

Evidence for differential effects of apoE3 and apoE4 on HDL metabolism

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Abstract We present a murine model that examines the effects of macrophage-produced apolipoprotein E3 (apoE3) and apoE4 on VLDL and high density lipoprotein (HDL) metabolism. Mice expressing apoE3 on the *ApoE*^{-/-} background had substantially lower VLDL levels than mice expressing apoE4. In addition, there were differences between the HDL of apoE3- and apoE4-expressing mice. *ApoE*^{-/-} mice have low levels of HDL. Low level expression of either apoE3 or apoE4 was able to restore near-normal HDL levels, which increased dramatically when the mice were challenged with a high-fat diet. ApoE4-expressing mice had smaller HDL than apoE3-expressing mice on both chow and high-fat diets. In addition, plasma from apoE4-expressing mice was less efficient at transferring apoA-I from VLDL to HDL and at generating HDL in vitro than that from apoE3-expressing mice. Thus, we present experimental evidence for differential effects of apoE3 and apoE4 on HDL metabolism that supports epidemiological observations made in humans, which suggested that individual homozygous for the $\epsilon 4$ allele had lower HDL than others.—Hopkins, P. C. R., Y. Huang, J. G. McGuire, and R. E. Pitas. Evidence for differential effects of apoE3 and apoE4 on HDL metabolism. *J. Lipid Res.* 2002. 43: 1881–1889.

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Apolipoprotein E (apoE) is a 34-kDa protein that participates in the mobilization and distribution of cholesterol and other lipids among various tissues of the body (1). In humans, there are three common isoforms of apoE: apoE2, apoE3, and apoE4. ApoE4 differs from apoE3, the most common isoform of apoE, at position 112, where apoE3 has cysteine and apoE4 arginine; apoE2 differs from apoE3 by an arginine to cysteine substitution at position 158. The $\epsilon 4$ allele, which is present in 14–22% of the population (2, 3), is one of the most frequent independent genetic risk factors for atherosclerosis. A single

$\epsilon 4$ allele is sufficient to increase the risk of developing atherosclerosis (3).

The $\epsilon 4$ allele results in slightly elevated plasma LDL cholesterol levels (by ~ 10 mg/dl) and a small but significant decrease in plasma HDL levels (2, 4). Furthermore, $\epsilon 4$ homozygotes have lower total levels of circulating apoE than $\epsilon 3$ homozygotes, and $\epsilon 3/\epsilon 4$ heterozygotes have a lower proportion of their circulating apoE as apoE4, reflecting the more rapid catabolism of apoE4-containing lipoproteins than apoE3-containing lipoproteins (5). Remnant lipoproteins containing apoE4 may also have a higher affinity for the LDL receptor than those containing apoE3 (6). Therefore, increased catabolism of lipoprotein remnants in apoE4 individuals could lead to the down-regulation of hepatic lipoprotein receptors (5), engendering an increase in circulating levels of atherogenic LDL, consistent with epidemiological observations.

As a ligand for the LDL receptors, the LDL receptor-related protein, and heparan sulfate proteoglycans, apoE participates in the hepatic clearance of triglyceride-rich lipoproteins (TRL) and their remnants from the circulation (7). In addition, apoE can accelerate TRL catabolism by acting as a cofactor for hepatic lipase and lipoprotein lipase, affecting their activities either positively or negatively depending on the plasma apoE level (8, 9). ApoE-deficient (*ApoE*^{-/-}) mice develop substantial hypercholesterolemia due to the accumulation of incompletely metabolized remnant lipoproteins in plasma (10) and develop extensive atherosclerotic lesions as early as 6 weeks of age, even on a low-fat diet (11, 12).

Although apoE is clearly important in the hepatic clearance of TRL, with consequent impacts on atherosclerosis, several lines of evidence suggest that apoE also plays important roles in lipoprotein metabolism and in the suscep-

Abbreviations: apo, apolipoprotein; apoE3^{+/-} and apoE4^{+/-} mice, *ApoE*^{-/-} mice hemizygous for the visna virus long terminal repeat-driven expression of human apoE3 or apoE4; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TRL, triglyceride-rich lipoprotein(s).

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tibility to atherosclerosis that are independent of its effects on TRL levels. The subendothelial accumulation of cholesterol-laden macrophages is an early feature of atherosclerosis, and apoE expression by macrophages increases substantially upon their differentiation from monocytes (13). When transplanted into *ApoE*^{-/-} mice, wild-type macrophages substantially reduce the development of atherosclerosis (14). However, such macrophages produce sufficient apoE to reestablish nearly normal clearance of TRL remnants (14). Nevertheless, low plasma levels of murine apoE, produced by the adrenal gland, are sufficient to prevent atherosclerosis independently of effects on remnant clearance (15). In transgenic *ApoE*^{-/-} mice, human apoE3 produced by macrophages at levels insufficient to normalize total plasma cholesterol, also prevents atherosclerosis (16).

TRL metabolism is closely linked to HDL metabolism (17–19). HDLs are formed mostly in the plasma compartment. In humans, it is estimated that at least 50% of the apoA-I of HDL is derived from TRL; nascent HDL also acquire apoCs and lipids from TRL (20). The TRL of *ApoE*^{-/-} mice contain significant amounts of apoA-I, the major protein component of HDL. *ApoE*^{-/-} mice have lower HDL levels than wild-type mice, and, unlike wild-type mice, their HDL levels do not increase in response to a high-fat diet (11, 21). Macrophage-derived murine apoE can restore both the plasma apoA-I distribution and the HDL profile of *ApoE*^{-/-} mice to normal (22).

Some, but not all, epidemiological studies have observed that the $\epsilon 4$ allele is over-represented in individuals with lower HDL cholesterol levels (2, 3, 23). However, the effect of apoE3 and apoE4 on HDL metabolism has not been examined in an animal model. In the current study, we investigated the effect of low plasma levels of the human apoE3 or apoE4, expressed in macrophages of *ApoE*^{-/-} mice, on HDL metabolism. Here we report that low plasma levels of either human apoE3 or apoE4 cause a shift of apoA-I from VLDL to HDL lipoprotein fractions and restore HDL to near normal levels, even while remnant levels remain elevated, and that apoE3-expressing mice have higher total HDL levels and produce larger HDL than apoE4-expressing mice.

MATERIALS AND METHODS

Materials

Regular mouse chow (Purina 5058; Ralston Purina Co., St. Louis, MO) or a high-fat Western-type diet (Adjusted-calories diet TD 88137; Harlan Teklad, Madison, WI, containing 21% fat by weight and 1.5 g/kg cholesterol) was used as indicated. Polyclonal goat anti-apoE and anti-apoA-I antibodies were purchased from Calbiochem (La Jolla, CA). ApoE knockout mice (*ApoE*^{tm1Unc}) and apoA-I knockout mice (*ApoA1*^{tm1Unc}) in the C57BL/6J background were purchased from Jackson Laboratory (Bar Harbor, ME).

Generation of transgenic mice

To obtain macrophage-specific expression of apoE, genomic DNA encoding either the human *APOE**3 or *APOE**4 genes was

cloned in front of the visna virus long terminal repeat (LTR), which directs macrophage expression (24), as previously described for apoE3 (16). Constructs containing the *APOE**3 or *APOE**4 genes were a gift of Dr. John Taylor (Gladstone Institutes). Fragments comprising the visna virus LTR and the *APOE* genes were excised by *SalI* and *HindIII* digestion and injected into the pronuclei of single-cell FVB/N mouse embryos, which were then implanted in foster mothers. Founder mice positive by Southern blot analysis were crossed with C57BL/6J mice, and the F1 generation was assessed by immunoblot analysis for the level of apoE expression in plasma (using purified recombinant apoE3 as a standard) and for tissue specificity by RNase protection assay. Selected lines of mice were bred for four or five generations with C57BL/6J *ApoE*^{-/-} mice. Study groups were generated by crossing these mice, which were hemizygous for the visna virus apoE3 or apoE4 alleles, with C57BL/6J *ApoE*^{-/-} mice to produce offspring hemizygous for either the *APOE**3 or *APOE**4 gene under control of the visna virus LTR promoter (apoE3^{+/-} or apoE4^{+/-}), and littermate controls having no functional *APOE* genes (*ApoE*^{-/-}).

Plasma lipid and lipoprotein analysis

Total cholesterol concentrations were determined using the coupled cholesterol esterase and oxidase assays (Cholesterol/HP; Boehringer Mannheim, IN). Protein concentrations were determined with the Lowry assay. Plasma lipoprotein profiles were determined by size-exclusion chromatography through a Superose 6 column (Pharmacia, Piscataway, NJ) with phosphate-buffered saline and 1 mM EDTA as the mobile phase. Purification of HDL was performed by centrifugation of samples through a potassium bromide density gradient ($d = 1.006$ to 1.21 g/ml) at 54,000 rpm for 18 h in a Beckman SW55 rotor. After centrifugation, 0.5 ml fractions were collected from the bottom of the tube. Each fraction was assayed for protein and cholesterol concentrations and for density by refractometry. Nondenaturing gel electrophoresis was carried out on 4–30% acrylamide gels as previously described (25), apoA-I was detected by immunoblot analysis, and HDL particle size was estimated by comparison with the size of known protein standards (Pharmacia high molecular weight calibration kit).

In vitro transfer of apoA-I from TRL to HDL

Blood was drawn by tail bleed from fasted wild-type, apoE3^{+/-}, apoE4^{+/-}, apoA-I^{-/-}, or *ApoE*^{-/-} mice and anticoagulated with EDTA, and the plasma was isolated. The in vitro transfer of apoA-I from TRL remnants was assessed by co-incubating 50 μ l *ApoE*^{-/-} plasma with an equal volume of plasma from either wild-type, apoE3^{+/-}, apoE4^{+/-}, *ApoE*^{-/-}, or apoA-I^{-/-} mice for 18 h. All incubations were performed at room temperature. The lipoproteins in the co-incubated plasmas were then fractionated by Superose 6 chromatography, and the apoA-I content of the fractions was analyzed by immunoblot.

Cholesterol efflux assays

HDL were isolated from pooled plasma by fractionation on a Superose 6 column. The fractions containing HDL were pooled and further purified by density gradient centrifugation. Purified HDL from wild-type, *ApoE*^{-/-}, apoE3^{+/-}, and apoE4^{+/-} mice were compared for their ability to promote cholesterol efflux from cholesterol-loaded human foreskin fibroblasts. The cells were cultured in Dulbecco's modified Eagle's medium containing 10% fetal calf serum in 35-mm dishes until almost confluent and labeled with 0.2 mCi of [1,2-³H]cholesterol (New England Nuclear, Boston, MA) for 72 h at 37°C (26). After washing with phosphate-buffered saline, HDL in serum-free medium was ap-

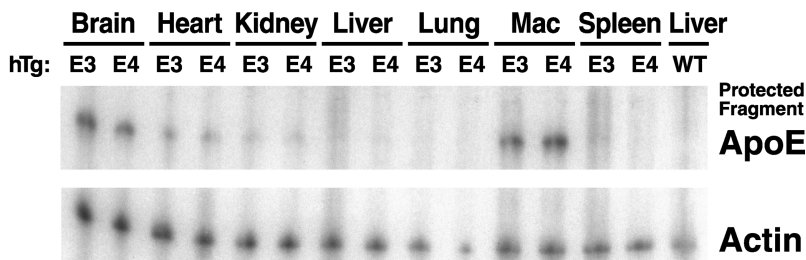


Fig. 1. RNase protection assay showing the specificity of apoE expression in the selected lines of apoE3^{+/-} and apoE4^{+/-} mice. RNA was isolated from various tissues and from thioglycollate-elicited peritoneal macrophages (Mac). A fragment of human apoE cDNA was used as a probe. Wild-type (WT) mouse liver RNA was included to demonstrate the specificity of the probe for human apoE mRNA.

plied to the cells. The amount of *ApoE*^{-/-} HDL added in each assay was 30 µg/ml as estimated by the Lowry assay, and the quantity of the HDL from apoE-expressing animals was normalized to this according to apoA-I content by densitometry of immunoblots. At the indicated times, aliquots of the medium were removed, cell debris was removed by centrifugation, and the radioactivity was counted by scintillation spectrometry. Efflux occurring in the absence of added HDL at each time point was subtracted before plotting the data.

RESULTS

Macrophage-specific expression of apoE in transgenic mice

Several lines of mice expressing human apoE3 or apoE4 in macrophages were generated. They were screened by RNase protection assay for tissue-specificity of expression and by immunoblot analysis of plasma to determine apoE expression levels. One line of each type was selected for further study. These lines of apoE3- and apoE4-expressing mice had similar patterns of mRNA production, with a high degree of specificity for apoE mRNA in macrophages and brain (**Fig. 1**). This expression pattern is consistent with the expression pattern of visna virus (24) and similar to that we observed previously (16). The selected lines also had similar plasma levels of apoE, as estimated by densitometry of Western blots using recombinant human apoE as a standard. The plasma of hemizygous apoE3- and apoE4-expressing mice contained 5.1 ± 1.4 µg/ml (n = 12) and 4.8 ± 1.5 µg/ml (n = 24) of apoE, respectively (**Table 1**). Mice hemizygous for the apoE3 or apoE4 genes were crossed with C57BL/6J *ApoE*^{-/-} mice for four or five generations to generate genetically similar inbred *ApoE*^{-/-} mice expressing apoE3 or apoE4. These hemizygous mice were then crossed with C57BL/6J *ApoE*^{-/-} mice to generate littermate offspring for the studies, which were hemizygous for the apoE3 or apoE4 gene or were completely deficient in apoE.

Effect of apoE3 or apoE4 expression on plasma cholesterol levels

Total cholesterol levels were measured in fasted mice four times over 20 weeks for each animal on chow diet. The plasma cholesterol levels of the *ApoE*^{-/-} littermates of

the apoE3- and apoE4-expressing mice were not significantly different (285 ± 69 vs. 320 ± 138 mg/dl, respectively, **Table 1**). Expression of either apoE3 or apoE4 resulted in a significant decrease in plasma cholesterol compared to their littermates not expressing apoE. Expression of apoE3 resulted in a decrease in plasma cholesterol levels from 285 ± 69 to 80.9 mg/dl, and expression of apoE4 resulted in a reduction of plasma cholesterol levels from 320 ± 138 to 175 ± 81 mg/dl. The mean plasma cholesterol levels of the apoE3-expressing mice was 80.9 ± 60 mg/dl; however, cholesterol levels were not evenly distributed about the mean and ranged from 35 to 181 mg/dl, clustering in the lower quartiles. These data indicate that both macrophage-derived apoE3 and apoE4 were capable of lowering plasma cholesterol levels, but that apoE4 was less efficient than was apoE3.

To compare the effect of apoE3 and apoE4 on the response to a high-fat Western-type diet, mice hemizygous for macrophage-specific expression of apoE3 or apoE4,

TABLE 1. Plasma apoE and cholesterol levels in *ApoE*^{-/-} mice with macrophage expression of human apoE3 or apoE4, and in *ApoE*^{-/-} littermate controls

Genotype	Plasma ApoE µg/ml ± SD (n)	Mean Total Plasma Cholesterol mg/dl ± SD	
		Chow Diet (n)	High-Fat Diet (n)
apoE3 ^{+/-}	5.1 ± 1.4 ^c (n = 12)	80.9 ± 60 ^a (n = 35)	348.6 ± 162.2 ^b (n = 16)
<i>ApoE</i> ^{-/-} littermates	0	285 ± 69 ^d (n = 19)	1,210 ± 531 ^e (n = 7)
apoE4 ^{+/-}	4.8 ± 1.5 ^c (n = 24)	175 ± 81 ^a (n = 83)	742.8 ± 140.8 ^b (n = 8)
<i>ApoE</i> ^{-/-} littermates	0	320 ± 138 ^d (n = 35)	1,101 ± 81 ^e (n = 5)

Blood was drawn from the tails of apoE3- and apoE4-expressing mice, and plasma apoE levels were determined by quantitative densitometry of Western blots. Recombinant human apoE served as a standard. In chow-fed mice, three or four cholesterol measurements were made over the period of the study (from weaning to 20 weeks of age), and the mean for each mouse was determined; the data presented are the means of these values. In mice on a high-fat diet, two or three cholesterol measurements were made over 10 weeks, and the mean for each mouse was determined; the data presented are the means of these values.

^{a,b} *P* < 0.001.

^{c,d,e} Differences not significant.

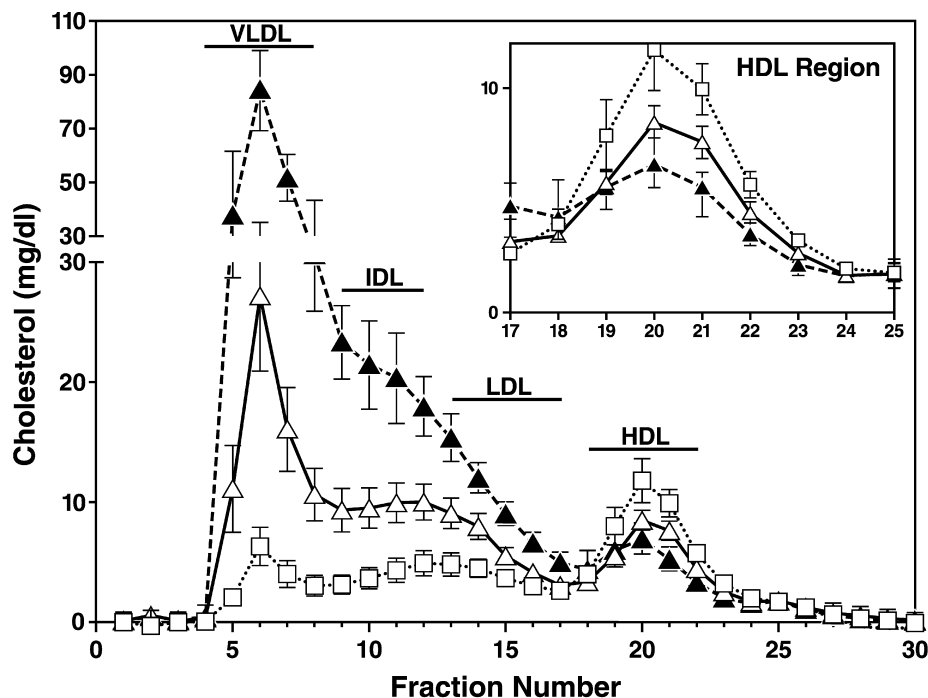


Fig. 2. The effect of apoE3 or apoE4 on plasma lipoprotein profiles of mice fed a chow diet. Plasma from individual apoE3^{+/+} (n = 10; square) or apoE4^{+/+} (n = 8; open triangle) mice or from *Apoe*^{-/-} littermate controls (n = 13; closed triangle) was separated by Superose 6 chromatography, and the cholesterol concentration in each fraction was determined. Bars indicate the SD. Fractions containing HDL are shown in the inset.

their *Apoe*^{-/-} littermates, and wild-type mice were fed the diet for 10 weeks after weaning. Total cholesterol levels were measured two or three times during this period. In response to the high-fat diet, total plasma cholesterol levels increased markedly in all mice. As with the chow diet, the plasma cholesterol levels were highest in *Apoe*^{-/-} littermate mice and lowest in apoE3-expressing mice (Table 1).

On both chow and high-fat diets, the variation in cholesterol levels of both *Apoe*^{-/-} mice and their apoE3^{+/+} or apoE4^{+/+} littermates was larger than previously reported for *Apoe*^{-/-} mice. The variability in genetic background likely accounts for this variation (27); nevertheless, statistical tests of significance exclude this variability as a significant modifier of our results.

Effect of apoE3 or apoE4 expression on plasma lipoprotein profiles

The plasma lipoprotein profiles of the apoE3- and apoE4-expressing mice and their *Apoe*^{-/-} littermates on the chow diet were determined by size fractionation using Superose 6 chromatography. The littermate *Apoe*^{-/-} mice had high levels of VLDL-, IDL-, and LDL-sized particles (Fig. 2), a profile similar to that previously reported for *Apoe*^{-/-} mice (10). Both apoE3 and apoE4 significantly reduced the levels of VLDL, IDL, and LDL; however, apoE4 did so less effectively. In addition, HDL was increased in the presence of apoE, this increase being less in the presence of apoE4 than apoE3 (Fig. 2, inset).

Size fractionation of plasma from mice on the high-fat

diet for 10 weeks by Superose 6 chromatography showed qualitative effects similar to those in mice fed the chow diet (Fig. 3). ApoE-expressing mice had lower VLDL, IDL, and LDL cholesterol levels than did their knockout litter-

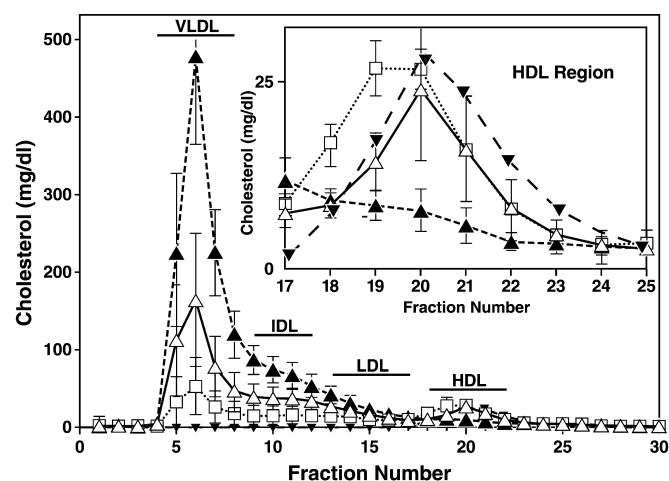


Fig. 3. The effect of apoE3 or apoE4 on plasma lipoprotein profiles of mice fed a high-fat diet. Plasma from individual apoE3^{+/+} (n = 7; square) and apoE4^{+/+} (n = 8; open triangle) mice, their *Apoe*^{-/-} littermates (n = 7; closed upright triangle), and WT mice (closed inverted triangle) was separated by Superose 6 chromatography, and the cholesterol concentration in each fraction was determined. Bars indicate the SD. The later elution of HDL from apoE4^{+/+} mice relative to apoE3^{+/+} mice indicates that apoE4^{+/+} mice have smaller HDL than apoE3^{+/+} mice (inset).

mates, with those expressing apoE4 having higher levels than those expressing apoE3. On a high-fat diet, the HDL were larger in mice expressing apoE; apoE3-expressing mice had a greater increase in HDL than apoE4-expressing mice. *ApoE*^{-/-} mice, however, showed almost no increase in HDL in response to the high-fat diet (Fig. 3, inset).

Examination of HDL size and apoA-I content by nondenaturing gelelectrophoresis

We examined the size difference in HDL produced in chow-fed apoE4 and apoE3-expressing mice by nondenaturing gel electrophoresis in which apoA-I was detected by immunoblot analysis (Fig. 4). Densitometric scanning of similar gels and calibration with the Pharmacia high-molecular-weight calibration kit yielded size estimates for HDL of 113 Å in apoE3^{+/-} mice and 107 Å in apoE4^{+/-} mice. In addition to the apoA-I present on HDL, more apoA-I was present on larger particles (likely VLDL) in the plasma of apoE4^{+/-} mice than in that of wild-type and apoE3^{+/-} mice. The intensity of total apoA-I-specific staining (larger particles plus HDL) and HDL-specific staining for apoE3^{+/-} and apoE4^{+/-} mice were compared. The ratio of total staining was 0.96:1.0 for apoE3^{+/-} mice and apoE4^{+/-} mice; however, the ratio of HDL-specific staining was 1.0:0.76 for apoE3^{+/-} mice and apoE4^{+/-} mice, indicating less HDL-associated apoA-I in apoE4^{+/-} mice than in apoE3^{+/-} mice.

Differential distribution of apoA-I in the plasma of apoE3 and apoE4 mice

Western blot analysis of whole plasma showed no difference in apoA-I levels between chow-fed wild-type, apoE3-expressing, apoE4-expressing, and *ApoE*^{-/-} mice (data not shown). However, there was an altered distribution of apoA-I between the lipoproteins of apoE3^{+/-} and apoE4^{+/-} mice (Fig. 5). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot analysis of plasma fractionated by Superose 6 chromatography showed that a substantial fraction of total apoA-I in *ApoE*^{-/-} mice was present on particles eluting in the VLDL and IDL regions. In mice expressing apoE3, no apoA-I was found in the VLDL and IDL regions; however, in mice expressing apoE4, apoA-I was distributed more widely among the lipoprotein fractions.

In vitro transfer of apoA-I from TRL to HDL

We demonstrated that apoE-containing plasma could cause the transfer of apoA-I from TRL remnants to HDL in vitro by performing reconstitution experiments. All apoE-containing plasmas (wild-type, apoE3^{+/-}, apoE4^{+/-}, and apoA-I^{-/-}) cause the loss of apoA-I from TRL remnants; however, apoE4^{+/-} plasma was notably less efficient at this than apoE3^{+/-} plasma (Fig. 6A). Quantitation of the amount of apoA-I transferred from TRL by densitometric scanning of immunoblots was performed. After incubation and fractionation, the densities of TRL apoA-I from *ApoE*^{-/-} mice were added to that from the apoE-containing plasma, and the apoA-I density remain-

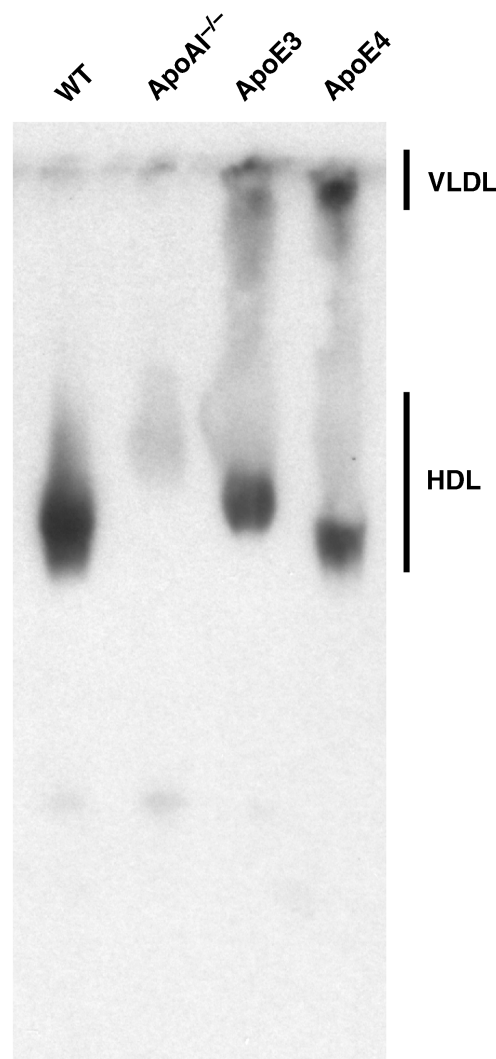


Fig. 4. Immunoblot for apoA-I of a 4–30% acrylamide nondenaturing gel of plasma from chow-fed WT, apoE3^{+/-}, apoE4^{+/-}, and apoA-I^{-/-} mice. HDL from apoE3^{+/-} were larger than HDL from apoE4^{+/-} mice. Calibration of these HDL against standards indicated a size of 113 Å for apoE3^{+/-} HDL, 107 Å for apoE4^{+/-} HDL, and 109 Å for WT HDL. Plasma from apoA-I^{-/-} mice was used as a negative control.

ing on TRL in the co-incubated plasmas was subtracted. This analysis showed that, during the period of the assay, plasma from wild-type or apoE3^{+/-} mice caused the transfer of 91.5% and 74.9% of the apoA-I present on initial TRL, respectively, whereas plasma from apoE4^{+/-} mice caused the transfer of only 38.3% of initial TRL apoA-I (Fig. 6B).

We then demonstrated that the apoA-I residing on VLDL in *ApoE*^{-/-} mice was transferred to an HDL-like particle in vitro. VLDL from *ApoE*^{-/-} mice was isolated by Superose 6 chromatography and incubated with plasma from apoA-I^{-/-} mice. The plasma from apoA-I^{-/-} mice proved to be capable of transferring apoA-I from VLDL to HDL; the lack of apoA-I in the plasma of these mice permitted the unambiguous determination that in this assay the apoA-I in the VLDL of *ApoE*^{-/-}

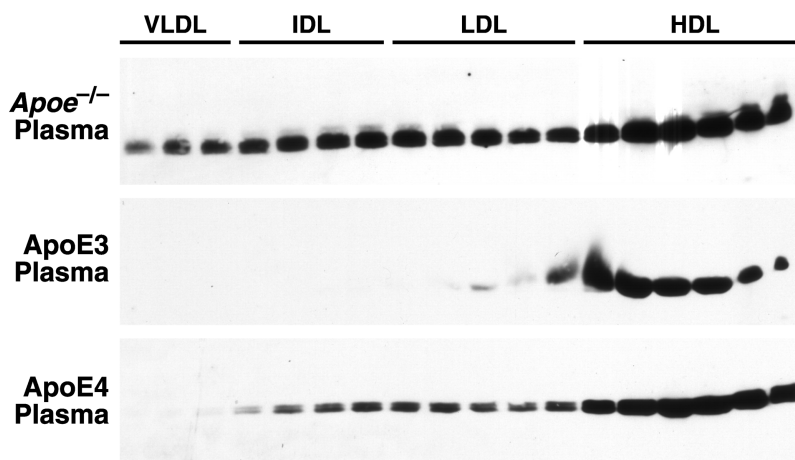


Fig. 5. Differential distribution of apoA-I in plasma lipoproteins from *Apoe*^{-/-}, apoE3^{+/-}, and apoE4^{+/-} mice. Plasma from each type of mouse was fractionated by Superose 6 chromatography. The fractions were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and apoA-I was detected by immunoblot analysis.

plasma was being transferred to an HDL-like particle (Fig. 7).

Effect of apoE on cholesterol efflux

The HDL fractions in pooled plasma from wild-type, apoE3, or apoE4 mice were isolated by Superose 6 chromatography and density centrifugation, and their ability to promote efflux from cholesterol-loaded human foreskin fibroblasts was determined. Measurement of the appearance of isotope in the media reflects a combination of isotope exchange and cholesterol efflux and is correlated with net mass efflux of cholesterol from cells to HDL (28). When normalized for apoA-I content, the HDL produced by wild-type, apoE3, and apoE4-expressing mice were similar in their ability to promote cholesterol efflux, and all were more competent than HDL from *Apoe*^{-/-}

mice (Fig. 8). The HDL isolated from apoE-expressing mice contained only trace amounts of apoE, as assessed by Western blot (data not shown). This assay therefore characterizes the relative abilities of HDL synthesized in apoE3- and apoE4-expressing mice, independent of any effect of apoE per se on the outcome.

DISCUSSION

In this study, we demonstrated that apoE displays isoform-specific effects in the transfer of apoA-I from TRL remnants to HDL in plasma and has isoform-specific effects on HDL in vivo. Although TRL metabolism differs significantly between apoE3- and apoE4-expressing mice (6, 29), the effects of these isoforms on HDL metabolism

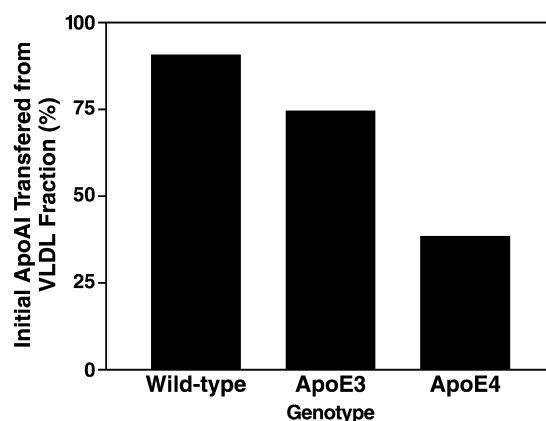
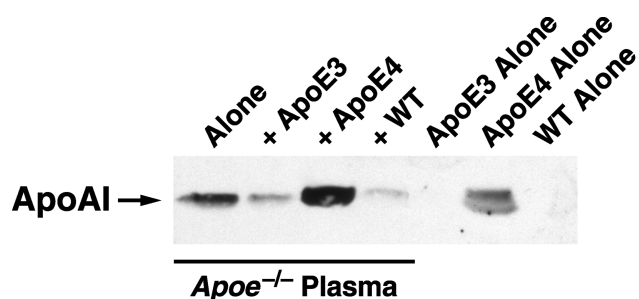


Fig. 6. A: ApoE4^{+/-} plasma is less effective at displacing apoA-I from triglyceride-rich lipoproteins (TRL) than is plasma from WT or apoE3^{+/-} mice. Plasma from *Apoe*^{-/-} mice was incubated at room temperature for 18 h either alone or with an equal volume of plasma from WT, apoE3^{+/-}, or apoE4^{+/-} mice. As controls, plasmas from apoE-expressing mice were incubated alone. The plasmas were then fractionated by Superose 6 chromatography and the VLDL fractions subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblot analysis for apoA-I. B: Quantitation of apoA-I transfer by densitometric scan. Each bar indicates the proportion (expressed as a percentage) of initial apoA-I lost from TRL during overnight co-incubation (i.e., the quantity of apoA-I present in the VLDL fraction of the co-incubated plasmas divided by the sum of the quantity of apoA-I in the VLDL of plasmas incubated alone).

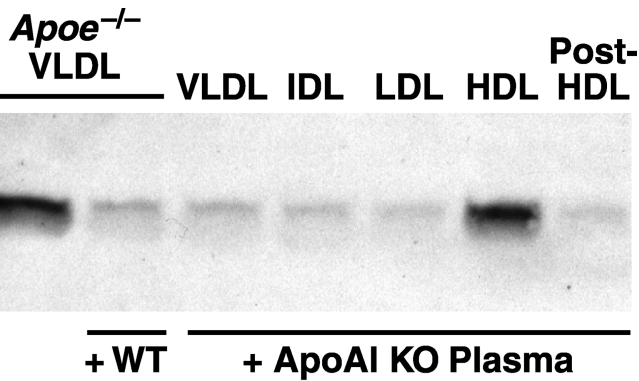


Fig. 7. Plasma from *apoA-I*^{-/-} mice is competent at displacing apoA-I from the VLDL of *ApoE*^{-/-} mice; ApoA-I is displaced to an HDL-like particle. Purified VLDL from *ApoE*^{-/-} mice was incubated for 18 h at room temperature either alone or with 50 μ l of plasma from WT or *apoA-I*^{-/-} mice. The co-incubated samples were then fractionated by Superose 6 chromatography. *ApoE*^{-/-} VLDL, the VLDL of *ApoE*^{-/-} plasma co-incubated with WT plasma, and all the fractions of *ApoE*^{-/-} plasma co-incubated with apoA-I knockout plasma were subjected to SDS-PAGE and immunoblot analysis for apoA-I.

in mice has not been studied previously. As chylomicrons pass from the mesenteric lymph to the blood stream, they acquire apoE and apoCs, which aid in their catabolism. During lipolysis of chylomicrons, surface components are shed and contribute to the formation of HDL. In this study, we demonstrate isoform-specific effects of apoE on this process.

Our examination of the lipoproteins in apoE3^{+/-} or apoE4^{+/-} mice with similar plasma levels of apoE showed a lower level of HDL in the apoE4^{+/-} mice. These data are

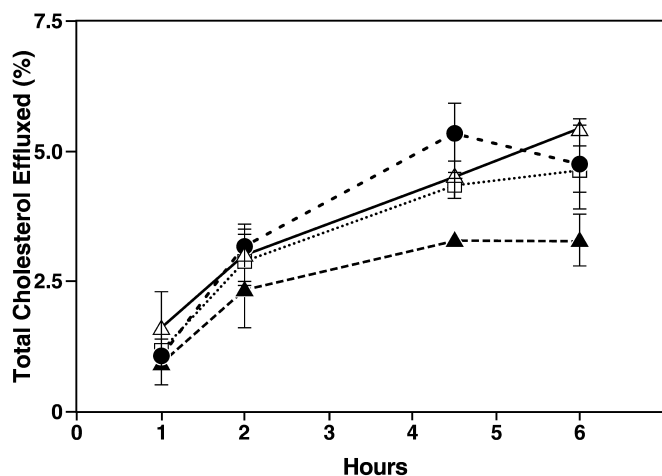


Fig. 8. HDL from apoE3^{+/-} mice and apoE4^{+/-} mice do not differ significantly in their ability to promote cholesterol efflux. HDL isolated from WT (circle), *ApoE*^{-/-} (closed triangle), apoE3^{+/-} (square) or apoE4^{+/-} (open triangle) mice, were assayed for their ability to promote cholesterol efflux from human foreskin fibroblasts as described under Materials and Methods. Each data point represents the mean of four experiments. Bars indicate the minimum and maximum values obtained.


consistent with the effects of apoE3 and apoE4 on HDL levels in humans (2, 3, 23). Furthermore, a difference in size of the HDL of the apoE3^{+/-} and apoE4^{+/-} mice fed a high-fat diet was readily apparent when the lipoproteins were separated by size-exclusion column chromatography. The HDL from apoE3^{+/-} mice was larger than the HDL from apoE4^{+/-} mice. When the mice were fed a normal chow diet, a size difference of the HDL was not as apparent on FPLC analysis. However, when the lipoproteins were separated by nondenaturing gel electrophoresis, a more sensitive technique for size discrimination, it was apparent that the HDL from the apoE3^{+/-} chow fed mice was also larger than that from the apoE4^{+/-} mice.

To determine if there are functional differences between the HDL found in the presence of apoE3 and apoE4, we examined the ability of the HDL to promote cholesterol efflux. HDL from wild-type, apoE3^{+/-}, and apoE4^{+/-} mice were similar in their ability to promote cholesterol efflux from cholesterol-loaded cells in vitro when normalized for apoA-I content, and all were significantly better than HDL from *ApoE*^{-/-} mice. The difference in efflux capacity between wild-type and *ApoE*^{-/-} HDL may reflect differences in their lipid content (30, 31). Our data therefore suggest that any differences in the ability of the plasma of apoE3^{+/-} or apoE4^{+/-} mice to serve as cholesterol acceptors for reverse cholesterol transport in vivo could be more affected by differences in the final equilibrium levels of HDL than by differences in their composition.

The apoE in our mice is macrophage derived, while normally most apoE is liver derived; however, we do not anticipate that the effect we have observed is macrophage-dependent. It is known that low levels of macrophage- or adrenal gland-derived apoE can normalize lipoprotein levels in *ApoE*^{-/-} mice similar to liver-derived apoE (14–16). Furthermore, it is known that HDL is formed largely in the plasma compartment where apoE derived from different tissues could serve similar functions. In addition, we have shown that HDL can be formed from VLDL components ex vivo if plasma from *ApoE*^{-/-} mice is co-incubated with plasma containing either normal or only macrophage-derived apoE. In this instance, therefore, the source of the apoE is not likely to be an important factor in HDL formation.

The mechanism by which apoE3 and apoE4 exert differential effects on HDL formation is under investigation. There are several possibilities. First, the known differential affinity of apoE3 and apoE4 for various plasma lipoproteins (5, 32, 33) could directly affect the transfer of HDL components from chylomicrons and their remnants to HDL. Second, the effect of apoE on HDL formation could be indirect. Many proteins that are not important structural components of chylomicrons, their remnants, or HDL are nonetheless important for the transfer of such components to and from HDL and among HDL subpopulations. In mice, hepatic lipase, lipoprotein lipase, phospholipid transfer protein, and lecithin:cholesterol acyltransferase can affect the equilibrium levels of HDL. Overexpression of hepatic lipase in transgenic mice de-

creases HDL levels (34), and there is considerable evidence for in vivo cooperativity between lipoprotein lipase and apoE in the processing of TRL (20). ApoE significantly accelerates the catabolism of TRL by lipoprotein lipase (8, 35) but, at high concentrations, may inhibit its activity in vivo (9). Phospholipid transfer protein affects HDL synthesis (36, 37), and its absence in mice profoundly alters plasma lipoprotein metabolism, including reduced plasma HDL levels (38), possibly due to an increase in HDL catabolism (39). Like apoE deficiency, deficiency in lecithin:cholesterol acyltransferase lowers HDL levels and causes TRL remnants to accumulate apoA-I. Our continuing investigations aim to include or exclude these proteins as candidates for an apoE-dependent factor involved in HDL metabolism.

In summary, our data show that, in C57BL/6 *ApoE*^{-/-} mice, human apoE3 and apoE4 differ in their effects on HDL metabolism. The presence of apoE3 results in a more efficient transfer of apoA-I from chylomicron remnants to HDL in higher HDL levels, and in larger HDL, than the presence of apoE4. These data provide experimental support for epidemiological observations suggesting that apoE exerts isoform-specific effects on HDL metabolism in humans (2, 3, 23). 

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