

Stimulation of the *in Vivo* Production of Very Low Density Lipoproteins by Apolipoprotein E Is Independent of the Presence of the Low Density Lipoprotein Receptor*

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Apolipoprotein (apo) E stimulates the secretion of very low density lipoproteins (VLDLs) by an as yet unknown mechanism. Recently, a working mechanism for apoE was proposed (Twisk, J., Gillian-Daniel, D. L., Tebon, A., Wang, L., Barrett, P. H., and Attie, A. D. (2000) *J. Clin. Invest.* 105, 521–532) in which apoE prevents the inhibitory action of the low density lipoprotein receptor (LDLr) by binding to it. We have first tested whether this newly described effect of the LDLr on VLDL secretion, obtained *in vitro*, is also observed *in vivo*. In LDLr knockout mice (LDLr^{-/-}), the production of VLDL triglycerides and apoB was 30% higher than that in controls. Also the ratio of apoB100:apoB48 secretion was increased in the LDLr^{-/-} mice. The composition of nascent VLDL was similar in both strains. To test whether the action of apoE depends on the presence of the LDLr, VLDL production was measured in LDLr^{-/-} and apoE^{-/-} LDLr^{-/-} mice. Deletion of apoE on a LDLr^{-/-} background still caused a 50% decrease of VLDL triglycerides and apoB production. The composition of nascent VLDL was again similar for both strains. We conclude that the effect of apoE on hepatic VLDL production is independent of the presence of the LDLr.

Apolipoprotein (apo)¹ E is a 34.2-kDa protein that acts as a ligand for receptor-mediated endocytosis of lipoproteins (1). The role of apoE in lipoprotein metabolism is not confined, however, to the clearance of lipoprotein particles from the circulation. ApoE inhibits lipolysis of lipoproteins by lipoprotein lipase (2, 3). More recently, it was demonstrated that apoE also affects the hepatic secretion of very low density lipoproteins (VLDLs): apoE-deficient (apoE^{-/-}) mice showed a reduction in VLDL secretion by some 50% (4), whereas adenoviral gene transfer of human apoE3 led to a gene dose-dependent increase in VLDL production (5). Similar results were obtained

in transgenic rabbits expressing human apoE3 (6). Hepatic overexpression of apoE2, apoE3, and apoE4 all stimulated VLDL secretion (7–9). In contrast, the mutant isoform apoE3Leiden is not capable of stimulating VLDL secretion (10).

The mechanism by which apoE affects VLDL assembly and secretion is poorly understood. A recent *in vitro* study by Twisk *et al.* (11) offered a potential mechanism by which apoE might affect VLDL secretion. These authors studied the role of the LDL receptor (LDLr) in production of VLDL. Experiments in cultured hepatocytes of LDLr^{-/-} and control mice showed that LDLr^{-/-} hepatocytes had a strongly increased apoB secretion. This was explained in part by a decreased intracellular degradation of apoB protein. It was concluded that the LDLr binds nascent apoB intracellularly during the course of VLDL assembly, thereby promoting its intracellular degradation. Because apoE and apoB are both ligands for the LDLr, it was suggested that apoE's stimulatory action on VLDL secretion might occur by preventing apoB from binding to the LDLr.

In this study, we tested the hypothesis that the effect of apoE on VLDL secretion is LDLr-dependent. After verification that the reported *in vitro* effects of the LDLr can also be observed in the *in vivo* situation, we tested whether the effect of apoE is LDLr-dependent by knocking out apoE on a LDLr-deficient background. Our results confirm that the LDLr modulates the VLDL production rate *in vivo*, but they also clearly indicate that the effect of apoE on VLDL production is independent of the presence of the LDLr.

EXPERIMENTAL PROCEDURES

Animals—all mice were housed under standard conditions with free access to water and regular lab chow. For the comparison between wild type and LDLr^{-/-} mice, male mice with a C57BL/6 background were used. Male animals were also used for comparison of apoE^{-/-} LDLr^{-/-} mice with LDLr^{-/-} mice. Because the apoE^{-/-} LDLr^{-/-} double knockout mice were on a mixed background of C57BL/6 and 129, LDLr^{-/-} mice on the same mixed genetic background as the double knockout mice were used in these experiments as controls. All experiments were approved by the institutional animal care committee.

VLDL Production and Composition—The *in vivo* VLDL production was measured after intravenous administration of 500 mg/kg Triton WR1339 as described previously (4, 12). To measure *de novo* synthesis of VLDL apoB, 100 μ Ci of ³⁵S-Tran label (ICN, Zoetermeer, The Netherlands) was injected i.v. 30 min before Triton WR1339 injection. Blood samples were withdrawn from the tail at regular intervals after Triton WR1339 injection. At *t* = 120 min, an additional large blood sample was withdrawn via the orbital plexus. In each sample, plasma triglycerides (TGs) were determined, and the rate of triglyceride accumulation in plasma was taken as the *in vivo* rate of VLDL TG production.

From the large blood sample at *t* = 120 min after Triton WR1339 administration, 200 μ l of plasma was brought to 1.063 g/ml with potas-

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¹ The abbreviations used are: apo, apolipoprotein; LDL, low density lipoprotein; LDLr, low density lipoprotein receptor; TG, triglyceride; VLDL, very low density lipoprotein; SDS-PAGE, SDS-polyacrylamide gel electrophoresis.

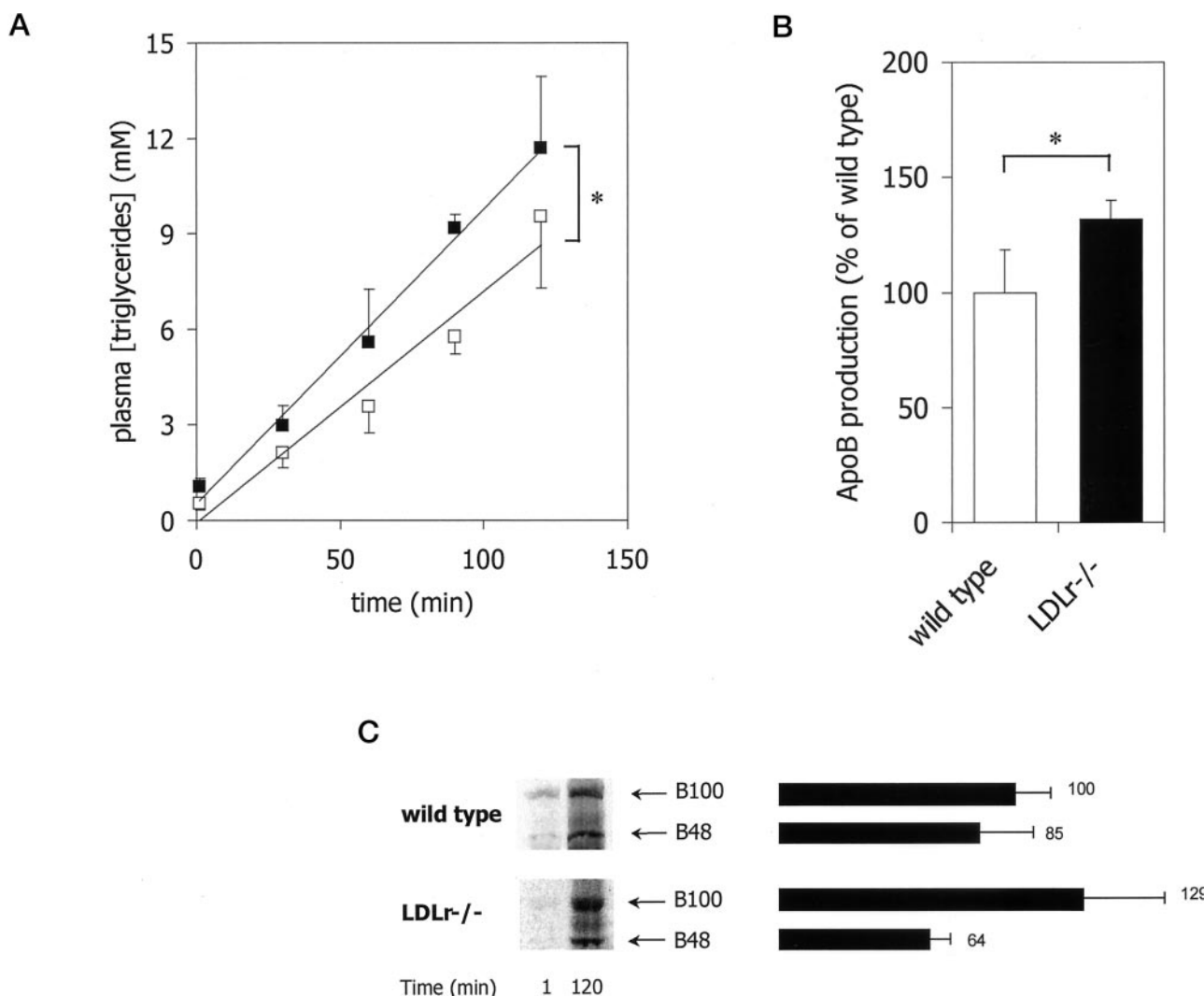


FIG. 1. VLDL production in C57BL/6 and LDLr^{-/-} mice measured by ³⁵S-Tran label and Triton WR1339 administration. *A*, accumulation of triglycerides in plasma. □, C57BL/6 mice; ■, LDLr^{-/-} mice. *B*, total VLDL apoB secretion. VLDL was isolated 2 h after Triton WR1339 injection, and label incorporation in apoB was counted after isopropanol precipitation. □, C57BL/6 mice; ■, LDLr^{-/-} mice. *C*, apoB100 and apoB48 production as analyzed by SDS-PAGE analysis of plasma. Plasma from 1 and 120 min after Triton WR1339 injection was delipidated and subjected to SDS-PAGE analysis. The *black bars* indicate the average label incorporation in the apoB100 and apoB48 bands. For *B* and *C*, label incorporation was normalized to the B100 incorporation in the C57BL/6 mice.

sium bromide in a volume of 2 ml, transferred to SW41 centrifuge tubes, and layered with a 1.006 g/ml salt solution. After 16 h of centrifugation at 37,000 rpm and 4 °C, the VLDL fraction was carefully removed by pipetting off 1.2 ml. ApoB in 0.2 ml of this VLDL fraction was precipitated with isopropanol (13), dissolved in 20% (w/v) SDS, and counted for assessment of total VLDL apoB production. Pilots with blood samples taken at $t = 1$ min after Triton WR1339 administration showed that basal activity was less than 10% of the value at $t = 120$ min, and therefore the $t = 120$ min point was taken as the total *de novo* VLDL apoB production. In the remaining VLDL, triglycerides, phospholipids, and cholesterol were measured with commercially available kits as described previously (4). This composition of VLDL is a mixture of VLDL that circulated before administration of Triton WR1339 and nascent VLDL produced during the 2-h period after Triton WR1339 administration. To obtain the composition of nascent VLDL, the contribution of circulating VLDL was determined and corrected for, as described previously (14).

For determination of apoB100 and apoB48 production, an aliquot of the blood samples at $t = 1$ and $t = 120$ min after Triton WR1339 administration (10 μ l) was delipidated by 1.8 ml of -20 °C diethyl ether:methanol (1:1), and, after centrifugation in an Eppendorf centrifuge (13,000 rpm, 10 min), the pellet was dissolved in sample buffer for SDS-PAGE analysis on a 5% (w/v) polyacrylamide gel. A volume corresponding to 5 μ l of plasma was loaded onto the gel. The gel was fixed by Coomassie staining (BioSafe Coomassie; Bio-Rad) and dried overnight between two cellophane sheets using a GradiDry gel drying solution (Gradipore). After drying, one sheet was carefully removed, and the

uncovered part of the gel was autoradiographed with phosphorimager technology.

Statistical Analysis—Nonparametric Mann-Whitney U tests were used for all statistical analyses. $p \leq 0.05$ was considered statistically significant.

RESULTS

Effect of the LDL Receptor on VLDL Secretion in Vivo—First we tested whether the LDLr affects the VLDL secretion rate *in vivo*. Hepatic VLDL triglyceride and apoB production rates were measured by intravenous injection of ³⁵S-Tran label and Triton WR1339 in 4-h-fasted mice. As shown in Fig. 1A, LDLr^{-/-} mice showed a 30% increase in the triglyceride production rate as compared with wild type animals (162 ± 42 and $211 \pm 12 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ for wild type and LDLr^{-/-} animals, respectively; $p < 0.005$). Total apoB production (Fig. 1B) was also significantly increased by ~30% in LDLr^{-/-} mice as compared with wild type mice ($100 \pm 19\%$ and $132 \pm 42\%$ for wild type and LDLr^{-/-} mice, respectively; $p < 0.05$). We also measured the *de novo* synthesis rate of B100 and B48 by SDS-PAGE analysis of plasma collected 1 and 120 min after Triton WR1339 injection (Fig. 1C). No significant differences could be observed between the LDLr^{-/-} and wild type mice for apoB100 and apoB48 production rates, although the former

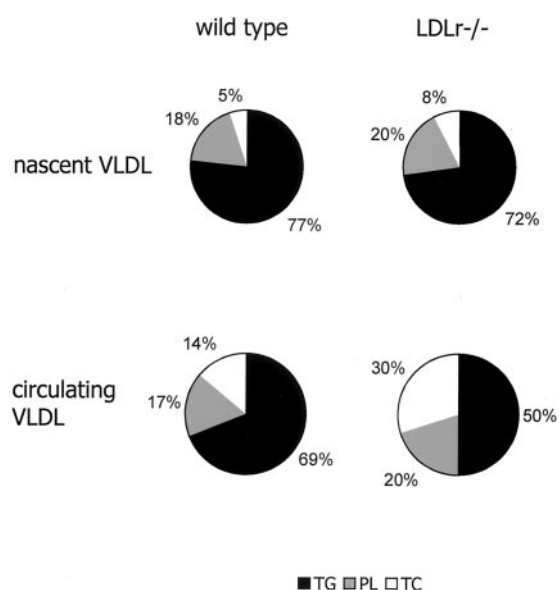


FIG. 2. Composition of nascent and circulating VLDL in C57BL/6 mice and LDLr^{-/-} mice. VLDL was isolated by ultracentrifugation from plasma after a 4-h fast (*circulating VLDL*) or 2 h after Triton WR1339 administration (*nascent VLDL*), and lipid composition was determined (in w/w% of total lipids).

strain secreted relatively more apoB100. This was evident from a significantly higher apoB100:apoB48 ratio (2.02 ± 0.39 and 1.26 ± 0.42 for LDLr^{-/-} and wild type mice, respectively; $p < 0.02$).

We have also analyzed the lipid composition of the nascent VLDL particles after Triton WR1339 injection (Fig. 2). We confirmed that Triton WR1339 itself did not affect the lipid composition of lipoprotein particles.² The composition of nascent VLDL was very similar between the LDLr^{-/-} and wild type mice; both groups had triglycerides comprising ~75% of total lipid mass. Circulating VLDL from LDLr^{-/-} mice was somewhat lower in triglyceride content than VLDL of the wild type mice (50% and 69%, respectively).

The Effect of apoE on VLDL Production in the Absence of the LDL Receptor—To determine whether the effect of apoE is dependent on the LDLr, we have characterized VLDL production and composition in apoE^{-/-} LDLr^{-/-} double knockout mice and LDLr^{-/-} mice. As seen in Fig. 3A, the VLDL TG production was approximately 2-fold lower in the apoE^{-/-} LDLr^{-/-} mice as compared with the LDLr^{-/-} mice (77 ± 16 and $132 \pm 39 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ for apoE^{-/-} LDLr^{-/-} mice and LDLr^{-/-} mice, respectively; $p < 0.005$). A similar result was obtained for the total VLDL apoB production (Fig. 3B). The rate of total apoB production was significantly lower in the apoE^{-/-} LDLr^{-/-} mice than in the LDLr^{-/-} mice ($53 \pm 17\%$ and $100 \pm 39\%$ for apoE^{-/-} LDLr^{-/-} mice and LDLr^{-/-} mice, respectively; $p = 0.01$). In Fig. 3C, SDS-PAGE analysis of plasma collected 1 and 120 min after Triton WR1339 administration is shown for the apoE^{-/-} LDLr^{-/-} double knockout mice and the LDLr^{-/-} mice. Most strikingly, apoB100 production in the apoE^{-/-} LDLr^{-/-} mice was severely diminished to only 10% of that of the LDLr^{-/-} mice.

In Fig. 4, the average composition of nascent and circulating VLDL is shown. Because of the high lipid levels in apoE^{-/-} LDLr^{-/-} mice before Triton WR1339 injection, the nascent VLDL composition is calculated using the differences in lipids between total VLDL 2 h after Triton WR1339 administration and the circulating VLDL lipid content (*i.e.* the lipids present

before Triton WR1339 administration). In both mouse models, nascent VLDL was rich in triglycerides. Large differences were found in circulating VLDL: VLDL of the apoE^{-/-} LDLr^{-/-} mice contained only 7% TGs as compared with 50% TGs in the LDLr^{-/-} mice.

DISCUSSION

In this study, we have tested the hypothesis that the effect of apoE on VLDL secretion is mediated via intracellular interaction with the LDL receptor. If this hypothesis were true, we reasoned that the 50% reduction of VLDL secretion as observed in the apoE^{-/-} mouse compared with the wild type mouse controls (4, 5) should not be observed on a LDLr^{-/-} background. Fig. 3 shows that the effect of deleting apoE in reducing VLDL secretion was independent of the LDL receptor: a 50% reduction of both VLDL apoB and TGs was observed in the apoE^{-/-} LDLr^{-/-} mouse as compared with the LDLr^{-/-} controls.

The conclusion that apoE acts on the VLDL assembly pathway irrespective of the presence of the LDLr is indirectly supported by other recent studies. On one hand, it was shown that apoE2, a variant of apoE that binds poorly to lipoprotein receptors (15), is still capable of stimulating VLDL secretion (3, 7, 9). On the other hand, truncation of apoE at the C-terminal lipid-binding domain abolishes the stimulatory effect of apoE on VLDL secretion (16, 17). This truncated form of apoE is properly transcribed and detectable in plasma associated with VLDL particles, and it still contains an intact LDLr binding domain as judged by rescue from hyperlipidemia in apoE^{-/-} mice. Thus, if competition of apoE with apoB for binding to the LDLr was the mechanism of apoE's action on VLDL secretion, the truncated apoE protein should still be able to increase VLDL secretion, whereas apoE2 should not be able to do so. Because the opposite is observed, we conclude that lipid binding, rather than receptor binding, is important for the stimulation of VLDL production by apoE.

Although apoE does not act via the LDL receptor, we did confirm *in vivo* that absence of the LDLr enhances VLDL apoB secretion as described previously for mouse hepatocytes (11). Thus, LDLr deficiency leads to a 30% increase in VLDL apoB production. Also, the rate of VLDL TG secretion was increased by 30% in LDLr^{-/-} mice. The effects of the LDL receptor on VLDL apoB100 and apoB48 secretion were too small for our analysis to give significant differences. However, we found a significant increase in the ratio of secreted apoB100:apoB48 particles in the LDLr^{-/-} mice. Thus, although the effect of the LDLr on VLDL apoB production *in vivo* is much smaller than that observed *in vitro*, it is in line with the previous *in vitro* observation that apoB100 secretion was increased 3–4-fold, and apoB48 was increased only 1.5–2-fold (11).

It has been reported that the genetic background can have a large impact on the rate of VLDL secretion (18). Because the apoE^{-/-} LDLr^{-/-} mice were on a mixed background of C57BL/6 and 129, we used LDLr^{-/-} mice that were on the same mixed background. Our data confirm the impact of genetic background: when the rate of VLDL TG secretion is compared between the LDLr^{-/-} mice on the two genetic backgrounds used in this study, the mice on a C57BL/6 background clearly had a higher VLDL TG secretion as compared with mice on the mixed background (211 ± 12 and $132 \pm 39 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$, respectively).

The composition of nascent VLDL was similar for all mouse models tested, comprising ~70–75% triglycerides (Figs. 2 and 4). The TG content of the apoE^{-/-} LDLr^{-/-} mice was higher, *i.e.* 88%, but this is likely the result of the large contribution of circulating VLDL to the total VLDL fraction after Triton WR1339 administration. In particular, the cholesterol content of nascent VLDL is prone to large error because of the enor-

² B. Teusink and H. van der Boom, unpublished observations.

FIG. 3. VLDL production in LDLr^{-/-} and apoE^{-/-} LDLr^{-/-} mice measured by ³⁵S-Tran label and Triton WR1339 administration. See the Fig. 1 legend for more details. **A**, accumulation of triglycerides in plasma. □, LDLr^{-/-} mice; ■, apoE^{-/-} LDLr^{-/-} mice. **B**, total VLDL apoB secretion. □, LDLr^{-/-} mice; ■, apoE^{-/-} LDLr^{-/-} mice. **C**, apoB100 and apoB48 production. For **B** and **C**, label incorporation was normalized to the B100 incorporation in the LDLr^{-/-} mice.

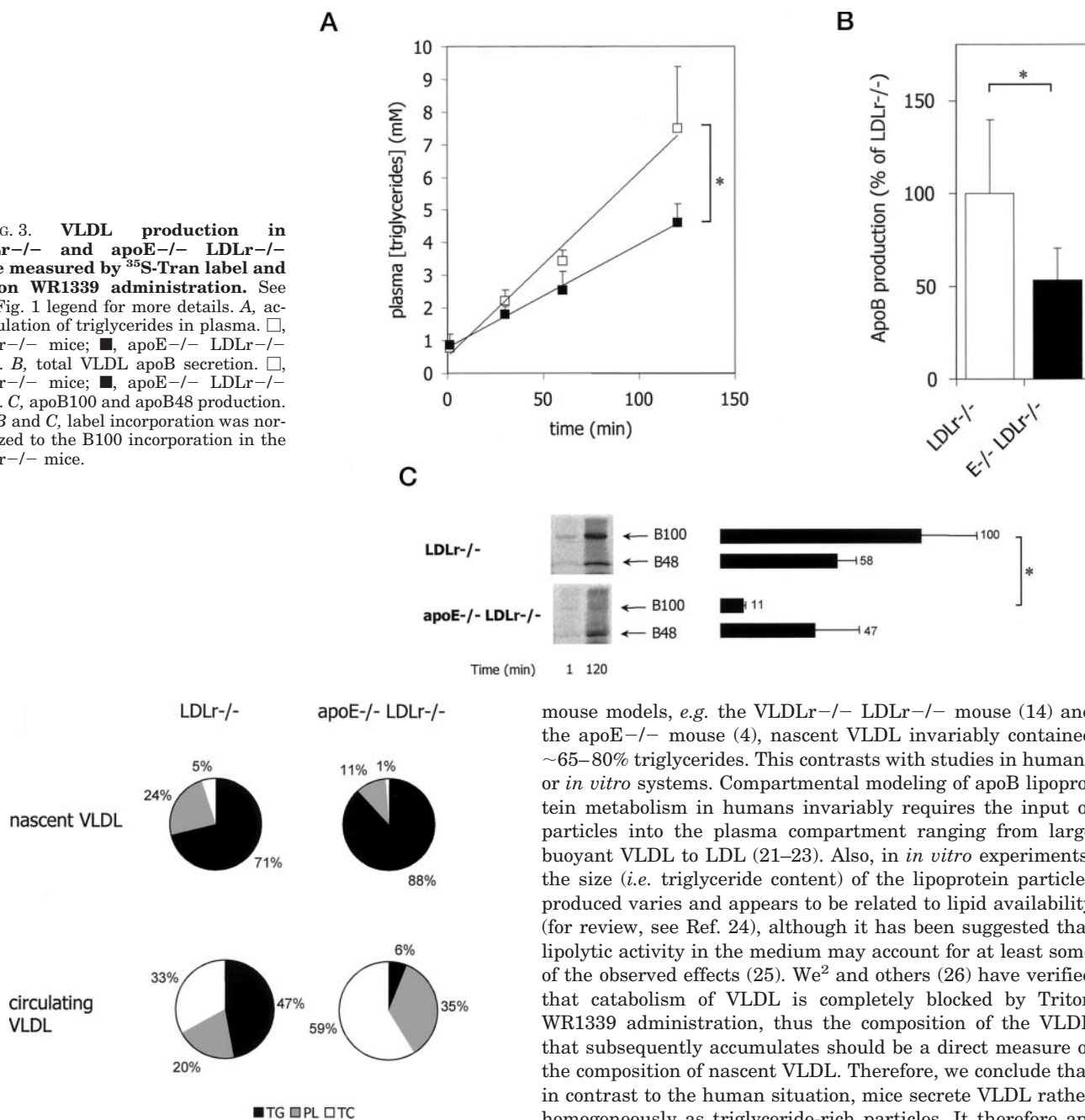
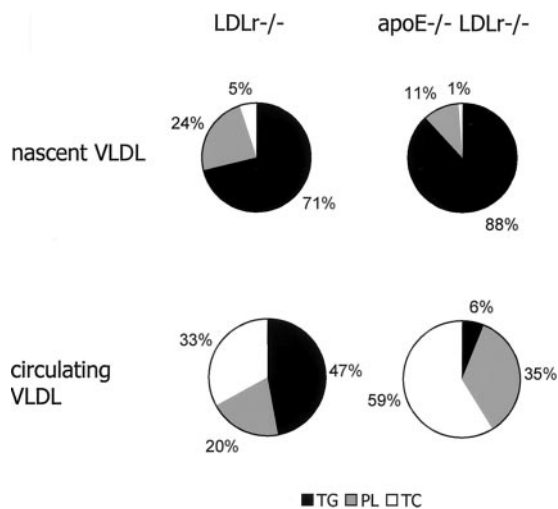


FIG. 4. Composition of nascent and circulating VLDL in LDLr^{-/-} and apoE^{-/-} LDLr^{-/-} mice. VLDL was isolated by ultracentrifugation from plasma after a 4-h fast (*circulating VLDL*) or 2 h after Triton WR1339 administration (*nascent VLDL*), and lipid composition was determined (in w/w% of total lipids). Values for nascent VLDL are corrected for circulating VLDL.



mouse amount of cholesterol already present in these mice (19, 20). Similar TG content of nascent VLDL is consistent with the fact that both total apoB and VLDL TG secretion were affected to the same extent in both Figs. 1 and 3. Maugeais *et al.* (7) previously reached a similar conclusion regarding the effect of apoE isoforms on VLDL production, *i.e.* that both apoB and TGs were affected. However, we have recently seen that the particles secreted by hepatocytes of apoE^{-/-} mice were smaller, although they were of similar composition (4). It appears that particle size and lipid composition are not always tightly correlated, but it remains to be seen whether this observation is specific for particles lacking apoE.

In the mouse models tested in this study and also in other

mouse models, *e.g.* the VLDLr^{-/-} LDLr^{-/-} mouse (14) and the apoE^{-/-} mouse (4), nascent VLDL invariably contained ~65–80% triglycerides. This contrasts with studies in humans or *in vitro* systems. Compartmental modeling of apoB lipoprotein metabolism in humans invariably requires the input of particles into the plasma compartment ranging from large buoyant VLDL to LDL (21–23). Also, in *in vitro* experiments, the size (*i.e.* triglyceride content) of the lipoprotein particles produced varies and appears to be related to lipid availability (for review, see Ref. 24), although it has been suggested that lipolytic activity in the medium may account for at least some of the observed effects (25). We² and others (26) have verified that catabolism of VLDL is completely blocked by Triton WR1339 administration, thus the composition of the VLDL that subsequently accumulates should be a direct measure of the composition of nascent VLDL. Therefore, we conclude that in contrast to the human situation, mice secrete VLDL rather homogeneously as triglyceride-rich particles. It therefore appears that in mice, the rate of VLDL production is regulated not so much by VLDL composition as by particle number, *i.e.* by degradation of nascent apoB protein and/or pre-VLDL particles during the second stage of VLDL assembly (24, 27). The large differences in the TG content of circulating VLDL as opposed to nascent VLDL (Figs. 2 and 4) reflect differences in VLDL catabolism (10).

We observed an unexpected effect of combined apoE and LDLr deficiency on the secretion of apoB100 particles: in the apoE^{-/-} LDLr^{-/-} mice, the secretion of apoB100 was reduced to only 10% of that in LDLr^{-/-} mice. In apoE^{-/-} mice, apoB100 production is at most 50% lower (5, 7), indicating that the severely reduced apoB100 production is a specific effect of combined apoE and LDLr deficiency. At this moment, we can only speculate on the mechanism that underlies this striking observation. Rather, we would like to discuss some fundamental differences between B100- and B48-containing lipoproteins that may be relevant in rationalizing our observations.

ApoB48 does not bind to the LDLr but rather requires the

LDLr-related protein for endocytosis (28, 29). LDLr-related protein-mediated uptake of lipoproteins depends on apoE (30). Therefore, in the apoE^{-/-} mouse, the LDLr and the LDLr-related protein are not able to take up apoB48 particles. However, secretion of apoB48 particles by the liver still continues (5, 7). Because the rate of apoB48 particle secretion should equal the rate of apoB48 particle uptake in steady state, this implies that other receptors should be present to clear these apoB48 particles. One receptor may be the recently cloned apoB48 receptor (31).

In the LDLr^{-/-} apoE^{-/-} mouse, apoB100 particles must also be cleared by receptors other than the LDLr and the LDLr-related protein. It appears that these back-up systems perform poorly because Ishibashi *et al.* (20) found that plasma B100 levels were well detectable (~50% of normal) in these mice, despite the very low production rate that we have observed in this study. The extremely low TG content of the circulating VLDL as compared with that in the wild type also suggests very poor clearance of VLDL particles. It may be envisaged that the reduced VLDL apoB100 secretion rate in apoE^{-/-} LDLr^{-/-} mice is the result of some unknown feedback mechanism to maintain steady state and prevent unrestrained accumulation of apoB100 particles in the circulation. Such a feedback on VLDL secretion is only hypothetical at this moment; yet it becomes apparent that factors such as apoE and the LDLr that are clearly involved in lipoprotein uptake are also involved in VLDL production. The concept of hepatic VLDL production as a merely substrate-driven process (32) thus requires considerable sophistication.

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REFERENCES

- Mahley, R. W., and Ji, Z. S. (1999) *J. Lipid Res.* **40**, 1–16
- Jong, M. C., Dahlmans, V. E., Hofker, M. H., and Havekes, L. M. (1997) *Biochem. J.* **328**, 745–750
- de Beer, F., van Dijk, K. W., Jong, M. C., van Vark, L. C., van Der Zee, A., Hofker, M. H., Fallaux, F. J., Hoeven, R. C., Smelt, A. H., and Havekes, L. M. (2000) *Arterioscler. Thromb. Vasc. Biol.* **20**, 1800–1806
- Kuipers, F., Jong, M. C., Lin, Y., Eck, M., Havinga, R., Bloks, V., Verkade, H. J., Hofker, M. H., Moshage, H., Berkel, T. J., Vonk, R. J., and Havekes, L. M. (1997) *J. Clin. Invest.* **100**, 2915–2922
- Mensenkamp, A. R., Jong, M. C., van Goor, H., van Luyn, M. J., Bloks, V., Havinga, R., Voshol, P. J., Hofker, M. H., van Dijk, K. W., Havekes, L. M., and Kuipers, F. (1999) *J. Biol. Chem.* **274**, 35711–35718
- Huang, Y., Ji, Z. S., Brecht, W. J., Rall, S. C., Taylor, J. M., and Mahley, R. W. (1999) *Arterioscler. Thromb. Vasc. Biol.* **19**, 2952–2959
- Maugeais, C., Tietge, U. J., Tsukamoto, K., Glick, J. M., and Rader, D. J. (2000) *J. Lipid Res.* **41**, 1673–1679
- Tsukamoto, K., Maugeais, C., Glick, J. M., and Rader, D. J. (2000) *J. Lipid Res.* **41**, 253–259
- Tsukamoto, K., Smith, P., Glick, J. M., and Rader, D. J. (1997) *J. Clin. Invest.* **100**, 107–114
- Mensenkamp, A. R., Teusink, B., Baller, J. F. W., Wolters, H., Havinga, R., Willems van Dijk, K., Havekes, L. M., and Kuipers, F. (2001) *Arterioscler. Thromb. Vasc. Biol.* **21**, in press
- Twisk, J., Gillian-Daniel, D. L., Tebon, A., Wang, L., Barrett, P. H., and Attie, A. D. (2000) *J. Clin. Invest.* **105**, 521–532
- Jong, M. C., Dahlmans, V. E., van Gorp, P. J., Breuer, M. L., Mol, M. J., van Der Zee, A., Frants, R. R., Hofker, M. H., and Havekes, L. M. (1996) *Arterioscler. Thromb. Vasc. Biol.* **16**, 934–940
- Egusa, G., Brady, D. W., Grundy, S. M., and Howard, B. V. (1983) *J. Lipid Res.* **24**, 1261–1267
- Tacke, P. J., Teusink, B., Jong, M. C., Harats, D., Havekes, L. M., van Dijk, K. W., and Hofker, M. H. (2000) *J. Lipid Res.* **41**, 2055–2062
- Mahley, R. W. (1988) *Science* **240**, 622–630
- Kypreos, K. E., Teusink, B., Willems, V. D., Havekes, L. M., and Zannis, V. I. (2001) *FASEB J.* **15**, 1598–1600
- Kypreos, K. E., Willems van Dijk, K., Van der Zee, A., Havekes, L. M., and Zannis, V. I. (2001) *J. Biol. Chem.* **276**, 19778–19786
- Voyiaziakis, E., Ko, C., O'Rourke, S. M., and Huang, L. (1999) *J. Lipid Res.* **40**, 2004–2012
- van Dijk, K. W., van Vlijmen, B. J., De Winther, M. P., van't Hof, B., van Der Zee, A., van Der Boom, H., Havekes, L. M., and Hofker, M. H. (1999) *Arterioscler. Thromb. Vasc. Biol.* **19**, 2945–2951
- Ishibashi, S., Herz, J., Maeda, N., Goldstein, J. L., and Brown, M. S. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91**, 4431–4435
- Packard, C. J. (1999) *Curr. Opin. Lipidol.* **10**, 237–244
- Packard, C. J., Demant, T., Stewart, J. P., Bedford, D., Caslake, M. J., Schwertfeger, G., Bedynek, A., Shepherd, J., and Seidel, D. (2000) *J. Lipid Res.* **41**, 305–318
- Packard, C. J., and Shepherd, J. (1997) *Arterioscler. Thromb. Vasc. Biol.* **17**, 3542–3556
- Davis, R. A. (1999) *Biochim. Biophys. Acta* **1440**, 1–31
- Arrol, S., Mackness, M. I., and Durrington, P. N. (2000) *Atherosclerosis* **150**, 255–264
- Aalto-Setälä, K., Fisher, E. A., Chen, X., Chajek-Shaul, T., Hayek, T., Zechner, R., Walsh, A., Ramakrishnan, R., Ginsberg, H. N., and Breslow, J. L. (1992) *J. Clin. Invest.* **90**, 1889–1900
- Olofsson, S. O., Asp, L., and Boren, J. (1999) *Curr. Opin. Lipidol.* **10**, 341–346
- Rohlmann, A., Gotthardt, M., Hammer, R. E., and Herz, J. (1998) *J. Clin. Invest.* **101**, 689–695
- Veniant, M. M., Zlot, C. H., Walzem, R. L., Pierotti, V., Driscoll, R., Dichek, D., Herz, J., and Young, S. G. (1998) *J. Clin. Invest.* **102**, 1559–1568
- Linton, M. F., Hastay, A. H., Babaev, V. R., and Fazio, S. (1998) *J. Clin. Invest.* **101**, 1726–1736
- Brown, M. L., Ramprasad, M. P., Umeda, P. K., Tanaka, A., Kobayashi, Y., Watanabe, T., Shimoyamada, H., Kuo, W. L., Li, R., Song, R., Bradley, W. A., and Gianturco, S. H. (2000) *Proc. Natl. Acad. Sci. U. S. A.* **97**, 7488–7493
- Lewis, G. F. (1997) *Curr. Opin. Lipidol.* **8**, 146–153

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