with apoE3/E3 subjects due to a 33.3% lower production rate (P = 0.28). There was no significant difference in the TRL apoB-48 fractional catabolic rate (5.1 ± 2.2 versus 5.0 ± 2.1 pools per day). The pool size for VLDL apoB-100 was 36% lower for apoE3/E4 subjects compared with apoE3/E3 subjects due entirely to a 30% lower production rate (P = 0.04). The LDL apoB-100 pool size was 57.8% higher (P = 0.003) for apoE3/E4 subjects compared with apoE3/E3 subjects due to a 35.5% lower fractional catabolic rate of LDL apoB-100 (P = 0.003), with no significant difference in production rate. In addition, 77% of VLDL apoB-100 in apoE3/E4 subjects compared with 58% in apoE3/E3 subjects (P = 0.05). In conclusion, the presence of 1 E4 allele was associated with higher LDL apoB-100 levels owing to lower fractional catabolism of LDL apoB-100 and a 33% increase in the conversion of VLDL apoB-100 to LDL apoB-100. (Arterioscler Thromb Vasc Biol. 2000;20:1807-1810.)

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cholesterol levels that are population. Persons with a single apoE2 allele have total cholesterol levels been found to influence cholesterol variability in the general population, whereas persons with a single apoE4 allele have total cholesterol levels lower than apoE3.12 ApoE4 differs from apoE3 by a cysteine-to-arginine amino acid substitution at amino acid residue 158.5 Human apoE4 preferentially associates with VLDL, and apoE3 associates with HDL.9–11 In addition, apoE4 has a lower binding affinity to heparan sulfate proteoglycans compared with that of apoE3.12

The polymorphism in the gene coding for apoE is probably the most important inherited trait modulating plasma cholesterol levels in the normal population.13–14 ApoE isoforms have been found to influence cholesterol variability in the general population. Persons with a single apoE2 allele have total cholesterol levels that are ∼10 mg/dL lower, and persons with a single apoE4 allele have total cholesterol levels ∼10 to 20 mg/dL higher than do persons with the apoE3/E3 genotype.13 Postulated mechanisms for the higher cholesterol levels in subjects with an apoE4 allele include more efficient intestinal absorption of cholesterol, which would suppress cholesterol synthesis and LDL apoB receptor activity and thus, result in a higher serum cholesterol level.14–16 In addition, an increased rate of clearance of chylomicron remnant lipoproteins has been observed by using retinyl palmitate techniques and has been postulated to lower hepatic LDL receptor activity and thus, elevate plasma LDL-cholesterol levels.17

In this study, we investigated the kinetics of apoB-100 and apoB-48 within lipoproteins in the constantly fed state in humans by using a primed-constant infusion of deuterated leucine and performing multicompartmental modeling to determine whether subjects with the apoE3/E4 genotype have differences in apoB metabolism compared with subjects with the apoE3/E3 genotype.

### Methods

Eighteen normolipidemic subjects, 8 females and 10 males, underwent stable-isotope studies. The baseline kinetics for these subjects were reported previously.18 The experimental protocol for in vivo stable-isotope kinetics, plasma lipid and lipoprotein characterization, quantification and isolation of the apolipoproteins, isotopic enrichment determinations, kinetic analysis, and statistical analysis were performed as previously described.18 ApoE genotypes were determined by polymerase chain reaction amplification as previously described.19

### Results

#### Characteristics of the Subjects

Table 1 shows the characteristics of the subjects. The apoE3/E4 subjects participating in this study were significantly younger than the apoE3/E3 subjects. Total cholesterol was 15 mg/dL higher and LDL cholesterol, 22 mg/dL higher in apoE3/E4 subjects compared with apoE3/E3 subjects. The nonfasting plasma apoB-100 concentrations in the VLDL, IDL, and LDL lipoprotein fractions and apoB-48 in chylomicrons (Table 2) represent means of measures at all 10 time points during the study period. Compared with the apoE3/E3 subjects, the apoE3/E4 subjects had significantly higher levels of total apoB, 100.1 ± 17.8 versus 135.4 ± 34.0 mg/dL (P = 0.009), and significantly higher levels of LDL apoB-100, 88.1 ± 19.2 versus 127.5 ± 32.7 mg/dL (P = 0.005), respectively.

#### In Vivo Kinetics of ApoB

During the kinetic studies, plasma apoB and lipid concentrations did not change significantly throughout the infusion period, indicating steady-state conditions as previously shown.18 Representative VLDL, IDL, LDL apoB-100, and apoB-48 leucine tracer-tracee ratios and model-predicted values have been shown previously.18 Table 3 shows the pool sizes, fractional catabolic rates, and production rates for apoB-48 and apoB-100 by apoE genotype. The pool size of triglyceride-rich lipoprotein apoB-48 was 17.4% lower for apoE3/E4 subjects compared with apoE3/E3 subjects due to a 33.3% decrease in production rate (P = 0.28). There was no significant difference in the triglyceride-rich lipoprotein...
apoB-48 fractional catabolic rate for apoE3/E3 subjects compared with apoE3/E4 subjects. The pool size for VLDL apoB-100 was 36% lower for apoE3/E4 subjects compared with apoE3/E3 subjects due to a 30% decrease in production rate ($P = 0.04$). The pool size for IDL apoB-100 was 14.7% lower for apoE3/E4 subjects compared with apoE3/E3 subjects due to a 16.9% decrease in production rate ($P = 0.49$).

The LDL apoB-100 pool size was 57.8% higher ($P = 0.003$) for apoE3/E4 subjects compared with apoE3/E3 subjects due to a 35.5% lower fractional catabolic rate for LDL apoB-100 ($P = 0.003$), with no effect on LDL apoB-100 production rate. In addition, 77% of VLDL apoB-100 was converted to LDL apoB-100 in apoE3/E4 subjects compared with only 58% in apoE3/E3 subjects ($P = 0.05$). Thus, the presence of 1 apoE4 allele was associated with significantly higher LDL apoB-100 levels owing mainly to a lower fractional catabolic rate of LDL apoB-100. In addition, there was increased conversion of VLDL apoB-100 to LDL apoB-100 in those subjects with 1 apoE4 allele.

Discussion

In the present study, we have shown that apoE3/E4 subjects have higher levels of LDL apoB compared with apoE3/E3 subjects due to a lower fractional catabolic rate of LDL apoB-100 and increased conversion of VLDL apoB-100 to LDL apoB-100. The lower LDL apoB fractional catabolic rate may result from increased intestinal absorption of cholesterol, which suppresses cholesterol synthesis and LDL receptor activity and thus, results in a higher serum cholesterol level.15,16

One prior study has examined VLDL, IDL, and LDL apoB-100 kinetics by using exogenous labeling with radioactivity in subjects homozygous for apoE3 and apoE4 genotypes. Demant et al20 observed that homozygous apoE4 subjects had a significantly lower LDL apoB-100 fractional catabolic rate of 0.20 pool/d, a rate identical to the results in the present study, which examined subjects heterozygous for the apoE4 genotype. The LDL apoB-100 fractional catabolic rate was 23% lower in the homozygous apoE4 subjects compared with homozygous apoE3 subjects. Demant et al20 postulated that apoE4/E4 subjects have a lower hepatic LDL receptor activity than do apoE3/E3 subjects. In addition, those authors showed that 70% of VLDL$_2$ (small VLDL) apoB was converted to LDL apoB in homozygous apoE4 subjects compared with only 50% in subjects with the E3/E3 genotype. These results are also quite comparable to those in the present study, in which apoE4/E3 subjects converted 77% of VLDL apoB-100 to LDL apoB-100 compared with 58% in the apoE3/E3 subjects.

The results of a previous study that used retinyl palmitate to follow chylomicron remnant clearance suggested that an increased rate of clearance of chylomicron remnant lipoproteins occurs in apoE4 subjects relative to apoE3 subjects. The authors concluded that this increased chylomicron remnant clearance may lead to decreased hepatic LDL receptor activity.17 However, the present study, which is the first to study both apoB-48 and apoB-100 kinetics simultaneously by using endogenous labeling of both species, suggests that the rate of clearance of chylomicron remnants is similar in apoE4 subjects compared with apoE3 subjects. Although our sample size may not be large enough to discern a difference in apoB-48 fractional catabolic rate, the similarity in fractional catabolic rates for apoE3 and apoE4 subjects suggests that this possibility is unlikely. ApoB-48 has been difficult to study owing to the difficulty in measuring its low protein concentration, especially in the fasting state.21,22 For this reason, retinyl esters have been used as a marker of chylomicron remnant clearance. Retinol (vitamin A) is absorbed in the intestine as retinyl ester, which is transported in the plasma within chylomicrons and can therefore serve as a marker for the metabolism of these lipoproteins. Once retinyl esters are taken up by the liver, they reenter the plasma as retinol bound to retinol-binding protein. Postprandial retinyl ester clearance has been reported to be much slower than that reported for chylomicron triglyceride, supporting the concept of slower clearance of remnant particles.23-25 However, it has been noted that retinyl ester can exchange between lipoproteins and therefore, may not be an ideal marker of chylomicron remnants.26,27 This finding can confound interpretation of the data. In contrast, apoB-48 appears to be a better marker for chylomicrons and their remnants in plasma because there is no exchange of apoB-48 between lipoproteins, and in humans, the small intestine is the only site of apoB-48 synthesis.2 In addition, endogenous labeling enables observation of the rate of incorporation of the isotope into protein and therefore, theoretically affords the opportunity to observe a more rapidly turning over remnant fraction; however, this was not observed. An additional advantage of our approach is that it allows for the simultaneous measurement of VLDL apoB-100 and triglyceride-rich lipoprotein apoB-48 metabolism.

The production of VLDL apoB-100 is significantly lower in apoE3/E4 subjects compared with that in apoE3/E3 subjects. This finding may be related to the smaller pool size and lower production rate of apoB-48 in subjects with the apoE3/E4 genotype compared with the apoE3/E3 genotype. We have previously shown a significant association of VLDL apoB-100 production rate with apoB-48 pool size and have hypothesized that this finding is related to the amount of lipid

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<th>TABLE 3. Continued</th>
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<tr>
<td>IDL ApoB-100</td>
</tr>
<tr>
<td>PS,$\text{ mg}$</td>
</tr>
<tr>
<td>75.8±40.6</td>
</tr>
<tr>
<td>66.1±35.7</td>
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<tr>
<td>−14.7%</td>
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TRL indicates triglyceride-rich lipoprotein.
delivered to the liver in the form of chylomicron remnants. The lower production of VLDL apoB-100 may also be related to suppression of cholesterol synthesis in the liver that has been postulated for apoE4 subjects. The increased conversion of VLDL apoB-100 to LDL apoB-100 in the apoE3/E4 subjects may be related to a lower LDL receptor activity, such that fewer remnants are removed via the LDL receptor and are thus converted to LDL. The increased conversion may also be related to the lower binding affinity of apoE4 for heparan sulfate proteoglycans, such that fewer VLDL-containing apoE4 particles will be removed through binding to heparan sulfate proteoglycans, and thus, a higher proportion of VLDL-containing apoE4 particles will be converted to LDL. A third potential cause for an increased conversion rate of VLDL apoB-100 to LDL in apoE3/E4 subjects may be the lower production of VLDL apoB-100 from the liver. The lower production would be predicted to result in smaller particles, which are more likely to be converted to LDL apoB-100 rather than being directly removed from the circulation, as is the case for larger VLDL apoB-100 particles as shown by Packard and Shepherd. In conclusion, the presence of 1 apoE4 allele is associated with higher LDL apoB levels due to decreased fractional catabolism of LDL apoB-100 and increased conversion of VLDL apoB-100 to LDL apoB-100. The similarity of our results to those of Demant et al, who studied homozygous apoE4 subjects by using exogenous labeling, indicates that the presence of even 1 apoE4 allele is associated with significant changes in VLDL and LDL apoB-100 metabolism. Therefore, kinetic studies investigating the effect of apoE4 could conceivably be done in heterozygous apoE4 subjects, who are much more common than are homozygous apoE4 subjects in the general population. In addition, the analytic and modeling approach described in the present study design should permit the use of stable isotopes to elucidate key features of both apoB-48 and apoB-100 metabolism in normal and pathological states in humans.

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References

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