Pathogenesis of type III hyperlipoproteinemia (dysbetalipoproteinemia): questions, quandaries, and paradoxes

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Abstract Type III hyperlipoproteinemia (HLP) is a genetic disorder characterized by accumulation of remnant lipoproteins in the plasma and development of premature atherosclerosis. Although receptor binding-defective forms of apolipoprotein (apo) E are the common denominator in this disorder, a number of apparent paradoxes concerning its pathogenesis still exist. However, studies in transgenic animals are resolving the mechanisms underlying this disorder. Paradox I: Defective apoE (commonly apoE2) is essential but not sufficient to cause overt type III HLP. In fact, most apoE2 homozygotes are hyperlipidemic. Studies in apoE2 transgenic models have demonstrated the impact of other genes or hormones in converting the hyperlipidemia to hyperlipidemia. Paradox II: Among apoE2 homozygotes, men are more susceptible than women to type III HLP. Transgenic studies have shown that estrogen affects both LDL receptor expression and lipolytic processing, explaining the resistance of women to this disorder until after menopause. Paradox III: ApoE deficiency is associated with hypercholesterolemia, whereas the type III HLP phenotype is characterized by both hypercholesterolemia and hypertriglyceridemia. The hypercholesterolemia is caused by impaired receptor-mediated clearance, whereas the hypertriglyceridemia is caused primarily by impaired lipolytic processing of remnants and increased VLDL production associated with increased levels of apoE. Paradox IV: ApoE2 is associated with recessive inheritance of this disorder, whereas other defective apoE variants are associated with dominant inheritance. Determinants of the mode of inheritance are the differential binding of apoE variants to the LDL receptor versus the HSPG/LRP complex and the preference of certain apoE variants for specific lipoproteins. Thus, the pathogenesis of this sometimes mysterious disorder has been clarified.—Mahley, R. W., Y. Huang, and S. C. Rall, Jr. Pathogenesis of type III hyperlipoproteinemia (dysbetalipoproteinemia): questions, quandaries, and paradoxes. J. Lipid Res. 1999. 40: 1933–1949.

Supplementary key words apolipoprotein E • lipoprotein metabolism • heparan sulfate proteoglycans • LDL receptor • lipolysis • β-VLDL • VLDL production • hypercholesterolemia • hypertriglyceridemia • remnant lipoproteins

Type III hyperlipoproteinemia (HLP), or dysbetalipoproteinemia, is a genetic disorder of lipid metabolism that predisposes affected subjects to the premature development of atherosclerosis (for review, see refs. 1, 2). The disorder is characterized by elevated plasma cholesterol and triglycerides, usually to approximately equal levels and usually ≥300 mg/dl. The hyperlipidemia is caused by the accumulation of chylomicron remnants derived from intestinal lipoproteins and very low density lipoprotein (VLDL) remnants derived from hepatic lipoproteins. Known collectively as β-VLDL, these abnormal cholesterol-, triglyceride-, and apoE-enriched lipoproteins are the diagnostic hallmark of the disease. Type III HLP is caused either by the expression of forms of apolipoprotein (apo) E that are defective in binding to lipoprotein receptors (1–5) or by apoE deficiency (6, 7). The early descriptions of the type III HLP lipid profile defined the biochemical characteristics of the disorder (8–11); later studies elucidated the molecular genetics (12–15).

The primary molecular cause of type III HLP is the presence of apoE2, which differs from the most common isoform of apoE (apoE3) by a single amino acid substitution (cysteine for arginine at residue 158) and is associated with recessive inheritance of the disorder (1, 2). In this manifestation, development of overt hyperlipidemia requires homozygosity for apoE2. However, less than 10% of apoE2 homozygotes develop the hyperlipidemia; despite the invariable presence of β-VLDL, most apoE2/2 subjects are either normolipidemic or even hypocholesterolemic (16–18). Therefore, additional genetic, hormonal, or environmental factors, such as obesity, hypothyroidism, estrogen status, or diabetes, are required to precipitate

Abbreviations: apo, apolipoprotein; HDL, high density lipoprotein(s); HL, hepatic lipase; HLP, hyperlipoproteinemia; HSPG, heparan sulfate proteoglycans; IDL intermediate density lipoprotein(s); LDL, low density lipoprotein(s); LPL, lipoprotein lipase; LRP, LDL receptor-related protein; VLDL, very low density lipoprotein(s).

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the hyperlipidemia (17). Type III HLP is much more common in men and tends to occur earlier in men than in women, who rarely develop the disease until after menopause (8). Other rare forms of apoE that cause type III HLP appear to be associated with dominant inheritance (1, 2). In this manifestation, the inheritance of a single defective apoE allele is usually sufficient to cause hyperlipidemia; secondary factors are not usually required to precipitate the accumulation of remnant lipoproteins in plasma. The rare apoE variants associated with dominant type III HLP result from single amino acid substitutions (Arg136–Ser/ Cys; Arg142–Cys/ Leu; Arg145–Cys; Lys146–Gln/Glu; Lys146–Asn, Arg147–Trp) or from the insertion of a tandem repeat of amino acids 121–127 (apoE-Leiden).

Type III HLP has turned out to be a dauntingly complex disorder surrounded by puzzling questions, quandaries, and paradoxes. However, unraveling the complexities of the disorder has shed new light on various roles for apoE in lipoprotein metabolism and has provided insights far beyond the disorder itself. Although apoE that is defective in receptor binding is the common denominator in type III HLP, the pathogenesis of the disorder is not fully explained by the overabundance of receptor binding-defective apoE. Because of recent data obtained primarily from transgenic animals, which may be applicable to human type III HLP, it is now possible to address several paradoxes and unanswered questions. Paradox I: Defective apoE (commonly apoE2) is essential but is not usually sufficient to cause type III HLP. Why don’t all apoE2 homozygotes develop hyperlipoproteinemia? Why are most apoE2 homozygotes hypolipidemic? What factors precipitate the accumulation of remnant lipoproteins? Paradox II: Among apoE2 homozygotes, men are more susceptible than women to the development of overt type III HLP. Paradox III: ApoE-deficient humans develop a hyperlipidemia unlike the type III HLP associated with defective apoE2. Why do apoE-null humans (and mice) primarily have hypercholesterolemia, whereas apoE2 homozygotes develop both hypercholesterolemia and hypertriglyceridemia? Paradox IV: ApoE2 is associated with recessive inheritance of the disorder, whereas the other defective apoE variants are associated with dominant inheritance.

In this review, we will summarize the role of apoE in lipoprotein metabolism and discuss recent studies that have provided insights into the pathogenesis of type III HLP and that have resolved most of the paradoxes.

Critical Roles for ApoE in Regulating Plasma Lipid and Lipoprotein Levels

Apolipoprotein E plays several critical roles in regulating plasma lipid and lipoprotein levels (for review, see refs. 1–5). First, it serves as a ligand that mediates the binding, uptake, and plasma clearance of lipoproteins by receptors of the low density lipoprotein (LDL) receptor gene family, including the LDL receptor and the LDL receptor-related protein (LRP). Likewise, apoE binds to cell-surface heparan sulfate proteoglycans (HSPG), which participate along with the LRP in the hepatic clearance of remnant lipoproteins (3). Certain apoE mutants are more defective in LDL receptor binding, while others are more defective in HSPG/LRP binding. Second, apoE modulates lipolytic activity. Elevated levels of apoE, as occur in type III HLP, can impair triglyceride hydrolysis and cause hypertriglyceridemia. Third, apoE has recently been shown to stimulate VLDL production by the liver, which is also associated with increased VLDL and plasma triglyceride levels. Thus, the plasma level and rate of hepatic biosynthesis of apoE can dramatically alter lipoprotein metabolism. In addition, the lipoprotein preferences of specific allelic forms of apoE can cause an enrichment of certain classes of lipoproteins with apoE (specifically, the preference of apoE4 for triglyceride-rich chylomicrons and VLDL and the preference of apoE2 and apoE3 for small phospholipid-rich HDL). All of these factors affect the expression of type III HLP and modulate remnant lipoprotein accumulation.

Mediating Plasma Clearance of Remnant Lipoproteins by the Liver

Apolipoprotein E is a multifunctional protein constituent of plasma lipoproteins. One of its major physiological roles is to mediate high-affinity binding of apoE-containing lipoproteins to the LDL receptor and the LRP and to cell-surface HSPG (1–3, 19). Thus, apoE serves as a critical ligand in the metabolism of remnant lipoproteins (Fig. 1), including chylomicron remnants, which are generated by lipolytic processing of intestinally derived chylomicrons, and VLDL and their remnants, which are derived from the liver. Some VLDL remnants are rapidly cleared from the plasma; others are converted to LDL by lipolysis. The binding and uptake of these remnant lipoproteins are mediated by hepatic LDL receptors and the HSPG/LRP pathway (for review, see refs. 1–3, 19).

Lipoproteins containing apoE can interact directly with the LDL receptor and can be taken up by cells via the classic LDL receptor pathway (2, 3, 5, 19–21). ApoE-containing lipoproteins can also be taken up via a second
pathway in a two-step process involving two cell-surface receptors, the LRP (22–25) and HSPG (26–30) (Fig. 2). Initially, apoE-containing lipoproteins bind to cell-surface HSPG. The lipoproteins become enriched in apoE, with the HSPG apparently serving as a reservoir for apoE. The apoE-enriched proteins are then transferred to the LRP, and the LRP either initiates uptake or, more likely, forms a complex with HSPG that is subsequently taken up by the cells. In addition, HSPG alone appear to mediate lipoprotein uptake directly (3, 30–32). Thus, a deficiency or a receptor-binding defect of apoE will cause remnants to accumulate in plasma, as occurs in type III HLP (1, 2). However, as apoE mediates remnant clearance via different pathways (LDL receptor, HSPG/LRP, or HSPG pathways), the relative defect of certain apoE mutants in terms of these various pathways will dramatically modify the extent or profile of the hyperlipidemia (see below).

Modulating VLDL and chylomicron triglyceride hydrolysis

The lipolysis of triglyceride-rich lipoproteins is regulated by several factors, including lipase activities and the plasma levels of apoC-II, which acts as a physiological inhibitor of lipoprotein lipase (LPL), and of apoC-II, which is a well-defined cofactor of LPL (for review, see refs. 33–36). In addition, apoE may inhibit LPL-mediated lipolysis, as the addition of purified apoE to VLDL or triglyceride-rich emulsion particles decreases their ability to serve as substrates for LPL-mediated lipolysis (5, 37–40). Furthermore, the apoE content of VLDL correlates inversely with the rate of LPL-mediated lipolysis in vitro (38, 39, 41). Recently, we demonstrated that overexpression of human apoE3 in transgenic mice and rabbits significantly inhibits LPL-mediated lipolysis of VLDL and causes hypertriglyceridemia (39, 42). As will be discussed later, overexpression of apoE2 in transgenic mice also causes hypertriglyceridemia, at least in part, to impaired lipolysis (38). Likewise, in hypertriglyceridemic humans, plasma and VLDL apoE levels correlate inversely with VLDL lipolysis (39). The inhibiting effects of apoE on lipolysis appear to be due primarily to displacement or masking of apoC-II, suggesting that the relative amount of apoE to apoC-II may be an important modulator of triglyceride-rich lipoprotein lipolysis (38, 39).

Conversely, apoE activates hepatic lipase (HL) (43, 44). HL functions primarily in the liver to catalyze the hydrolysis of phospholipids and triglycerides in the final processing of chylomicron remnants and in the conversion of intermediate density lipoproteins (IDL) to LDL. When added to lipoprotein in an in vitro assay, apoE3 or apoE4 enhances HL-mediated lipolysis to a much greater extent than apoE2 does (43, 44).

Fig. 2. Remnant lipoprotein clearance pathways. Remnant particles are sequestered in the space of Disse, where they can undergo final lipolytic processing by LPL or HL. Lipoprotein uptake can occur by any of three mechanisms: 1) the LDL receptor, 2) the HSPG/LRP pathway, and 3) HSPG alone. In HSPG/LRP uptake, lipoproteins could first bind to HSPG and then be transferred to LRP for uptake (2a) or bind to and be taken up by a HSPG/LRP complex (2b). (Reproduced with permission from J. Lipid Res. 1999. 40: 1–16.)
Stimulating VLDL production

Intracellular assembly of VLDL in hepatocytes appears to involve two separate steps: 1) cotranslational binding of apoB to a small quantity of triglycerides to form a nascent lipid-poor complex in the rough endoplasmic reticulum, and 2) transport of the lipid-poor particles to the smooth endoplasmic reticulum, where more triglycerides are added to form the larger, triglyceride-rich VLDL particles (45–49). The assembly and secretion of VLDL in the liver can be regulated by several factors, including the availability of intracellular lipids (especially triglycerides) (50, 51), expression levels of microsomal triglyceride transfer protein (52–55), and dietary and hormonal effects (56–58).

Recently, we demonstrated that overexpression of human apoE3 in transgenic mice causes hypertriglyceridemia, at least partially by stimulating hepatic VLDL triglyceride production (5, 39). This stimulatory effect of apoE3 has also been confirmed in vitro in transfected rat hepatic cells expressing human apoE isoforms (39) and in vivo in apoE3 transgenic rabbits (42) and LDL receptor-null mice receiving the apoE gene via adenoviral infection (59). The stimulatory effect of apoE on VLDL assembly and/or secretion is due to enhanced triglyceride synthesis and preferential transport of the newly synthesized triglycerides to the endoplasmic reticulum in apoE-transfected McA-RH7777 cells (Y. Huang and R. W. Mahley, unpublished observation). Thus, apoE appears to be important in modulating hepatic VLDL assembly and secretion. Support for this possibility comes from the observation that apoE-deficient mouse hepatocytes, in vitro and in vivo, have impaired secretion of VLDL triglycerides (60).

Overexpression of apoE may also contribute to the development of hypertriglyceridemia in humans. Epidemiological studies show that the plasma level of apoE is an important determinant of plasma triglyceride and VLDL metabolism (61). In fact, changes in plasma apoE concentrations account for 20–40% of the variation of plasma triglyceride and VLDL levels (61), independent of apoE polymorphism (62). Interestingly, plasma and VLDL levels of apoE are determined by the hepatic apoE production rate, not by plasma apoE residence time (63). Thus, the apoE expression level is an important determinant of VLDL metabolism that acts by modulating VLDL production rates, lipolytic processing, and plasma clearance, as discussed above. As will be discussed later, apoE2 overexpression also stimulates VLDL production.

PARADOX I: DEFECTIVE APOE IS ESSENTIAL BUT NOT SUFFICIENT TO CAUSE TYPE III HLP

Type III HLP is not simply caused by defective apoE2 leading to remnant accumulation. Apolipoprotein E2 homozgyosity is essential but not sufficient to cause overt hyperlipidemia. In fact, as mentioned above, less than 10% of apoE2 homozygotes are hyperlipidemic; the majority are normolipidemic or hypolipidemic (2). The development of hyperlipoproteinemia therefore requires apoE2 (the susceptibility gene) plus a secondary genetic or environmental factor (a “second hit”) to precipitate the overt phenotype of type III HLP. What, then, causes hypolipidemia, and what precipitates the hyperlipidemia?

Factors contributing to hypolipidemia

In humans, the hypolipidemia associated with apoE2 homozgyosity is usually characterized by a marked decrease in the plasma LDL level and by a smaller decrease in the plasma high density lipoprotein (HDL) level (62). Recently, we demonstrated that transgenic mice expressing medium plasma levels of human apoE2 (~10–30 mg/dl) were hypolipidemic (64), whereas expression of low levels of apoE2 (~<10 mg/dl) had no significant effect on plasma lipids (64, 65). The hypolipidemic apoE2 mice had decreased plasma cholesterol, normal triglyceride, and slightly increased remnant levels. When the hypolipidemic apoE2 mice were cross-bred with human apoB transgenic mice, the resulting double transgenic mice had significantly decreased plasma LDL cholesterol compared with apoB-only mice, mimicking a situation seen in most apoE2 homozygous humans (2). Similarly, the hypolipidemic effect of apoE2 has also been confirmed in transgenic rabbits expressing low levels of plasma apoE2 (~<10 mg/dl) (Y. Huang and R. W. Mahley, unpublished observations).

There are at least three hypotheses to explain the LDL cholesterol-lowering effect of apoE2. The first is up-regulation of hepatic LDL receptors caused by decreased delivery of cholesterol to the liver resulting from the defective binding of apoE2-containing lipoproteins to the LDL receptors (2, 18). The second is poor competition between defective apoE2-containing remnants and apoB-100-containing LDL for hepatic LDL receptors, leading to enhanced clearance of the LDL (66). Both hypotheses suggest a role for the hepatic LDL receptor. The third hypothesis is that apoE2 impairs the lipolytic conversion of VLDL to LDL, leading to decreased LDL formation (67–70).

To address the role of the LDL receptor, we crossed transgenic mice expressing various low levels (5–10 mg/dl) of apoE2 with LDL receptor-null mice (38). The plasma lipoprotein profiles of these mice are shown in Fig. 3. In the LDL receptor-null mouse lacking apoE2 expression, the plasma LDL cholesterol level was significantly increased. In mice expressing apoE2 at 14 or 29 mg/dl, the plasma LDL cholesterol level decreased progressively. In contrast, the plasma triglyceride levels increased concomitantly with apoE2 expression, mostly in the VLDL and LDL fractions. These results indicate that human apoE2 expression leads to a reduction in LDL cholesterol even in the absence of the LDL receptor. This suggests that LDL receptor-related activity is not responsible for the LDL cholesterol-lowering effect of apoE2.

To evaluate the role of impaired lipolytic conversion of VLDL to LDL, we correlated the apoE2 level with LDL cholesterol and plasma triglyceride levels in LDL receptor-null male mice expressing apoE2 at various levels (Fig. 4). As the apoE2 level increased, LDL cholesterol decreased progressively, whereas triglycerides increased markedly. These results suggest that apoE2 affects lipolysis directly.
This suggestion is not a new one. Twenty years ago, Chait et al. (68) showed that conversion of apoB-containing VLDL to LDL is impaired in patients with type III HLP. Likewise, in vitro studies, including one of our own, have demonstrated that apoE2 impairs the conversion of β-VLDL to LDL (69, 70).

We assessed the ability of normal and apoE2-containing VLDL and IDL (obtained from our transgenic models) to serve as substrates in an in vitro lipolytic assay (38). Both LPL and HL were used as enzymes, and in some studies, purified human apoC-II was added to the assay to determine its effect on lipolysis. The release of free fatty acids was assessed by an enzymatic colorimetric assay. Figure 5 shows the results of this assay performed with VLDL from nontransgenic, LDL receptor-null, and LDL receptor-null apoE2 transgenic mice expressing apoE2 at three levels. Increasing apoE2 levels progressively inhibited LPL-mediated lipolysis of apoE2-containing VLDL compared with VLDL from nontransgenic or LDL receptor-null mice. The inhibition was about 80% at the highest apoE2 level. Thus, for LPL-mediated lipolysis, apoE2-enriched VLDL are a poorer substrate than normal VLDL. Essentially identical results were obtained with IDL (38).

In considering the mechanism by which apoE2 impairs LPL-mediated lipolysis, we found a significantly decreased apoC-II content in the remnants of the apoE2 mice (Table 1) (38). Nontransgenic VLDL contained 19 μg of mouse apoE and 32 μg of apoC-II per mg of triglycerides. In contrast, the VLDL from the LDL receptor-null mice expressing apoE2 contained an abundance of apoE2 (51 μg) but only 5 μg of apoC-II. These results suggest that the accumulation of apoE2 in the transgenic VLDL or remnants may displace apoC-II, which is a well-defined co-factor for LPL. In fact, we have demonstrated that adding back apoC-II to apoE2-containing VLDL stimulated LPL-mediated lipolysis 3-fold and that increasing the amount of apoC-II on the particles partially corrected the apoE2-impaired lipolysis of VLDL (38).

Thus, these studies of LDL receptor-null mice overexpressing apoE2 led to the conclusion that inhibition of lipolysis, and not increased LDL receptor levels, causes the low LDL. As described previously, apoE can enhance HL activity in vitro (43, 44); however, apoE2 is less effective than apoE3. Therefore, the low LDL could result from the combination of two factors: 1) the reduced lipolytic processing of VLDL to IDL caused by inhibition...
Factors precipitating hyperlipidemia

We have hypothesized that secondary genetic or environmental factors can convert the hypolipidemia to overt type III HLP characterized by dramatically increased levels of \( \beta \)-VLDL and by markedly reduced levels of LDL (1, 2). For example, factors that increase remnant lipoprotein production could overwhelm the clearance pathway. In combination with the defective receptor binding of apoE2, factors that decrease receptor number or activity or decrease lipolytic processing could also induce the remnant accumulation and hyperlipidemia.

Valuable information about the factors precipitating hyperlipidemia in type III HLP patients has been obtained in transgenic animals. Crossing the hypolipidemic apoE2 transgenic mice with mice overexpressing human apoB converted the hypolipidemic phenotype of the apoE2 mice to a hyperlipidemic phenotype characterized by a pronounced accumulation of remnants and decreased LDL.

Of LPL-mediated lipolysis due to high levels of apoE2 accumulation and the displacement of apoC-II, and 2) the lesser ability of apoE2 to enhance HL-mediated lipolysis of IDL to LDL.

TABLE 1. Decreased apoC-II content in VLDL/remnants 
(\( d < 1.006 \) g/ml) from transgenic mice

<table>
<thead>
<tr>
<th>Human</th>
<th>Mouse</th>
<th>Mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>ApoE2</td>
<td>ApoE</td>
<td>ApoE2</td>
</tr>
<tr>
<td>14.6</td>
<td>19</td>
<td>32</td>
</tr>
<tr>
<td>9.4</td>
<td>30</td>
<td>26</td>
</tr>
<tr>
<td>1.2</td>
<td>11</td>
<td>5</td>
</tr>
</tbody>
</table>

For a further description of these data, see ref. 38; hE2, human apoE2; LDLR, LDL receptor; TC, total cholesterol; TG, triglycerides.

Thus, overproduction of apoB or VLDL is a secondary factor that precipitates overt type III HLP in the hypolipidemic apoE2 mice (71), and this has also been suggested in humans (72).

To determine the role of the LDL receptor in precipitating overt type III HLP, we also crossed the hypolipidemic apoE2 mice with LDL receptor-null mice. Removing the LDL receptor also converted the hypolipidemic phenotype of the apoE2 mice to a hyperlipidemic phenotype characterized by sharply increased apoE levels (from 23 to 61 mg/dl; contrast these results with those in Fig. 3) and increased plasma cholesterol and triglyceride levels, marked accumulation of remnants, and decreased LDL cholesterol levels (Table 2) (71). Therefore, a decrease in LDL receptors is another secondary factor that precipitates overt type III HLP in the hypolipidemic apoE2 mice, as reported previously in humans (73).

It has also been shown in humans that estrogen levels modify the hyperlipidemic phenotype of type III HLP (2). As will be discussed (Paradox II), low estrogen levels can convert hypolipidemic apoE2 transgenic rabbits to a hyperlipidemic phenotype.

PARADOX II: RESISTANCE OF APOE2 HOMOZYGOUS FEMALES TO DEVELOPING OVERT TYPE III HLP

One of the interesting questions in type III HLP is why men are susceptible and women resistant to developing type III HLP (2). Among apoE2 homozygotes, men can develop the hyperlipidemia after adolescence, whereas women almost never develop the disorder until after menopause. It was therefore hypothesized that estrogen increases LDL receptor levels and lipolytic activity (74, 75). Both of these effects would be expected to mask or overcome the detrimental effects of apoE2 on lipoprotein metabolism.

Recently, we developed an apoE2 transgenic rabbit model that has allowed us to explore the gender difference in type III HLP (75). Although male and female transgenic rabbits had very similar plasma levels of defective apoE2 (\( \sim 48 \) mg/dl), the males had dramatically higher levels of cholesterol (\( \sim 290 \) versus 140 mg/dl) and triglycerides (697 versus 174 mg/dl) than the females. We hypothesized that estrogen treatment would protect male apoE2 rabbits from developing the hyperlipidemia. In fact, that is exactly what happened. Before treatment with estrogen, the apoE2 male rabbits had markedly hypercholesterolemia and hypertriglyceridemia and an accumulation of typical \( \beta \)-VLDL. After 10 days of treatment with estrogen, the apoE2 male rabbits were normolipidemic and had no \( \beta \)-VLDL (Table 3). We also demonstrated that estrogen treatment significantly increased both LPL and HL activities.

Next, we tested the hypothesis that ovariectomy of female apoE2 transgenic rabbits would accentuate the hyperlipidemia and induce a more profound type III HLP phenotype (75). As expected, within 10 days after ovariectomy, cholesterol and triglyceride levels had increased approximately 2-fold, and typical \( \beta \)-VLDL had accumulated in the
plasma (Table 3). Accompanying these changes were reductions in LPL and HL activities. Thus, estrogen modulates lipid levels in the context of the apoE2 allele, probably by altering both receptor expression and lipolytic activity (75).

TABLE 3. Effects of estrogen on plasma lipid levels in male and female apoE2 transgenic rabbits

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Males (n = 3)</th>
<th></th>
<th>Females (n = 3)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before E-estrone</td>
<td>After E-estrone (10 days)</td>
<td>Before Ovariectomy</td>
<td>After Ovariectomy (10 days)</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>235 ± 57 mg/dl</td>
<td>63 ± 12</td>
<td>127 ± 8</td>
<td>222 ± 20</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>445 ± 152 mg/dl</td>
<td>52 ± 38</td>
<td>159 ± 24</td>
<td>347 ± 21</td>
</tr>
<tr>
<td>β-VLDL</td>
<td>+++++</td>
<td>0</td>
<td>+</td>
<td>+++++</td>
</tr>
<tr>
<td>Lipoprotein lipase</td>
<td>195 ± 14 nmol/min</td>
<td>377 ± 56</td>
<td>296 ± 41</td>
<td>192 ± 36</td>
</tr>
<tr>
<td>Hepatic lipase</td>
<td>50 ± 13</td>
<td>97 ± 18</td>
<td>94 ± 6</td>
<td>61 ± 3</td>
</tr>
</tbody>
</table>

*P < 0.05 versus pre-estrone values.

*The elevation of both plasma cholesterol and triglyceride levels and the accumulation of cholesterol- and triglyceride-rich remnant lipoproteins in type III HLP patients have been readily ascribed to impaired clearance of remnant lipoproteins caused by defective receptor binding of apoE2. Likewise, overexpression of apoE2 in mice causes both increased cholesterol and triglyceride levels (71). However, apoE deficiency (absence) in humans is associated with hypercholesterolemia and an increase in cholesterol-rich remnants without a significant increase in triglycerides (6, 7, 76, 77). Similarly, apoE-null mice have increased plasma levels of cholesterol and cholesterol-rich remnants (78, 79). Thus, there is an additional paradox: Why is defective apoE2 associated with a combined hypercholesterolemia and hypertriglyceridemia, whereas apoE deficiency is associated primarily with hypercholesterolemia? These observations suggest that the absence of apoE or the presence of receptor binding-defective apoE2 is associated with hypercholesterolemia, whereas apoE2 also affects plasma triglyceride metabolism independently of its effect on cholesterol metabolism. The hypertriglyceridemia in the apoE2 overexpressers appears to be independent of the receptor-binding defect.

TABLE 2. Mean plasma lipid levels (mg/dl ± SD) of various apoE2 transgenic mice

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Males</th>
<th></th>
<th>Females</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ApoE2</td>
<td>Cholesterol</td>
<td>Triglyceride</td>
<td>HDL-C</td>
</tr>
<tr>
<td>hE2 Tg</td>
<td>n</td>
<td>12</td>
<td>0</td>
<td>93 ± 15</td>
</tr>
<tr>
<td>Nontransgenic</td>
<td>11</td>
<td>24</td>
<td>40 ± 16</td>
<td>65 ± 11</td>
</tr>
<tr>
<td>hE20/0, LDLR</td>
<td>7</td>
<td>0</td>
<td>137 ± 17</td>
<td>138 ± 22</td>
</tr>
<tr>
<td>hE20/0</td>
<td>6</td>
<td>25</td>
<td>95 ± 9</td>
<td>157 ± 21</td>
</tr>
<tr>
<td>hE20/0, LDLR</td>
<td>10</td>
<td>0</td>
<td>203 ± 29</td>
<td>99 ± 16</td>
</tr>
<tr>
<td>hE20/0, LDLR</td>
<td>14</td>
<td>61</td>
<td>288 ± 51</td>
<td>356 ± 72</td>
</tr>
</tbody>
</table>

For further description of the data, see ref. 71; HDL-C, HDL cholesterol; hE2, human apoE2; Tg, transgenic; mE, mouse apoE; hB, human apoB; (hypo-), hypolipidemic mice expressing medium levels of apoE2. Differences were evaluated by t test: a P < 0.001 versus nontransgenic mice; b P < 0.001 versus hE20/0, LDLR mice.
PARADOX IV: APOE2 IS ASSOCIATED WITH A RECESSIVE MODE OF INHERITANCE, WHEREAS THE OTHER APOE VARIANTS ARE ASSOCIATED WITH DOMINANT INHERITANCE

Type III HLP is inherited in either a recessive or a dominant mode, depending on the specific mutations of apoE (1, 2). Why is the apoE2 isoform associated with recessive inheritance, whereas other rare mutations, such as apoE-Leiden and those involving residues 136, 142, 145, and 146, are associated with dominant inheritance? All of these mutants are defective in receptor binding. The answer lies in the structure of apoE.

Receptor-binding region of apoE

Primarily as a result of studies by Gladstone Institute investigators (for review, see refs. 1–5), it has been established that the region of apoE that directly interacts with the LDL receptor encompasses amino acids 136–150. Most of the apoE variants associated with dominant inheritance of type III HLP have single amino acid mutations in this region (residues 136, 142, 145, 146, 147) and invariably result in hyperlipidemia (2) (see Table 4 for a summary of dominant variants). ApoE-Leiden, with the tandem repeat of the seven-amino acid sequence at residues 121–127, likely disrupts receptor binding by altering the conformation of the receptor-binding region (this tandem repeat occurs at the loop between helices 3 and 4 of the amino-terminal domain) (Fig. 6) (106). Apolipoprotein E2, which is associated with recessive inheritance and low penetrance, has a mutation at residue 158, which lies outside the receptor-binding region. As a result, apoE2 affects receptor binding indirectly by modulating the receptor-binding region (107).

X-ray crystallographic studies (108) have shown that the amino-terminal two-thirds of the apoE molecule forms a four-helix bundle (Fig. 6). The receptor-binding region lies in helix 4. The basic amino acids in the 136–150 region are largely solvent exposed, extend away from the backbone of the helix, and form a 20 Å (area) basic field of charge that may be available to interact with the receptor. The backbone structures of apoE2 and apoE3 are essentially identical; however, there are local changes in the region of residue 158 (Fig. 7). In apoE3, there is a salt bridge between Arg-158 and Asp-154. In apoE2, however, which has cysteine rather than arginine at residue 158, that salt bridge cannot form, and Asp-154 interacts instead with Arg-150, forming a new

<table>
<thead>
<tr>
<th>Causative Mutation</th>
<th>Receptor-binding Activity</th>
<th>LDL</th>
<th>HSPG/LRP</th>
<th>Other Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arg136→Ser</td>
<td>Moderate</td>
<td>—</td>
<td>—</td>
<td>Mutant E: normal E ratio is as high as 4:1 in plasma of heterozygous subjects; one homozygous subject identified; extensive pedigree analyses; influence of age, body mass, gender, but not second apoE allele; increased frequency in Spanish population (2, 83, 84).</td>
</tr>
<tr>
<td>Arg136→Cys</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>Variable expression of hyperlipidemia (85–87).</td>
</tr>
<tr>
<td>Arg142→Leu</td>
<td>Low</td>
<td>—</td>
<td>—</td>
<td>Only two subjects identified (88).</td>
</tr>
<tr>
<td>Arg142→Cys</td>
<td>Low</td>
<td>Very low</td>
<td>—</td>
<td>Severe hyperlipidemia; 100% penetrance; very early and severe expression of hyperlipidemia (possibly at birth); VLDL preference for isoform due to second mutation of Cys112→Arg; mutant E: normal E ratio in β-VLDL is 3:1 (80, 82, 89, 90).</td>
</tr>
<tr>
<td>Arg145→Cys</td>
<td>Moderate</td>
<td>Low</td>
<td>—</td>
<td>Many similarities to apoE2 homozygosity, including influence of secondary factors; homozygous subjects identified; homozygosity in combination with a second mutation (Glu13→Lys) associated with severe hyperlipidemia; increased frequency in blacks (16, 91–94).</td>
</tr>
<tr>
<td>Lys146→Gln</td>
<td>Moderate</td>
<td>—</td>
<td>—</td>
<td>Extensive pedigree analyses; variable expression of the hyperlipidemia; some influence of secondary factors; very inefficient lipolysis of β-VLDL by LPL (95–98).</td>
</tr>
<tr>
<td>Lys146→Glu</td>
<td>Low</td>
<td>Very low</td>
<td>—</td>
<td>High degree of penetrance (82, 99–102).</td>
</tr>
<tr>
<td>Lys146→Asn, Arg147→Trp</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>Very early expression of hyperlipidemia (103).</td>
</tr>
<tr>
<td>Seven-amino acid duplication of residues 121–127</td>
<td>Low</td>
<td>Very low</td>
<td>—</td>
<td>Extensive pedigree analyses; 100% penetrance; some influence of second apoE allele, body mass, age, but not gender; VLDL preference for isoform due to second mutation of Cys112→Arg; mutant E: normal E ratio in plasma is &gt;4:1; commonly referred to as apoE-Leiden (81, 82, 104, 105).</td>
</tr>
</tbody>
</table>

For a further discussion of these data, see refs. 2, 118.

1 Changes compared to apoE3 structure (e.g., Arg136→Ser, arginine at residue 136 changed to serine at that site).
2 LDLr, LDL receptor.
3 Dash indicates not yet determined.
salt bridge. This interaction swings the side chain of Arg-150 into a new plane outside the receptor-binding region and disrupts receptor binding (109). Arginine-150 is part of the receptor-binding region (110). Thus, the substitution at residue 158 in apoE2 appears to have a secondary effect on the receptor-binding domain of apoE and modulates binding indirectly. We had previously hypothesized on the basis of biochemical studies that the substitution at residue 158 affects the receptor-binding domain indirectly (111).

Although all dominant apoE mutations cause defective LDL receptor binding, their LDL receptor-binding activities are higher than that of the recessive apoE2. This is just the opposite of what we might predict. The apoE2 variant that is most defective in LDL receptor binding might be expected to have the most detrimental effects on remnant metabolism. But this turns out not to be the case. Although apoE2 has <2% of normal LDL receptor-binding activity, it is the rare apoE variants, which have 20–50% of normal receptor-binding activity, that are, paradoxically, associated with the dominant mode of inheritance and the invariable presence of hyperlipidemia (Table 4). Thus, reasons other than LDL receptor-binding activity must be sought to explain this phenomenon.

Fig. 6. Three-dimensional structure of the amino-terminal region of apoE. The four-helix bundle is shown as a ribbon diagram. The receptor-binding domain in helix 4 (residues 136 - 150) is highlighted, and the positions of side chains of the critical basic residues, including arginine-158, are superimposed on the ribbon backbone. (Courtesy of Karl H. Weisgraber, Gladstone Institute of Cardiovascular Disease.)
To explore the mechanisms responsible for the recessive versus dominant expression of type III HLP (as detailed in the following sections), we created transgenic mice expressing apoE(Cys142) (112), a dominant variant of apoE, and compared them with apoE2 transgenic mice. Mice expressing intermediate levels of apoE2 (~20 mg/dl) have hypocholesterolemia and normal triglyceride levels, whereas mice expressing similar levels of apoE(Cys142) have hypercholesterolemia and severe hypertriglyceridemia (Fig. 8). Thus, in these mice, we can at least partially mimic the lipid profiles of humans with apoE2 (recessive type III HLP) or apoE(Cys142) (dominant type III HLP). Another mouse model of dominant type III HLP has also been established by overexpressing apoE-Leiden in transgenic mice (113, 114). These mice also develop hyperlipidemia.

**Isoform-specific preferences for lipoproteins**

Two major factors may contribute to the recessive versus dominant expression of type III HLP. The first is the lipoprotein preference of apoE variants (for review, see ref. 107). Apolipoprotein E2, which is associated with the recessive mode of inheritance, has a preference for HDL (115, 116), whereas some apoE variants associated with dominant inheritance, such as apoE(Cys142) and apoE-Leiden, have a preference for VLDL due to the presence of Arg-112 (i.e., these variants result from mutations in the E4 allele) (80, 81). Thus, apoE(Cys142) and apoE-Leiden would be predicted to be overrepresented in VLDL. In fact, the ratio of mutant to normal apoE in the β-VLDL particles is 3:1 in subjects with apoE(Cys142) (80) and up to 7:1 in subjects with apoE-Leiden (81). Analysis of the apoE distribution in various lipoproteins from apoE2 and apoE(Cys142) transgenic mice, each expressing apoE at ~20 mg/dl, showed that only about 10% of the apoE2 is found in the VLDL and IDL fractions (>60% in HDL), whereas more than 60% of the apoE(Cys142) is found in those fractions (only 25% in HDL). Therefore, at similar plasma apoE levels, more apoE(Cys142) accumulates in the remnants, as would be expected.

We predicted that even low levels of apoE(Cys142),
preferentially distributed in VLDL, would displace more apoC-II from the particles and impair lipolysis more severely. This prediction was borne out in studies of LPL-mediated lipolysis of VLDL from apoE2 and apoE(Cys142) transgenic mice with similar plasma apoE levels (20, 257 mg/dl). Lipolysis of VLDL containing apoE2 was 30% lower than nontransgenic VLDL, whereas lipolysis of VLDL containing apoE(Cys142) was 75% lower (Fig. 9). These results indicate that the preference of apoE(Cys142) for VLDL impairs lipolysis more severely, exacerbating the hypertriglyceridemia. Thus, lipoprotein preference probably contributes to the hyperlipidemia in subjects with certain dominant apoE mutations.

Isoform-specific differential binding to heparin and HSPG

The affinity of the apoE variants for HSPG is the second factor that may contribute to recessive versus dominant expression of type III HLP. HSPG binding is the initial step in remnant uptake by the liver (sequestration and capture in the space of Disse) and is a component of the HSPG/LRP pathway (3). Apolipoprotein E2 has 50–90% of normal (apoE3) HSPG-binding affinity, whereas apoE(Cys142) has only 5% (2, 3, 5, 82). Likewise, apoE2 retains significant LRP binding (40–50% as effective as apoE3 in binding to the LRP on a ligand blot) (117). Apolipoprotein E2-containing β-VLDL (remnants) can bind and be internalized by cultured cells lacking the LDL receptor (82, 117) and by cultured cells lacking the LDL and LRP receptors (3, 30). Thus, apoE2 (recessive type III HLP) binds fairly normally to HSPG and the HSPG/LRP, which may, under some circumstances, compensate for the very defective binding to the LDL receptor.

To assess the importance of HSPG-binding affinity in the dominant expression of type III HLP, we examined the effects of apoE2 and apoE(Cys142) expression on plasma lipid levels of mice lacking both endogenous murine apoE and the LDL receptor. The absence of the LDL receptor allowed us to determine the relative importance of the HSPG/LRP pathway versus the LDL receptor pathway in remnant clearance. In the absence of LDL receptors, the plasma cholesterol and triglyceride levels each doubled in the apoE2-expressing mice, but increased 8-fold and more than 10-fold, respectively, in the mice expressing apoE(Cys142) (Fig. 10). The lower cholesterol and triglyceride levels in the apoE2 mice lacking the LDL receptor may reflect the ability of apoE2 to use the HSPG/LRP pathway as an alternative means of remnant clearance more efficiently than apoE(Cys142)
Thus, apoE2 is associated with lower lipid levels, while apoE(Cys142) is associated with a more severe hyperlipidemia.

We have demonstrated variability in the binding of apoE variants to heparin-Sepharose and hepatic matrix or membrane-bound HSPG and in cellular binding that is heparinase-susceptible (82). Apolipoprotein E3 (the common form of apoE) binds very well to HSPG, and apoE2 is only somewhat impaired. However, the apoE142, 145, 146, and Leiden variants, all of which are associated with dominant transmission of type III HLP, bind very poorly to HSPG (82). In fact, defective HSPG binding is the most striking common property of these dominant variants (Table 4). The cell-surface binding of 125I-labeled β-VLDL or β-VLDL plus various forms of apoE to McA-RH7777 cells is shown in Fig. 11. Adding apoE3 to β-VLDL enhanced the binding severalfold. All of the enhanced binding was abolished by pretreating the cells with heparinase to remove the sulfated carbohydrate side chains from cell-surface HSPG. Heparinase plus an LDL receptor antibody abolished the remaining activity. ApoE2-enriched β-VLDL also displayed significantly enhanced binding, reflecting the ability of apoE2 to bind to HSPG despite markedly impaired LDL receptor binding, and the enhanced binding was abolished by heparinase. In contrast, β-VLDL enriched with apoE(Cys142) or apoE-Leiden did not show enhanced HSPG binding, reflecting the poor heparin-binding activity of these variants.

The relative contributions of the LDL receptor and HSPG/LRP pathways to remnant clearance are summarized in Fig. 12. Apolipoprotein E2 cannot use the LDL receptor pathway efficiently, but it can use the HSPG/ LRP pathway fairly normally. In contrast, apoE(Cys142) uses the LDL receptor pathway more efficiently than apoE2 but the HSPG/ LRP pathway less efficiently. In the animal models lacking LDL receptors, however, apoE(Cys142)-containing remnants cannot be cleared effectively because of the severely defective HSPG binding of apoE(Cys142), leading to a severe type III HLP. In contrast, because apoE2 can still use the backup HSPG/ LRP pathway effectively, plasma lipids are maintained at lower levels, affording protection against overt hyperlipoproteinemia. A corollary in humans with normal LDL receptor expression and the apoE(Cys142) variant, which is only partially impaired in LDL binding but markedly defective in HSPG binding, is that LDL receptor-binding activity alone is not...
sufficient to mediate normal remnant clearance, and type III HLP occurs. Thus, the HSPG-binding affinity of apoE variants appears to be an important determinant of recessive versus dominant expression of type III HLP. Recall that HSPG appear to function not only in the initial sequestration (capture) of apoE-containing remnants in the space of Disse, but also as part of the HSPG/LRP pathway or acting alone as a receptor mediating uptake (3, 5, 118).

Clearly, studies in transgenic models have provided insights into why apoE2 behaves as a recessive variant, whereas other variants are dominant. As discussed above and outlined in Table 4, various properties of the mutant forms of apoE interact to modulate remnant lipoprotein metabolism. The differential binding of the variants to the LDL receptor versus the HSPG/LRP pathway appears to play a key role. Defective HSPG binding of the dominant variants of apoE associated with type III HLP could impair even the initial sequestration or capture step in remnant metabolism, as well as receptor-mediated clearance. Likewise, the preferential enrichment of remnant lipoproteins with the defective apoE, secondary to the mutation occurring on the E4 allele, could also contribute to the dominant expression. In subjects with apoE2, which is associated with the recessive mode of inheritance, the relatively normal HSPG binding protects against overt hyperlipidemia except when secondary environmental or hormonal factors stress remnant metabolism and saturate or impair normal clearance pathways. The HSPG sequestration pathway has been shown to be readily saturated (119). On the other hand, in subjects with apoE variants associated with dominant transmission, in which secondary factors are usually not required, the defective HSPG binding, even in the context of significant LDL receptor binding, invariably results in hyperlipidemia.

ACCELERATED ATHEROSCLEROSIS

As a consequence of the derangements in lipoprotein metabolism, subjects with type III HLP are at increased risk of accelerated atherosclerosis (2). We have used our apoE2 transgenic rabbit model to explore this association (75). When fed a normal chow diet, apoE2 transgenic rabbits had plasma cholesterol levels in the 250–500 mg/dl range. At 11 months of age, the transgenic rabbits had significant spontaneous lesions in both the aortic arch and in the abdominal aorta, which were more extensive in males, whereas nontransgenic rabbits on the chow diet had essentially no atherosclerotic lesions. Mapping of suda-roaphilic lesions in the aortas of the male apoE2 transgenic rabbits demonstrated the extensive distribution of the lesions in these rabbits (the area of lipid staining was 24% in the aortic arch and 10% in the abdominal aorta). The atherogenic hyperlipidemia in the apoE2 rabbits was very similar to the lipid profile seen in humans with overt type III HLP, and these results further establish the role of remnant lipoproteins in lesion formation.

Apolipoprotein E(Cys142) transgenic mice, which had typical β-VLDL and cholesterol levels of about 300 mg/dl, did not have significant lipid-stained aortic lesions at 4 months of age on a normal chow diet (120). However, after consuming a high-fat, high-cholesterol diet for 3 months, they had elevated cholesterol levels (≈400 mg/dl) and extensive complicated lesions characterized by increased cellularity and fibrous cap formation involving the base of the aortic valves and the aortic wall. The mean lesion area was 10-fold higher in the apoE(Cys142) mice than in nontransgenic mice (120). Likewise, diet-induced atherosclerosis has been reported in transgenic mice coexpressing the defective apoE-Leiden with apoCI (114).

One of the important lessons learned from studies of type III HLP is the atherogenic potential of remnant lipoproteins. Even in the context of the low plasma LDL levels, typical of most patients with overt type III HLP, atherosclerosis of coronary and peripheral arteries occurs frequently, and undoubtedly it is the remnants that are responsible for the vascular disease (121, 122). Remnant lipoproteins are uniquely taken up by macrophages and result in cholesterol loading and foam cell formation (123–125).
125). In addition, several recent reports have provided important insights into the role of remnant lipoproteins as atherogenic lipoproteins in clinical studies in humans (126, 127).

SUMMARY

Transgenic animal studies have allowed us to gain insights into some of the questions and paradoxes of type III HLP. Studies in apoE2 transgenic mice have shown that secondary factors, in this instance, other gene interactions, can precipitate type III HLP in otherwise hypolipidemic apoE2 mice. Studies in apoE2 transgenic rabbits have established the importance of estrogen in protecting females against developing type III HLP and the role of estrogen deficiency in precipitating hypercholesterolemia, hypertriglyceridemia, and remnant accumulation. The combined hypercholesterolemia and hypertriglyceridemia that are characteristic of overt type III HLP may be explained by a combination of factors: 1) defective apoE impairing receptor-mediated clearance of remnants by the liver, 2) increased levels of apoE impairing lipolytic processing, and 3) increased levels of apoE stimulating VLDL production. These factors combine to alter lipoprotein metabolism in ways characteristic of the type III HLP phenotype. Unique properties of the rare apoE variants associated with dominant inheritance, compared with the properties of apoE2, explain the mode of inheritance of type III HLP. Of major importance is the differential binding of the variants to the LDL receptor and to the HSPG/LRP pathway. The dominant variants tend to be more defective in HSPG/LRP binding than apoE2 and thus are less capable of being sequestered in the space of Dissé and tethered to cell-surface HSPG for further processing and uptake. In addition, the dominant mode of inheritance for certain apoE variants may be partly mediated by their preferential association with remnant lipoproteins, enriching these particles in defective apoE. Overall, transgenic animal models of type III HLP have afforded an opportunity to achieve a better understanding of the complexities of this fascinating human lipid disorder.

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