Hepatic apolipoprotein E expression promotes very low density lipoprotein-apolipoprotein B production in vivo in mice

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Abstract In addition to its role in the uptake of apolipoprotein B (apoB)-containing lipoproteins, apoE promotes hepatic very low density lipoprotein-triglyceride (VLDL-TG) production in animal models. However, it is not known if apoE increases the amount of TG per VLDL particle or the number of VLDL particles secreted. VLDL-apoB production is a measure of the rate of VLDL particle secretion. We determined the effects of apoE deficiency and apoE overexpression on VLDL-apoB production in mice. [^{35}S]methionine was injected into endogenously label VLDL-apoB and Triton WR-1339 was simultaneously injected to block the catabolism of VLDL. Compared with wild-type mice, the VLDL-apoB production rate was decreased by 33% in apoE-deficient mice. Conversely, VLDL-apoB production was increased by 48% in mice overexpressing apoE compared with controls. Nascent VLDL, obtained from post-Triton plasma, had a decreased, not increased, content of TG per apoB in the apoE-overexpressing group compared with the control group. This study demonstrates that hepatic apoE expression increases the output of VLDL triglyceride by increasing the production rate of VLDL-apoB, suggesting that hepatic apoE influences the number of VLDL particles secreted by the liver.—Maugeais, C., U. J. F. Tietge, K. Tsukamoto, J. M. Glick, and D. J. Rader. **Hepatic apolipoprotein E expression promotes very low density lipoprotein-apolipoprotein B production in vivo in mice.** ***J. Lipid Res.*** **2000.** 41: **1673–1679.**

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Apolipoprotein E (apoE) has a well-established role in the receptor-mediated uptake of apoB-containing lipoproteins (1). More recently, it has been established that apoE plays a role in promoting the hepatic secretion of very low density lipoprotein triglycerides (VLDL-TG) (2). VLDL-TG secretion is impaired in apoE-deficient mice compared with wild-type mice and was not normalized by extrahepatic apoE expression (3). The livers of apoE-deficient mice have increased content of TG as well as cholesterol (4), consistent with a defect in hepatic lipid mobilization. Overexpression of human apoE in apoE-deficient mice in vivo resulted in increased production of VLDL-TG (3, 5, 6).

Changes in VLDL-TG production can be due to changes in the amount of TG per VLDL particle, the number of VLDL particles secreted, or both. The production of VLDL-TG and VLDL-apoB is differentially modulated by diet (7), genetic disorders (8), obesity (9), and non-insulin-dependent diabetes (10). The role of apoE in promoting VLDL-TG production could be to facilitate the loading of TG onto nascent VLDL particles in the hepatocyte, resulting in the production of TG-enriched VLDL particles. Alternatively, apoE could increase the VLDL-TG production by increasing the number of secreted VLDL particles. Because one apoB molecule is secreted per VLDL particle, VLDL-apoB production provides an assessment of the number of VLDL particles secreted.

To test the hypothesis that apoE expression promotes VLDL-apoB production in vivo, we simultaneously measured VLDL-TG and VLDL-apoB production in apoE-deficient mice and apoE-overexpressing mice compared with controls. We showed that VLDL-apoB production is decreased in apoE-deficient mice and increased in apoE-overexpressing mice. These findings indicate that apoE modulates hepatic VLDL-apoB production and VLDL particle secretion.

MATERIALS AND METHODS

Mouse experimental protocols

Female apoE-deficient mice (6–8 weeks old) crossed at least six times onto the C57BL/6 background and female wild-type C57BL/6 mice (6–8 weeks old) were obtained from the Jackson Laboratory (Bar Harbor, ME). In the first series of experiments,

Abbreviations: AdhapoE3, adenovirus encoding the human apoE3 cDNA; Adnull, adenovirus without a transgene; EC, esterified cholesterol; FC, free cholesterol; HDL, high density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; LDLR, low density lipoprotein receptor; LRP, LDL receptor-related protein; PL, phospholipids; RAP, receptor-associated protein; TG, triglyceride; VLDL, very low density lipoprotein.

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chow-fed apoE-deficient mice were compared with chow-fed wild-type mice with regard to VLDL-TG and VLDL-apoB production, using simultaneous injection of Triton WR-1339 and -[^{35}S]methionine as described below. In the second series of experiments, wild-type mice overexpressing human apoE3 were compared with control wild-type mice, using the same protocol as described below. For the second series, apoE-overexpressing mice were generated by intravenous injection of recombinant adenovirus. Second-generation adenoviruses encoding the human apoE3 cDNA (AdhapoE3) or no transgene (Adnull) were used as previously described (11). Adenoviruses were injected by tail vein at a dose of 1×10^{11} particles (approximately 2×10^{9} PFU). Blood was obtained from the retroorbital plexus before injection and 5 days after adenovirus injection to quantitate the plasma human apoE level. Kinetic studies were performed on day 5 after adenovirus injection as reported (12).

For determination of VLDL-TG and VLDL-apoB production rates, the following protocol was used for both series of experiments. On the morning of the kinetic study, 4 h prior to injection, mice were changed from a normal chow diet to a fat-free diet (dry cereal) and the supply of fat-free food was continued for the period of the experiment. Twenty milligrams of Triton WR-1339 (Sigma, St Louis, MO) was mixed with 500 μCi of L-[^{35}S]methionine (DuPont NEN, Boston, MA), and this mixture was injected in a volume of 200 μl through the tail vein. Blood was drawn from the retroorbital plexus before injection (50 μl) and at 5 min (10 μl), 1 h (50 μl), 2 h (50 μl), and 4 h (50 μl) after injection.

VLDL triglyceride production measurement

Plasma triglyceride levels were assayed prior to and 30 min, 1 h, 2 h, and 4 h after Triton WR-1339 injection, using an enzymatic method (Wako, Osaka, Japan). The VLDL-TG production rate was calculated by the increase in plasma TG level from baseline to 1 h after Triton WR-1339 injection. We used the 1-h time point to estimate VLDL-TG production, assuming a linear increase of plasma triglyceride concentrations during this time period. The values obtained under such conditions correspond to an apparent rate of VLDL-TG production based on the dose of Triton WR-1339 used. The data were expressed as micromoles of TG produced per hour per kilogram of body weight, assuming a plasma volume of 3.5% (liters per kilogram).

VLDL-apoB production determination

Twenty-microliter samples of plasma collected 1, 2, and 4 h after radioactivity injection were subjected to sequential ultracentrifugation using 1-ml polycarbonate tubes (Beckman, Fullerton, CA) at 90,000 rpm in a Beckman TLA 100.2 rotor for 3 h at 10°C (TL-100 centrifuge; Beckman). The VLDL ($d < 1.006$) and intermediate density lipoprotein/low density lipoprotein (IDL/LDL) ($1.006 < d < 1.063$) were isolated by tube slicing in a volume of 250 μl. ApoB was precipitated, and resolved in a 3–20% linear gradient soldium dodecyl sulfate (SDS)-polyacrylamide gel as previously described (12, 13). Radioactivity in apoB-48 and apoB-100 was determined as described (12). The counts per minute in apoB bands were normalized for the 5-min plasma counts per minute of [^{35}S]methionine. To assess the protein labeling precursor curve, 2 μl of plasma was submitted to trichloroacetic acid (TCA) precipitation and the supernatant radioactivity was counted to obtain the free plasma methionine radioactivity curve. No significant differences in the shape of this curve were observed between groups, indicating that the tissue uptake of the tracer was the same. Furthermore, we compared this radioactivity precursor curve with and without Triton WR-1339 to check for any inherent effect of Triton WR-1339 on the radioactivity uptake, and no differences were found.

When VLDL-apoB catabolism is blocked with Triton WR-1339, the radioactivity accumulation is directly proportional to the production rate of VLDL-apoB. Virtually no radioactivity was detected in IDL/LDL-apoB ($1.006 < d < 1.063$) in either wild-type or apoE-deficient mice (data not shown), demonstrating that Triton WR-1339 efficiently blocked the conversion of VLDL to LDL and indicating that direct production of IDL-apoB or LDL-apoB is not significant in these mice under our experimental conditions. The rate of radioactivity increase in VLDL-apoB was linear for 2 h after tracer injection (see Fig. 3). The VLDL-apoB production was expressed as cpm/μl/h, using the radioactivity from the 2-h time point.

Analysis of nascent VLDL composition

Mice were injected with AdhapoE3 or Adnull at the dose of 1×10^{11} particles as described above. Five days after adenovirus injection, Triton WR-1339 was injected into isolate nascent VLDL. VLDL ($d < 1.006$) was individually isolated from 200 μl of plasma from each mouse obtained 2 h after Triton WR-1339 injection. Enzymatic methods were used to quantitate cholesterol, free cholesterol, triglycerides, and phospholipids (Wako). The mass of esterified cholesterol, including the fatty acid mass, was calculated by multiplying the amount of esterified cholesterol by 1.67, which corresponds to the ratio of the average molecular weight of human cholesteryl ester to that of free cholesterol. The bicinchoninic acid (BCA) method (Pierce, Rockford, IL) was used to quantitate protein. ApoB-100 and apoB-48 were quantified in VLDL by densitometric analysis of Coomassie-stained SDS-polyacrylamide gels, using human LDL as standard.

Analytical methods

Pooled plasma samples (25 μl of plasma from five mice) from each group were subjected to fast protein liquid chromatography (FPLC) gel filtration (Pharmacia LKB Biotechnology, Uppsala, Sweden) on two Superose 6 columns (11). Samples were chromatographed at a flow rate of 0.5 ml/min and 0.5-ml fractions were collected. Lipid concentrations in the FPLC fractions were determined by enzymatic assays (Wako). The Student's *t*-test was used for comparison between two groups and a $P < 0.05$ was considered significant. Values are given as means ± SEM.

RESULTS

VLDL-apoB production is significantly reduced in apoE-deficient mice

Consistent with a previous report (3), the VLDL-TG production rate was significantly decreased by 43% ($P < 0.001$) in apoE-deficient mice compared with wild-type mice (**Fig. 1**). The lipoprotein profile was obtained by FPLC gel filtration before and 1 h after Triton WR-1339 injection. The increase in TG after Triton WR-1339 injection was limited to the VLDL peak (**Fig. 2**), indicating that the triglyceride production measured by the Triton WR-1339 method corresponds to VLDL-TG production.

The appearance of endogenously labeled VLDL-apoB in plasma is shown in **Fig. 3** for apoE-deficient and wild-type mice. The rate of total VLDL-apoB production was significantly decreased by 33% ($P < 0.001$) in apoE-deficient mice compared with wild-type mice (**Fig. 4**). A decrease of 41% ($P < 0.001$) and 28% ($P < 0.01$) was observed for production of VLDL-apoB-100 and of VLDL-apoB-48, respectively.

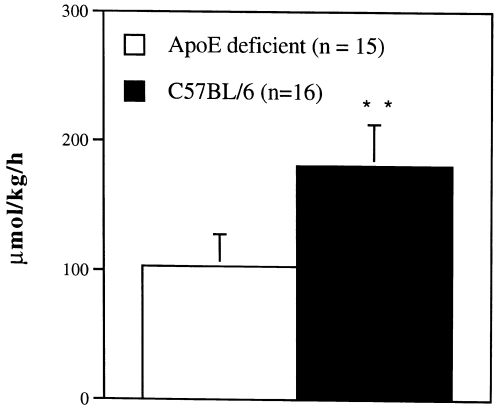

Fig. 1. Effect of apoE deficiency on hepatic triglyceride production. Triton WR-1339 was injected into C57BL/6 and apoE-deficient mice. The difference between baseline and 1-h plasma triglyceride levels was measured after Triton WR-1339 injection. The VLDL-TG production rate is expressed as micromoles of triglyceride produced per kilogram of body weight per hour (µmol/kg/h). Data represent means ± SEM. ** Different from apoE-deficient mice, $P < 0.01$.

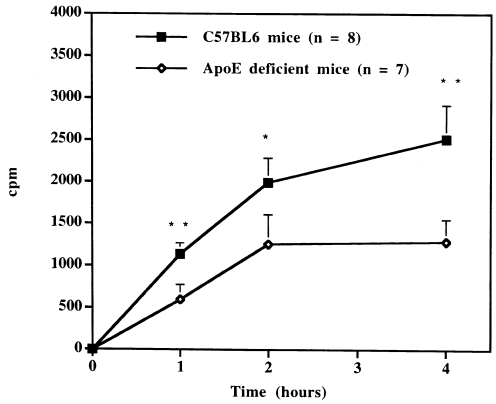

Fig. 3. Incorporation of [^{35}S]methionine in VLDL-apoB of C57BL/6 mice (n = 8) and apoE-deficient mice (n = 7) injected with Triton WR-1339 and [^{35}S]methionine. Data (means ± SEM) represent the sum of counts per minute recovered in VLDL-apoB-100 and VLDL-apoB-48. Asterisks indicate a significant difference from apoE-deficient mice: * $P < 0.05$; ** $P < 0.01$.

VLDL-apoB production is significantly increased in mice overexpressing apoE

To test the hypothesis that apoE overexpression increases VLDL-apoB production, adenovirus encoding human apoE3 (AdhapoE3) was injected via the tail vein into wild-type mice. The endogenous labeling experiment was performed 5 days after adenovirus injection.

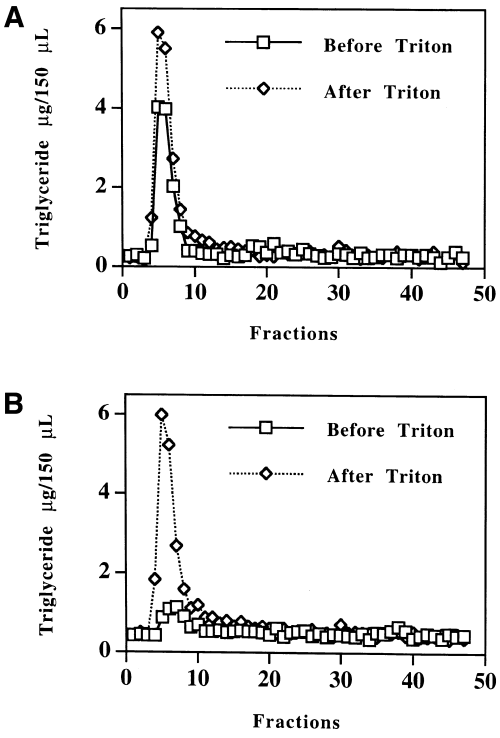

Fig. 2. Plasma lipoprotein triglyceride distribution as determined by fast protein liquid chromatography. Each profile represents pooled plasma from five mice before (squares) and 1 h after (diamonds) Triton WR-1339 injection. (A) ApoE-deficient mice; (B) wild-type mice.

The mean plasma human apoE level on day 5 was 67 ± 23 mg/dl (**Table 1**), indicating expression of human apoE3 as previously reported (11). The plasma cholesterol concentration was significantly reduced (47%, $P < 0.05$) by apoE overexpression whereas the plasma triglyceride concentration was significantly increased (27%, $P < 0.05$) (Table 1). The VLDL-TG production was significantly increased by 63% ($P < 0.01$) in the AdhapoE3 group compared with the control adenovirus group (Adnull) (**Fig. 5**).

Total VLDL-apoB production was increased by 48% ($P < 0.001$) in mice overexpressing apoE compared with control mice (**Fig. 6**). VLDL-apoB-48 production was significantly increased by 55% ($P < 0.01$); a trend toward increased VLDL-apoB-100 production by 20% was noted but did not reach statistical significance ($P = 0.17$).

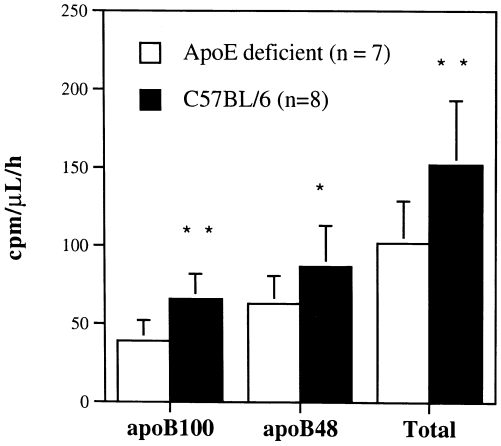

Fig. 4. Effect of apoE deficiency on hepatic VLDL-apoB production assessed by endogenous labeling. The rate of VLDL-apoB production was expressed as counts per minute per microliter per hour, using radioactivity measured 2 h after tracer injection. Data represent means ± SEM. * $P < 0.01$; ** $P < 0.001$.

TABLE 1. Lipid and human apoE steady state concentrations in plasma of apoE-deficient mice and C57BL/6 or in C57BL/6-mice before (day 0) and 5 days after (day 5) injection of recombinant adenovirus encoding human apoE (AdhapoE) or no transgene (Adnull)

	Cholesterol		Triglyceride		Human apoE	
	Day 0	Day 5	Day 0	Day 5	Day 0	Day 5
			mg/dl			
ApoE deficient	—	508 ± 62	—	80 ± 10	—	—
C57BL/6	—	72 ± 5	—	103 ± 12	—	—
C57BL/6 + Adnull	68 ± 4	66 ± 10	110 ± 15	104 ± 15	—	—
C57BL/6 + AdhapoE	77 ± 6	41 ± 14[a]	97 ± 8	123 ± 14[a]	—	67 ± 23

Data represent means ± SEM.
[a] Significantly different from baseline ($P < 0.05$).

Triglyceride content of nascent VLDL is not increased by apoE overexpression

Nascent VLDL was isolated 2 h after Triton injection from apoE-expressing and control mice and analyzed for lipid and protein composition. VLDL triglyceride, cholesteryl ester, free cholesterol, phospholipid, and protein mass were all significantly increased in apoE-overexpressing mice compared with control mice (**Table 2**). However, the components of VLDL were not all increased to the same degree. Whereas VLDL triglyceride was increased by 31%, VLDL protein was increased by 64% and VLDL cholesteryl ester by 103%. When these data are expressed as a percentage of total VLDL mass, apoE expression decreased the relative amount of triglyceride in VLDL (63 ± 3 vs. 54 ± 3%, $P < 0.01$), and did not change the protein in VLDL (12 ± 1 vs. 9 ± 2%, $P = 0.06$) (Table 2). VLDL-apoB-48 and apoB-100 mass were increased (150 and 58%, respectively) in apoE-overexpressing mice compared with control mice (**Table 3**). The content of triglyceride per VLDL particle, assessed by calculating the number of TG molecules per molecule of apoB, was significantly decreased by apoE overexpression (3,719 ± 617 vs. 4,948 ± 219, $P < 0.05$) (Table 3).

DISCUSSION

We demonstrate in this study that decreased VLDL-TG production in apoE-deficient mice is accompanied by decreased VLDL-apoB production. Conversely, hepatic apoE overexpression in wild-type mice resulted in increased VLDL-TG production associated with increased VLDL-apoB production. The amount of triglyceride per nascent VLDL particle was not increased and in fact was significantly decreased by apoE overexpression. These data are consistent with a primary effect of hepatic apoE expression being an increase in secretion of VLDL particles and not simply an increase in the TG content of VLDL. These results suggest that the level of hepatic apoE expression modulates hepatic VLDL-apoB production rate in vivo and extend our understanding of the effects of apoE expression on hepatic VLDL production.

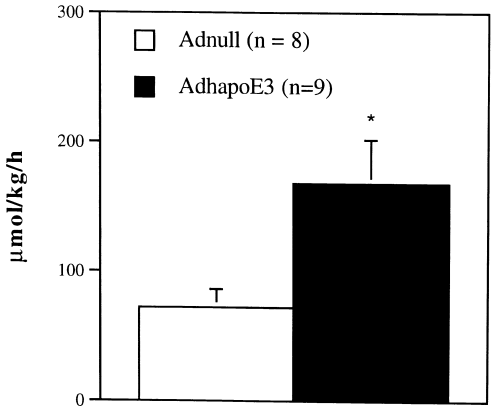

Fig. 5. Effect of apoE overexpression on hepatic triglyceride production. Triton WR-1339 was injected into C57BL/6 mice 5 days after injection of adenovirus encoding human apoE3 (AdhapoE3) or no transgene (Adnull). The difference between baseline and 1-h triglyceride levels was measured after Triton WR-1339 injection. The VLDL-TG production rate is expressed as micromoles of triglyceride produced per kilogram of mouse body weight per hour (μmol/kg/h). Data represent means ± SEM. * $P < 0.01$.

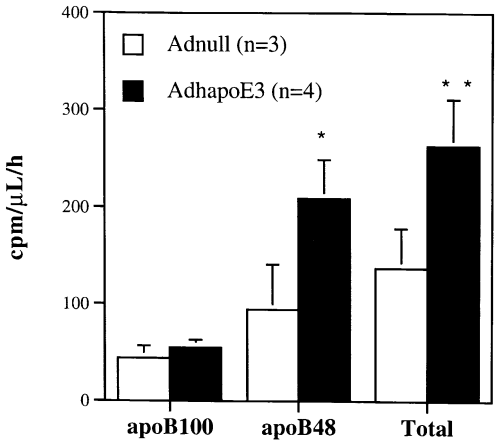

Fig. 6. Effect of human apoE3 overexpression on VLDL-apoB production. The endogenous labeling of apoB, using coinjection of Triton WR-1339 and [^{35}S]methionine, was performed 5 days after injection into C57BL/6 mice of adenovirus encoding human apoE3 (AdhapoE3) or no transgene (Adnull). The rate of VLDL-apoB production was expressed as counts per minute per microliter per hour, using radioactivity measured 2 h after tracer injection. Data represent means ± SEM. ** $P < 0.001$; * $P < 0.01$.

TABLE 2. Lipid and protein content of VLDL isolated from mouse plasma collected 2 h after Triton WR-1339 injection in apoE-expressing group (AdhapoE) compared with control group (Adnull)

	TG		EC[a]		FC		PL		PROT	
	mg/dl	%	mg/dl	%	mg/dl	%	mg/dl	%	mg/dl	%
Adnull	346 ± 46	63 ± 3	33 ± 16	6 ± 1	34 ± 5	4 ± 1	101 ± 12	18 ± 2	47 ± 5	9 ± 1
AdhapoE	454 ± 33[b]	54 ± 3[b]	67 ± 18[b]	7 ± 1	62 ± 8[b]	8 ± 2	166 ± 16[b]	20 ± 2	100 ± 15[b]	12 ± 1

Data represent means ± SEM; TG, triglyceride; EC, esterified cholesterol; FC, free cholesterol; PL, phospholipids; PROT, protein.

[a] Values of esterified cholesterol correspond to the difference between those of total cholesterol and free cholesterol multiplied by a coefficient of 1.67.

[b] $P < 0.01$ (Student's *t*-test); significantly different from control Adnull group.

Our data suggest that apoE may be directly involved in the VLDL assembly process within the hepatocyte. Although some studies reported that apoB and apoE were independently secreted by the liver (14, 15), other studies observed intracellular association of apoE with apoB-containing lipoproteins (16). ApoE has been found in different cellular compartments including the endoplasmic reticulum (ER) and Golgi stacks (17, 18). An in vivo apoE recycling process has been identified in mouse hepatocytes, in which apoE is taken up and then resecreted in association with apoB-containing lipoproteins (19).

One regulatory mechanism for apoB secretion is adequate lipidation of apoB versus intracellular degradation of poorly lipidated apoB (20–22). Reduced availability of neutral lipids as well as reduced activity of the microsomal transfer protein result in increased apoB degradation and reduced apoB secretion in vitro (20–22). Primordial lipidated apoB particles coalesce with lipid droplets within the ER to form mature TG-rich lipoprotein particles (23–26). ApoE could potentially enhance the intracellular lipidation of apoB by stabilizing the lipid droplets and facilitating their coalescence with nascent apoB-containing lipoprotein particles, thus protecting apoB and promoting secretion of the mature particle. This action of apoE would be expected to inhibit apoB degradation occurring throughout the secretory pathway (23, 27, 28).

Alternatively, apoE may act as a chaperone to directly protect apoB from degradation independent of lipids. A substantial portion of apoB degradation occurs on the cytosolic side of the ER (23, 29, 30). ApoE could directly associate with apoB, thereby inhibiting the interaction of apoB with ER proteins that lead to its cytosolic degradation. A similar mechanism was demonstrated for the interaction of receptor-associated protein (RAP) and the LDL receptor-related protein (LRP), which results in protection of LRP against intracellular degradation and increased expression of LRP on the cell surface (31). In that case, apoE competes with RAP for intracellular binding to LRP and results in increased degradation and reduced expression of LRP (31). Interestingly, an in vitro study demonstrated the role of the LDLR in mediating intracellular degradation of apoB (32). The authors suggested that apoE could inhibit apoB degradation by competing with apoB for the binding to LDLR. Further experiments will be required to determine the molecular and cellular mechanisms by which apoE promotes apoB secretion.

ApoE3 overexpression resulted in a greater increase in VLDL-apoB-48 production compared with apoB-100. Wild-type mice produce substantially more VLDL-apoB-48 than VLDL-apoB-100 and apoE may have a proportionally greater effect in facilitating apoB-48 production. Further studies of apobec-1 (cytidine deaminase responsible for apolipoprotein B mRNA editing)-deficient mice will be required to determine the effect of apoE on VLDL-apoB-100 production in the absence of hepatic VLDL-apoB-48 production.

The measurement of VLDL-apoB production in mice is a difficult problem because of the high turnover and low plasma concentration of VLDL-apoB as well as the limited amount of plasma. To overcome this problem, the use of Triton WR-1339 enables investigation after blocking the lipolysis and clearance of nascent VLDL. In this experimental setting, the rate of increase in VLDL-apoB mass after Triton WR-1339 injection is proportional to the production rate of VLDL-apoB. This approach was taken by Li, Grundy, and Patel (33), who used densitometric analysis of Coomassie-stained SDS-polyacrylamide gels of VLDL-apoB isolated before and after Triton WR-1339 injection. The limit of this method is the imprecision of the apoB measurement and the low levels of VLDL-apoB, which require pooling of plasma samples from multiple mice (33). An alternative approach, as used in our study, is injection of a radiolabeled amino acid that becomes incorporated into VLDL-apoB (endogenous labeling) (12, 34). The rate of tracer incorporation in VLDL-apoB after Triton injection is proportional to the rate of VLDL-apoB production; therefore the amount of radioactivity recovered in VLDL-apoB at different time points after Triton WR-1339 injection can be used to assess the production rate of VLDL-

TABLE 3. Plasma concentration of apoB-100 and apoB-48 of VLDL isolated from mouse plasma collected 2 h after Triton WR-1339 injection in apoE-expressing group (AdhapoE) compared with control group (Adnull)

	ApoB-100	ApoB-48	TG/[B-100 + B-48][a]
	nmol/l	*nmol/l*	
Adnull	236 ± 28	419 ± 196	4,948 ± 219
AdhapoE	373 ± 56[b]	1,048 ± 182[c]	3,719 ± 617[b]

[a] The ratio was calculated using molecular weights of 885, 512,772, and 240,855 for TG, apoB-100, and apoB-48, respectively.

[b] $P < 0.05$; [c] $P < 0.01$ (Student's *t*-test); significantly different from control Adnull group.

apoB. This method is more sensitive than the Coomassie-staining method and does not require the determination of baseline VLDL-apoB. On other hand, the endogenous labeling method does not allow an actual quantification of the VLDL-apoB production rate expressed in milligrams of apoB produced per unit of time.

Two reports, published since we originally submitted this manuscript, addressed the effect of apoE expression on VLDL-apoB production rate in mice (35) and rabbits (36). One study, performed in human apoE3 transgenic rabbits, suggested that TG production was increased compared with control rabbits (36). The VLDL-apoB steady state plasma concentration was increased as well as the VLDL-apoB plasma clearance rate, indicating that the production rate of VLDL-apoB was probably increased, although it was not directly measured. The other study (35) was performed in mice, using Triton injection. The triglyceride production rate was decreased in apoE-deficient mice compared with control mice and, when human apoE3 was overexpressed, the TG production was increased comparable to our findings. The investigators used Coomassie staining of SDS gels to estimate VLDL-apoB mass before and after Triton injection and concluded that VLDL-apoB production rates were not changed by apoE expression. However, this method requires accurate quantitation of small differences in apoB mass before and after Triton injection. Furthermore, these studies were only performed in apoE transgenic mice with plasma apoE levels of only 0.06 ± 0.004 mg/dl compared with 67 ± 23 mg/dl in our study. Therefore, this low level of apoE expression may not have been adequate to promote VLDL-apoB production, explaining the differences from our results.

In summary, this study demonstrates that, in addition to promoting VLDL-TG production, hepatic apoE expression promotes VLDL-apoB production and therefore the number of VLDL particles secreted by the liver. The role of apoE as a facilitator of apoB production may provide new insights into VLDL assembly and secretion and could possibly be relevant to human dyslipoproteinemia associated with apoB overproduction.

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