Overexpression of Apolipoprotein E3 in Transgenic Rabbits Causes Combined Hyperlipidemia by Stimulating Hepatic VLDL Production and Impairing VLDL Lipolysis

Yadong Huang, Zhong-Sheng Ji, Walter J. Brecht, Stanley C. Rall, Jr, John M. Taylor, Robert W. Mahley

Abstract—The differential effects of overexpression of human apolipoprotein (apo) E3 on plasma cholesterol and triglyceride metabolism were investigated in transgenic rabbits expressing low (<10 mg/dL), medium (10 to 20 mg/dL), or high (>20 mg/dL) levels of apoE3. Cholesterol levels increased progressively with increasing levels of apoE3, whereas triglyceride levels were not significantly affected at apoE3 levels up to 20 mg/dL but were markedly increased at levels of apoE3 >20 mg/dL. The medium expressers had marked hypercholesterolemia (up to 3- to 4-fold over nontransgenics), characterized by an increase in low density lipoprotein (LDL) cholesterol, while the low expressers had only slightly increased plasma cholesterol levels. The medium expressers displayed an 18-fold increase in LDL but also had a 2-fold increase in hepatic very low density lipoprotein (VLDL) triglyceride production, an 8-fold increase in VLDL apoB, and a moderate decrease in the ability of the VLDL to be lipolyzed. However, plasma clearance of VLDL was increased, likely because of the increased apoE3 content. The increase in LDL appears to be due to an enhanced competition of VLDL for LDL receptor binding and uptake, resulting in the accumulation of LDL. The combined hyperlipidemia of the apoE3 high expressers (>20 mg/dL) was characterized by a 19-fold increase in LDL cholesterol but also a 4-fold increase in hepatic VLDL triglyceride production associated with a marked elevation of plasma VLDL triglycerides, cholesterol, and apoB100 (4-, 9-, and 25-fold over nontransgenics, respectively). The VLDL from the high expressers was much more enriched in apoE3 and markedly depleted in apoC-II, which contributed to a >60% inhibition of VLDL lipolysis. The combined effects of stimulated VLDL production and impaired VLDL lipolysis accounted for the increases in plasma triglyceride and VLDL concentrations in the apoE3 high expressers. The hyperlipidemic apoE3 rabbits have phenotypes similar to those of familial combined hyperlipidemia, in which VLDL overproduction is a major biochemical feature. Overall, elevated expression of apoE3 appears to determine plasma lipid levels and may represent an important determinant of specific types of hyperlipoproteinemia. (Arterioscler Thromb Vasc Biol. 1999;19:2952-2959.)

Key Words: apoB ■ lipoprotein lipase ■ VLDL clearance ■ hypertriglyceridemia

Apolipoprotein (apo) E, a 299–amino acid glycoprotein with a molecular weight of 34 200, has a central role in plasma lipid metabolism in both physiological and pathophysiological processes. It is a major protein constituent of triglyceride-rich lipoproteins, including chylomicrons, VLDL, and their remnants. A major function of apoE is to serve as a high-affinity ligand for several hepatic lipoprotein receptors, including the LDL receptor and the LDL receptor–related protein, and for cell-surface heparan sulfate proteoglycans. By interacting with these receptors or with heparan sulfate proteoglycans, apoE mediates the clearance of chylomicrons, VLDL, and their remnants from the circulation. There are 3 common isoforms of apoE—apoE2, apoE3, and apoE4.1 ApoE3 is by far the most frequently occurring and binds normally to lipoprotein receptors. Although apoE4 also binds normally to receptors, other properties cause it to be associated with increased LDL cholesterol levels and sometimes with increased triglyceride levels. Because it is defective in binding to the LDL receptor, apoE2 causes a significant disorder in the metabolism of triglyceride-rich lipoproteins, ie, type III hyperlipoproteinemia, which is characterized by both hypercholesterolemia and hypertriglyceridemia, that is caused by the plasma accumulation of chylomicron and VLDL remnants, ie, β-VLDL.2,5 The plasma level of apoE is also an important determinant of triglyceride-rich lipoprotein levels.6 In fact, changes in
plasma apoE concentrations account for 20% to 40% of the variation of plasma triglyceride levels that is independent of apoE polymorphism. In human subjects, hypertriglyceridemia is correlated positively with increased levels of apoE in both plasma and VLDL. We have demonstrated in transgenic mice that overexpression and accumulation of human apoE3 cause hypertriglyceridemia, especially on an LDL receptor–null background, by stimulating VLDL triglyceride production and by impairing VLDL lipolysis. Furthermore, apoE3 overexpression (10 to 15 mg/dL) in transgenic rabbits also causes hyperlipidemia, largely due to an accumulation of LDL and a concomitant increase in plasma cholesterol. Since lipoprotein metabolism in humans is more similar to that in rabbits than in mice, hyperlipidemic apoE3 rabbits are valuable for studying the relationship between apoE overexpression and hyperlipidemia. Here, we investigated the effects of various levels of human apoE3 overexpression on the development of hyperlipidemia in transgenic rabbits and specifically on the pathways controlling triglyceride-rich lipoprotein metabolism. Our data indicate that increased expression of human apoE3 in transgenic rabbits leads to normolipidemia at low apoE3 levels (<10 mg/dL), hypercholesterolemia at medium apoE3 levels (10 to 20 mg/dL), and combined hypercholesterolemia and hypertriglyceridemia at high apoE3 levels (>20 mg/dL).

Methods

Materials
New Zealand White rabbits were purchased from Charles River (Hollister, CA). A Superox 6 column, purchased from Pharmacia, was used on a Pharmacia fast protein liquid chromatography system. Centrifugation concentration filters were from Amicon. Cholesterol standard was from Abbott. Triglyceride standard and assay kits were from Boehringer Mannheim. An automated system (Kinetic Microplate Reader) was used for lipid analysis. Triton WR1339, oleic acid, BSA without free fatty acids, bovine milk lipoprotein lipase (LPL), and heparinase I were from Sigma. The ECL chemiluminescence detection kit for Western blots was from Amersham Life Science.

Transgenic Rabbits
Transgenic rabbits expressing different plasma levels of human apoE3 were generated previously at the Gladstone Institute of Cardiovascular Disease with a DNA construct containing the human apoE3 gene and its hepatic control region. Transgene expression was detected by immunoblotting rabbit plasma (1 μL) with human-specific anti-apoE antiseraum. In the Western blot assay, human apoE3 was semiquantitated by comparing the densitometric readings of the sample bands with those of different concentrations of purified human apoE. Antibodies and apoE standards were provided by K.H. Weisgraber (Gladstone Institute of Cardiovascular Disease, San Francisco, Calif). All experiments were performed under protocols approved by the Committee on Animal Research, University of California, San Francisco.

Lipoprotein Separation and Analysis
Blood was collected from the intermedial auricular artery of 8- to 12-month-old rabbits that had been fasted overnight. EDTA was used as the anticoagulant (final concentration 10 mmol/L). Plasma was obtained by centrifugation at 14 000 rpm (microcentrifuge) for 10 minutes at 4°C, and samples were stored for no more than 2 days at 4°C in the presence of 1 mmol/L PMSF, a protease inhibitor.

Lipoproteins in 200 μL of plasma were separated by chromatography on a Superox 6 column, as described previously. The major lipoprotein classes eluted from the column were pooled and concentrated with Centricon filters (fractions 16 to 18, VLDL; fractions 19 to 22, IDL; fractions 23 to 27, LDL and a subclass of high density lipoproteins [ie, HDL]); and fractions 28 to 33, HDL). Cholesterol and triglycerides were measured on total plasma and on chromatographic fractions by an enzymatic colorimetric method adapted for use with a microplate reader. Cholesterol and triglycerides in VLDL, IDL, and LDL were calculated from the Superose 6 chromatographic profiles of plasma lipoproteins by summing the individual fractions.

For analysis of apolipoprotein composition or lipolysis assays, VLDL (d<1.006 g/mL), IDL (d=1.006 to 1.02 g/mL), and LDL (d=1.02 to 1.06 g/mL) were isolated from rabbit plasma by ultracentrifugation at 100 000 rpm for 2 hours at 4°C in a Beckman TL100 ultracentrifuge. Cholesterol and triglyceride levels were measured as described above. Apolipoproteins were separated on 3% to 20% polyacrylamide-SDS gradient gels and detected by Coomassie blue staining. There was no detectable apoB48 in the d<1.006 g/mL fractions.

VLDL Triglyceride Production In Vivo
Hepatic VLDL triglyceride production was determined with the Triton WR1339 method. In brief, nontransgenic or apoE3 transgenic rabbits were injected intravenously with 500 mg of Triton WR1339 (400 mg/mL NaCl) per kilogram of body weight after an overnight fast. Blood samples (1 mL) were collected 0, 15, 30, 60, and 90 minutes later. Plasma triglyceride concentrations were measured as described above. The hepatic VLDL triglyceride production rate was calculated from the slope of the curve and presented as micromoles per kilogram per hour.

Lipolysis of VLDL and IDL In Vitro
The suscepsibility of VLDL (d<1.006 g/mL) and IDL (d=1.006 to 1.02 g/mL) to lipolysis was determined by incubating 30 μg of lipoprotein triglycerides with 1 μL of LPL in PBS (pH 7.4) without serum for 30 minutes at 37°C. The levels of released free fatty acids were determined before and after incubation by an enzymatic colorimetric method (Wako Pure Chemical Industries). Lipolysis was calculated by subtracting the values before incubation from the values after incubation. As reported previously, the intra-assay and interassay coefficients of variation for this assay were ~7% and ~9%, respectively.

VLDL Clearance
The VLDL (d<1.006 g/mL) isolated from the plasma of 4 or 5 rabbits from each of the nontransgenic and apoE3 transgenic groups were pooled and iodinated by the method of Bilheimer et al. The 125I-labeled VLDL (5 μg of protein in 100 μL of saline) were injected into the tail vein of normal mice. At each time interval (0, 5, 10, and 20 minutes), 3 mice were euthanized, blood was collected via heart puncture, and the liver was removed. The removal of 125I-VLDL from plasma was determined as described previously. A liver sample was taken for quantitation of uptake of the 125I-VLDL. Plasma clearance and liver uptake were calculated on the basis of the percent of the injected dose of labeled material at different time points after injection. A plasma volume of 3.5% of body weight was used for the calculation.

Cell Association of VLDL
Cultured HepG2 cells were grown to ~100% confluence, washed 3 times with fresh serum-free medium, and incubated at 37°C with 125I-VLDL (5 μg of protein). In some cases, the cells were pretreated at 37°C with heparinase I (10 U/mL) for 1 hour. The cells were then incubated in the presence of the heparinase with 125I-VLDL for 2 hours and washed 5 times on ice with 0.1 mol/L PBS containing 0.2% BSA and once with 0.1 mol/L PBS. The cell-associated radioactivity (from both cell-surface bound and internalized lipoproteins) was then counted, as described previously.

Statistical Analysis
Mean lipid levels are reported as the mean±SD. Differences in lipids, apolipoproteins, or VLDL triglyceride production were evaluated by the t test. Correlation of plasma apoE3 with VLDL triglyceride production or VLDL and IDL lipolysis was assessed by regression analysis.
Plasma Lipid Levels of ApoE3 Transgenic Rabbits

<table>
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<th>Total Cholesterol, mg/dL</th>
<th>Triglycerides, mg/dL</th>
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<td>ApoE3</td>
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<tr>
<td>Male</td>
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<tr>
<td>Nontransgenic</td>
<td>4</td>
<td>26±5</td>
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<tr>
<td>ApoE3 low (&lt;10 mg/dL)</td>
<td>6</td>
<td>8±2</td>
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<tr>
<td>ApoE3 medium (10–20 mg/dL)</td>
<td>5</td>
<td>15±4</td>
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<tr>
<td>ApoE3 high (&gt;20 mg/dL)</td>
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<td>28±4</td>
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<tr>
<td>Female</td>
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<tr>
<td>Nontransgenic</td>
<td>4</td>
<td>38±8</td>
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<td>ApoE3 high (&gt;20 mg/dL)</td>
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Rabbits were analyzed at 8 to 12 months of age and divided arbitrarily into groups based on their plasma apoE levels. ApoE, cholesterol, and triglycerides were measured as described in Methods.

*P<0.05 vs nontransgenics (t test).
†P<0.005 vs nontransgenics (t test).

Results

Effects of ApoE3 Overexpression on Plasma Lipids and Lipoproteins

The offspring (F1, hemizygotes) of 2 previously generated transgenic rabbit lines9 that expressed low (<10 mg/dL) or medium (10 to 20 mg/dL) levels of plasma human apoE3 were used in this study. To generate a high-expresser line (>20 mg/dL), F2 homozygous transgenic rabbits were established from the medium-expresser line. As reported previously,10 overexpression of the human apoE transgene in rabbits did not significantly alter endogenous rabbit apoE gene expression (data not shown).

The Table summarizes the plasma lipid levels in various apoE3 transgenic rabbit lines and nontransgenic rabbits at 8 to 12 months of age. In transgenic males and females, plasma total cholesterol levels were 3- to 4-fold higher in medium expressers (10 to 20 mg/dL) and 5- to 9-fold higher in high expressers (>20 mg/dL) than in nontransgenic rabbits. However, plasma triglyceride levels showed little change in low and medium expressers but were markedly increased in high expressers (5-fold). Since no significant sex difference was observed (the Table), male rabbits were used for subsequent studies.

The correlation between plasma lipid and apoE3 levels for the 21 male rabbits indicated in the Table is shown in Figure 1. Plasma total cholesterol increased proportionally with increasing levels of apoE3, whereas plasma triglycerides remained unchanged (or decreased slightly) at apoE3 levels <20 mg/dL but increased sharply at apoE3 levels >20 mg/dL. Thus, apoE3 overexpression differentially affects plasma cholesterol and triglyceride levels, leading to hypercholesterolemia in the medium expressers and to combined hyperlipidemia in the high expressers. The changes in specific lipoproteins in response to apoE3 expression levels, as analyzed by gel filtration chromatography on a Superose 6 column, are shown in Figure 2. A typical transgenic rabbit expressing a low amount of human apoE3 (6.5 mg/dL) (Figure 2B) had a lipoprotein profile that was not significantly different from that in nontransgenic rabbits (Figure 2A). However, a transgenic rabbit with an apoE3 concentration of 9.4 mg/dL had significantly higher LDL cholesterol and lower VLDL triglyceride (Figure 2C). At an apoE3 level of 15 mg/dL (Figure 2D), LDL cholesterol was dramatically increased, and VLDL cholesterol and triglyceride were further decreased. At apoE3 levels >20 mg/dL (Figures 2E and 2F), VLDL and LDL cholesterol and triglyceride levels were significantly increased, and LDL cholesterol remained at high levels.

Changes in the cholesterol and triglyceride content of the various lipoproteins as a consequence of increased apoE3 expression are shown in Figure 3. With the elevation of

![Figure 1. Correlation of plasma lipids with apoE3 levels. Total cholesterol (TC) and triglycerides (TG) were determined in whole plasma of 21 individual male rabbits (4 nontransgenic and 17 transgenic). The lines represent the best-fit curve, as determined by regression analysis. Plasma total cholesterol increased proportionally with increasing levels of apoE3, whereas plasma triglycerides remained unchanged (or decreased slightly) at apoE3 levels <20 mg/dL but increased sharply at apoE3 levels >20 mg/dL.](image-url)

![Figure 2. Superose 6 chromatography of 200 μL of rabbit plasma. The cholesterol and triglyceride distributions in the plasma of individual male rabbits were analyzed as described previously. Each panel is 1 representative profile selected from plasma profiles of several rabbits in each group. The bars in A indicate the plasma lipoprotein classes. The LDL fraction also contained a small quantity of HDL1. TC indicates total cholesterol; TG, triglyceride. The units for apoE3, TC, and TG are mg/dL.](image-url)
plasma apoE3 levels from 10 to 20 mg/dL, there was a nearly stepwise increase in LDL cholesterol (≈18-fold over nontransgenic controls; Figure 3E), only slight increases in VLDL and IDL cholesterol (Figures 3A and 3C), and no significant changes in VLDL and IDL triglycerides (Figures 3B and 3D). Thus, the hypercholesterolemia associated with medium levels of apoE3 overexpression (the Table and Figure 1) is due to a dramatic accumulation of LDL cholesterol. In contrast, at plasma apoE3 levels >20 mg/dL, there was no further change in LDL cholesterol (≈19-fold over nontransgenic controls; Figure 3E), whereas VLDL and IDL cholesterol and triglycerides increased progressively with increasing levels of plasma apoE3 (Figures 3A through 3D), as did LDL triglycerides (Figure 3F). These results indicate that the hypercholesterolemia associated with medium levels of apoE3 overexpression (10 to 20 mg/dL) is due to the accumulation of cholesterol-rich LDL, whereas the combined hyperlipidemia associated with high levels of apoE3 overexpression (>20 mg/dL) is due to the accumulation of cholesterol and triglycerides in VLDL and IDL.

**Effects of ApoE3 Overexpression on Hepatic VLDL Triglyceride Production**

At least 3 mechanisms could explain the hyperlipidemia associated with apoE3 overexpression: increased VLDL production, impaired VLDL lipolysis, and decreased clearance of apoB-containing lipoproteins. Previously, we demonstrated in transgenic mice that overexpression of apoE3 stimulates hepatic VLDL triglyceride production. However, ≈2/3 of the apoB secreted by the mouse liver is apoB48, whereas the rabbit liver secretes the apoB100-containing VLDL only, raising the possibility that apoE overexpression has differential effects on apoB48 and apoB100 particles. To ascertain whether apoE3-overexpressing rabbits have increased hepatic VLDL triglyceride production, we determined VLDL triglyceride production rates in apoE3 transgenic rabbits in which Triton WR1339 was administered intravenously to inhibit lipolysis. The hepatic VLDL triglyceride production rate increased 2- and 4-fold in the medium and high expressers, respectively, but only slightly in the low expressers (Figure 4A). The VLDL triglyceride production rate was correlated positively with plasma apoE3 levels (Figure 4B). These results suggest that the apoE expression level is an important determinant of VLDL triglyceride production in rabbits.

Next, we determined whether the apoE overexpression-induced changes in triglyceride levels were correlated with plasma apoB levels. Apolipoproteins in the VLDL and LDL fractions from nontransgenic and transgenic rabbits were separated by polyacrylamide-SDS gradient gel electrophoresis (Figure 5). Densitometric quantitation showed 8- and 25-fold increases in VLDL apoB100 in the medium and high expressers, respectively. Consistent with the similar LDL cholesterol levels in the medium- and high-expressor transgenic rabbits (Figure 3E), the LDL apoB levels were also similar, 21- and 23-fold higher than in the nontransgenic rabbits, respectively (Figure 5). The proportional increase in...
VLDL and LDL cholesterol and apoB100, together with our previous observation that the mean particle sizes of VLDL and LDL from nontransgenic rabbits and medium expressers are similar, suggests that apoE overexpression may result in a large increase in the number of apoB-containing lipoprotein particles produced by the liver.

Effects of ApoE3 Overexpression on VLDL and IDL Lipolysis In Vitro

A second mechanism to explain the hyperlipidemia, especially the hypertriglyceridemia associated with apoE3 overexpression, could be an impairment of lipolysis caused by apoE3 accumulation in triglyceride-rich lipoproteins, as previously demonstrated both in vitro and in vivo. Since there was no significant difference in LPL activity of postheparin plasma between nontransgenic and transgenic rabbits, even in the high expressers (data not shown), we assessed the susceptibility of VLDL (d<1.006 g/mL) and IDL (d=1.006 to 1.02 g/mL) to LPL-mediated lipolysis. The lipolysis of both classes of lipoproteins was correlated negatively with plasma apoE3 levels, suggesting a dose-dependent inhibitory effect of apoE3 on LPL-mediated lipolysis (Figure 6).

Previously, we demonstrated that the impairment of lipolysis caused by apoE accumulation in transgenic VLDL is due mainly to a displacement of apoC-II, a well-defined cofactor for LPL activity. To test whether the displacement of apoC-II is also involved in the impairment of lipolysis caused by apoE3 accumulation in transgenic rabbits, we determined the apoC levels in VLDL from nontransgenic and transgenic rabbits by polyacrylamide-SDS gradient gel electrophoresis. Compared with VLDL from controls and apoE low expressers, VLDL from high expressers had a much lower content of all of the apoCs (apoC-apoB ratios were 2.76, 1.66, and 0.44 for controls, low expressers, and high expressers, respectively) and a substantially higher content of apoE (apoE-apoB ratios were 0.44, 0.77, and 1.75 for controls, low expressers, and high expressers, respectively). These data indicate that accumulation of apoE3 in transgenic rabbit VLDL either displaces the apoCs from the particles or prevents their association with the particles initially, an effect that may be the primary cause of impaired lipolysis.

Effect of ApoE3 Overexpression on VLDL Clearance In Vivo

In addition to impaired lipolysis, the accumulation of VLDL in the plasma of apoE3 high expressers raises the possibility that the clearance of VLDL in these transgenic rabbits might be impaired. To address this issue, we determined plasma turnover (Figure 7A) and liver uptake (Figure 7B) of 125I-labeled control and transgenic rabbit VLDL after intravenous injection.
inhibiting lipolysis of VLDL.

Discussion

We have demonstrated in this study that overexpression of human apoE3 at plasma levels of ≈6 to 34 mg/dL differentially affects plasma total cholesterol and triglyceride metabolism: plasma cholesterol levels increased linearly with increasing levels of apoE3, whereas plasma triglyceride levels remained normal at apoE3 levels <20 mg/dL but increased sharply at higher levels (>20 mg/dL). Thus, the effects of apoE3 overexpression on plasma cholesterol and triglyceride metabolism lead to normolipidemia at low apoE3 levels (<10 mg/dL), hypercholesterolemia at medium apoE3 levels (10 to 20 mg/dL), and combined hypercholesterolemia and hypertriglyceridemia at high apoE3 levels (>20 mg/dL). Our data show a striking dose-dependent effect of apoE expression levels on plasma cholesterol and triglyceride metabolism. Supporting this conclusion is the observation that in humans, both plasma cholesterol and triglyceride levels are correlated positively with plasma and VLDL apoE concentrations.6–8

Three major factors act in concert to determine the steadystate levels of triglyceride-rich plasma lipoproteins: production rate, lipolytic processing, and plasma clearance. By altering these factors, apoE can modulate the metabolism of triglyceride-rich lipoproteins (Figure 8).

Low-Level ApoE3 Expression

At low levels of plasma human apoE3 expression (<10 mg/dL; Figure 8, apoE3 low expresser), the VLDL clearance rate increased 70% (Figure 7A), VLDL production increased 42% (Figure 4A), and VLDL lipolysis decreased 24% (Figure 6A) compared with the levels in nontransgenics. Increased VLDL clearance (caused by more apoE3 on the particles and enrichment of apoE at the hepatic surface in the space of Disse) appeared to compensate for increased particle production and slightly impaired conversion of VLDL to LDL. Thus, VLDL steady-state levels are somewhat lower than in nontransgenic rabbits. Therefore, we conclude that enhanced VLDL clearance is the predominant effect of apoE at levels up to ≈10 mg/dL and leads to slightly decreased VLDL cholesterol and triglycerides (Figures 2 and 3).

On the other hand, LDL cholesterol and apoB increased modestly at low levels of apoE3 expression. In this case, the slight impairment of lipolysis was probably not sufficient to offset the increased production of VLDL, resulting in more particles transiting the lipolytic cascade to become LDL. Furthermore, the increased clearance of VLDL, which is the predominant effect at low levels of apoE overexpression, appeared to compete favorably with LDL catabolism, resulting in delayed clearance and accumulation of LDL in plasma.6,26

Medium-Level ApoE3 Expression

At medium levels of apoE3 expression (10 to 20 mg/dL; Figure 8, apoE3 medium expresser), VLDL production increased an average of 135% (Figure 4A) and VLDL lipolysis decreased an average of 35% (Figure 6A). VLDL clearance was estimated to be increased by ≈105%. The additional increase in clearance over that found in apoE3 low expressers did not appear to be sufficient to compensate for the substan-
tial increase in production and the greater impairment of lipolysis. Thus, in medium expressers, VLDL overproduction becomes more important in determining VLDL steady-state levels. However, since VLDL lipolysis is not dramatically impaired, at least a portion of the overproduced VLDL can be effectively converted to LDL, leading to only slightly increased VLDL (Figures 2 and 3).

The LDL cholesterol and apoB increased markedly at medium levels of apoE3 expression. Since VLDL lipolysis is not dramatically impaired, the enhanced VLDL production, especially apoB, indicates that many more VLDL particles will transit the lipolytic cascade to become LDL (even though the percentage of conversion may be decreased). The further increase in VLDL and remnant clearance probably comes close to saturating the receptor-mediated removal system, and since the apoE3-enriched VLDL have a distinct competitive advantage because of their higher affinity for receptors, LDL catabolism becomes severely hampered. In fact, LDL clearance was slower in the medium expressers than in nontransgenic rabbits.9

High-Level ApoE3 Expression
At high levels of apoE3 expression (>20 mg/dL; Figure 8, apoE3 high expresser), VLDL production increased 318% (Figure 4A), VLDL clearance increased 160% (Figure 7A), and VLDL lipolysis decreased 64% (Figure 6A). The increased clearance rate does not appear to be sufficient to compensate for the dramatically increased production of VLDL and the more severe impairment of VLDL lipolysis. Therefore, markedly enhanced VLDL production and severe impairment of lipolysis are the predominant effects at high levels of apoE3, leading to both increased VLDL cholesterol and triglycerides (Figures 2 and 3).

The LDL cholesterol and apoB levels were similar to those at medium levels of expression, probably because the more severe impairment of VLDL lipolysis compensates for the increased VLDL production. Thus, the number of VLDL particles that transit the lipolytic cascade does not increase. Since LDL catabolism is already maximally inhibited by the competition of enhanced VLDL clearance, the steady-state levels of LDL in high expressers do not differ from those of medium expressers.

Effects of ApoE on VLDL Production
The stimulatory effect of apoE on VLDL production—first demonstrated in transgenic mice8 and confirmed in transgenic rabbits in this study—suggests a physiological role for apoE in VLDL assembly, secretion, or both. Supporting this hypothesis is the observation that apoE-deficient mouse hepatocytes, in vitro and in vivo, have impaired secretion of VLDL triglycerides that leads to the accumulation of these particles in the liver.15 Intracellular assembly of VLDL involves 2 steps: cotranslational binding of apoB to a small quantity of triglycerides to form a nascent lipid-poor complex in the rough endoplasmic reticulum (ER) and the subsequent transport of the lipid-poor particles to the smooth ER, where more triglycerides are added to form triglyceride-rich VLDL particles.27,28 Thus, apoE may function as a chaperone to help transport the lipid-poor, apoB-containing particles from the rough ER to the smooth ER for further lipidation. Alternatively, apoE may be involved in the transfer of newly generated triglycerides to a “secretion-coupled” pool in the smooth ER, which is directly available for VLDL assembly.29 In support of this possibility, apoE has been found to be distributed within the ER, Golgi apparatus, and trans-Golgi apparatus structures30 and to be incorporated into VLDL particles before secretion.31

Comparison of rabbit data obtained in this study with mouse data from our previous studies makes it clear that similar levels of apoE overexpression stimulated VLDL triglyceride production to different extents. For example, apoE overexpression at 30 mg/dL stimulated VLDL triglyceride production by 318% in rabbits but only by ~50% in mice. In humans and rabbits, the liver secretes VLDL containing apoB100 only, whereas in rats and mice, the liver secretes particles containing predominantly apoB48 and substantially less apoB100.32,33 Therefore, we hypothesize that apoE preferentially (or exclusively) affects the assembly and/or secretion of apoB100-containing VLDL. Supporting this hypothesis is the observation that VLDL apoB was increased 25-fold in transgenic rabbits expressing plasma apoE3 levels at ~30 mg/dL but only 3-fold in transgenic mice expressing similar levels of plasma apoE3.9 Since hepatically derived human VLDL, like rabbit VLDL, contains only apoB100, one could expect that apoE overexpression would have a profound effect on VLDL production in humans.

ApoE Levels as Determinants of the Lipoprotein Phenotype
We have demonstrated that overexpression of human apoE3 in transgenic rabbits stimulates VLDL production and clearance, impairs VLDL lipolysis, and delays LDL clearance9 in a dose-dependent manner. The plasma level of apoE appears to modulate those 4 metabolic pathways to determine plasma lipid levels: normolipidemia (low or physiological levels of apoE3), hypercholesterolemia (medium levels of apoE3), or combined hyperlipidemia (high levels of apoE3). Clearly, there appear to be 2 narrow ranges of apoE3 concentrations in transgenic rabbits that sharply affect LDL and VLDL levels and that determine the lipoprotein phenotype. Levels of 10 to 20 mg/dL have a large effect on LDL cholesterol (Figure 3E) and lead to normolipidemia or hypercholesterolemia (type IIa). Levels of 20 to 30 mg/dL have a much greater effect on VLDL cholesterol and triglycerides (Figures 3A and 3B) and lead to a shift between hypercholesterolemia (type IIa) and combined hyperlipidemia (type IIb). Thus, the hyperlipidemic apoE3 rabbits represent phenotypes similar to familial combined hyperlipidemia in humans, in which VLDL overproduction is a major biochemical feature34–37 and in which various hyperlipidemic phenotypes (type IIa, IIb, or IV) occur in the same family.38,39 Significantly increased levels of plasma and VLDL apoE have been observed in patients with familial combined hyperlipidemia.7 The hyperlipidemic apoE3 rabbits may serve as an important model for understanding the etiology of this disorder and may prove useful for studying the molecular mechanisms that control the lipoprotein phenotype.

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References


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