Normal Production Rate of Apolipoprotein B in LDL Receptor–Deficient Mice

John S. Millar, Cyrille Maugeais, Ilia V. Fuki, Daniel J. Rader

Abstract—The low density lipoprotein (LDL) receptor is well known for its role in mediating the removal of apolipoprotein B (apoB)-containing lipoproteins from plasma. Results from in vitro studies in primary mouse hepatocytes suggest that the LDL receptor may also have a role in the regulation of very low density lipoprotein (VLDL) production. We conducted in vivo experiments using LDLR−/−, LDLR+/−, and wild-type mice (LDLR indicates LDL receptor gene) in which the production rate of VLDL was measured after the injection of [35S]methionine and the lipase inhibitor Triton WR1339. Despite the fact that LDLR−/− mice had a 3.7-fold higher total cholesterol level and a 2.1-fold higher triglyceride level than those of the wild-type mice, there was no difference in the production rate of VLDL triglyceride or VLDL apoB between these groups of animals. Experiments were also conducted in apobec1−/− mice, which make only apoB-100, the form of apoB that binds to the LDL receptor. Interestingly, the apobec1−/− mice had a significantly higher production rate of apoB than did the wild-type mice. However, despite significant differences in total cholesterol and triglyceride levels, there was no difference in the production rate of total or VLDL triglyceride or VLDL apoB between LDLR−/− and LDLR+/− mice on an apobec1−/− background. These results indicate that the LDL receptor has no effect on the production rate of VLDL triglyceride or apoB in vivo in mice. (Arterioscler Thromb Vasc Biol. 2002;22:989-994.)

Key Words: triglycerides ■ very low density lipoproteins ■ familial hypercholesterolemia ■ Triton WR1339 ■ apobec1

The regulation of the production of apoB from liver is thought to occur intracellularly and at the cell surface. Intracellular regulation occurs in the endoplasmic reticulum and targets misfolded or poorly lipidated apoB. A second site for the intracellular regulation of apoB production is between the endoplasmic reticulum and Golgi and is responsive to insulin and n-3 fatty acids. At the cell surface, a portion of the newly secreted VLDL with high affinity for lipoprotein receptors is also targeted for uptake and degradation. In this case, nascent lipoproteins are captured by receptors, taken up into the hepatocyte by endocytosis, and degraded.

The role of the LDL receptor in mediating the hepatic uptake of apoB-containing lipoproteins from plasma is well established. A new role for the LDL receptor in regulating the hepatic production of VLDL apoB has been recently proposed based on in vitro studies that used primary hepatocytes from LDLR−/− mice (LDLR indicates LDL receptor gene). Horton et al showed that compared with primary hepatocytes from wild-type mice, primary hepatocytes from LDLR−/− mice had an increased secretion of triglycerides in vitro. This in vitro finding was confirmed by Twisk et al, who determined that primary hepatocytes from LDLR−/− mice had a lower intracellular degradation of apoB and lower rate of uptake of newly secreted apoB than did hepatocytes from wild-type mice, resulting in an increased secretion of apoB into the culture medium. This led to the hypothesis that the LDL receptor influences the posttranslational fate of apoB by 2 mechanisms: (1) by promoting intracellular degradation of apoB by forming LDL receptor–apoB complexes within the secretory pathway and (2) by promoting the uptake of newly secreted apoB-containing lipoproteins at the cell surface. The targeting of the LDL receptor–apoB complex for degradation within the secretory pathway, if confirmed, would be a novel mechanism for modulating the production rate of apoB from the liver. However, the in vivo relevance of this proposed mechanism is unknown.

In vivo studies in which the hepatic production rate of apoB in homozygous familial hypercholesterolemia (FH) has been measured have generated conflicting results. Radiotracer studies in humans in which the kinetic data were analyzed by using multicompartmental analysis showed that total apoB production rates in homozygous FH patients were increased compared with those in the control subjects. Other radiotracer studies in humans, analyzed by integration of the area under the VLDL apoB clearance curve, found that VLDL apoB production rates in homozygous FH patients were similar to those in control subjects. Uauy et al concluded that there was no evidence of apoB overproduction in kinetic studies in receptor-negative homozygous FH subjects. Studies in the Watanabe heritable hyperlipidemic (WHHL) rabbit, an animal model of homozygous FH,
showed no effect of the LDL receptor on VLDL apoB or triglyceride production. However, because the WHHL rabbit produces receptors with defective transport to the cell surface that have partial ligand-binding properties, there may be enough receptor activity, particularly intracellular receptor activity, to reduce triglyceride and apoB production rates to levels similar to those in control rabbits.  

The reasons for the discrepancy in results regarding apoB production in human studies may be related to differences in the methods used to analyze kinetic data as well as genetic heterogeneity among study subjects. There has been some debate as to the existence of direct production of LDL apoB-100, and this could be a source of variation because of the assumptions made in the analysis of the kinetic data. However, elimination of direct LDL production by substituting a rapid conversion pathway from VLDL to LDL has a minimal effect (<10%) on the total apoB production rate. Therefore, this is not a likely reason for the discrepancy. Another possibility would be that there are differences in the intracellular degradation and/or uptake of newly secreted VLDL between subjects homozygous for FH. The diagnosis of homozygous FH was made on the basis of clinical criteria in many of these subjects, and the precise molecular defects in the LDL receptor gene were not determined. Homozygous FH subjects with LDL receptor mutations that maintain partial or normal ligand-binding activity would presumably have lower, and possibly normal, rates of apoB production if uptake of apoB alone or in combination with LDL receptor targeting of apoB, as hypothesized, can occur to any significant extent.

The lipase inhibitor Triton WR1339 is a nonionic detergent that inhibits VLDL clearance by preventing access of triglyceride lipases to their lipid core. Intravenous injection of this detergent results in the accumulation of lipoproteins in plasma, thus allowing the calculation of the production rate for triglyceride and apoB when timed samples are collected. This method is frequently used to measure the production rate of triglyceride and apoB when timed samples are collected. This method is frequently used to measure the production rate of triglyceride and apoB when timed samples are collected. In the present study, we used Triton WR1339 to test the hypothesis that LDL receptor deficiency is associated with increased VLDL triglyceride and apoB production rates in mice. The LDLR−/− mouse, with a completely absent LDL receptor, is an excellent model for addressing the role of the LDL receptor in VLDL production in vivo. Our results indicate that hepatic production rates of VLDL triglyceride and apoB in vivo are not increased in LDL receptor−deficient mice under these experimental conditions.

Methods

Animals
C57Bl/6 LDLR−/− and wild-type mice were obtained from Jackson Laboratories (Bar Harbor, Me). C57Bl/6 apobec1−/− mice were obtained from Dr Lyn Powell-Braxton at Genentech, Inc (San Francisco, Calif). Mice were bred to generate the LDLR−/− and LDLR+/− genotypes on the apobec1−/− background. Female LDLR−/−/apobec1−/− and female LDLR+/−/apobec1−/− mice aged 6 to 8 weeks and LDLR−/− and wild-type mice aged 21 to 22 weeks were fed a regular chow diet (diet 5010, PMI Nutrition International). Before the kinetic studies, the mice were fasted for 4 hours and bled from the retro-orbital plexus with the use of heparinized capillary tubes, and serum was assayed for total cholesterol and triglyceride levels. All procedures conducted in mice were in accordance with the University of Pennsylvania Institutional Animal Care and Use Committee guidelines.

Determination of Hepatic Triglyceride and ApoB Production

Hepatic triglyceride and VLDL apoB production rates were determined as previously described. Mice were injected via the tail vein with 100 μL of a sterile PBS solution containing 500 μCi of [35S]methionine (NEN Life Science Products, Inc) and 10 mg Triton WR1339 (Tyloxapol, Sigma Chemical Co). Before injection (0 hours) and at 30 minutes, 1 hour, and 2 hours after injection, either 80 μL (30-minute and 2-hour time points) or 40 μL (0- and 1-hour time points) of blood was drawn by retro-orbital bleeding into heparinized capillary tubes, and plasma was separated by centrifugation at 4°C.

Total cholesterol and triglyceride levels were measured enzymatically with the use of Wako reagents (Wako Chemicals USA, Inc). A delay in the rise in triglycerides has been described as occurring in some mice in the first 30 minutes after Triton WR1339 injection in wild-type mice. We noted an increase in total triglyceride levels in apobec1−/− mice in the first 30 minutes after Triton WR1339 injection that was greater than the linear rate of increase in total triglyceride levels in the period between the 30-minute and 4-hour time points (Figure 1). For these reasons, the linear increment between 30 minutes and 2 hours was used to determine the triglyceride production rate, which was similar to the approach used by other investigators. Total and VLDL triglyceride concentrations were expressed as milligrams per kilogram, assuming a plasma volume of 3.5% of body weight. Triglyceride production rates were calculated by subtracting the 30-minute value from the 2-hour value and then dividing this result by 1.5 to obtain production rates expressed in milligrams per kilogram body weight per hour.

Plasma for the baseline, 30-minute, and 2-hour samples was subjected to ultracentrifugation to isolate VLDL and IDL/LDL fractions for the calculation of the VLDL triglyceride production rate and the VLDL and IDL/LDL apoB production rates for these fractions. VLDL (density <1.006 g/mL) was separated from plasma by ultracentrifugation at 90,000 rpm for 3 hours in a TL 100 ultracentrifuge (Beckman) by using a TLA 100.2 rotor (Beckman). The infranatant (density >1.006 g/mL) was adjusted to 1.063 g/mL with KBr and subjected to a second ultracentrifugation at 90,000 rpm for 3 hours, after which the IDL/LDL fractions were collected in the supernatant fraction (density 1.006 to 1.063 g/mL).

ApoB-100 and apoB-48 were separated from VLDL in the baseline, 30-minute, and 2-hour time points by SDS-PAGE with the use of a 3% to 20% linear gradient. Protein bands were visualized by staining with Coomassie blue R250. ApoB-100 and apoB-48 bands were cut from the gels and placed in glass scintillation vials (Fisher Scientific) along with 500 μL of H2O and 500 μL of Solvable (Packard). Vials were capped and incubated at 50°C for 3 hours. At the end of the incubation, the vials were cooled, and 5 mL of Scintiverse (Fisher Scientific) was added. Vials were recapped, vortexed, and then counted on a scintillation counter (Beckman LS6500). ApoB production rates in counts per minute per microliter.
plasma per hour were calculated as the background-corrected counts in the 30-minute time point subtracted from the 2-hour time point, divided by 1.5, and expressed relative to plasma volume. Throughout the present study, the term “production rate” is used as a measure of the amount of triglyceride or apoB that enters the cell culture medium per unit time from in vitro studies, and the term “secretion rate” refers to the amount of triglyceride or apoB that enters the cell culture medium per unit time from in vitro studies.

Statistical Analysis
Values are presented as mean±SD. Results were analyzed by the Student’s t test for independent samples (2-tailed). Statistical significance for comparisons was assigned at \( P<0.05 \).

Results

Studies in Apobec1+/+ Mice
Total cholesterol and triglyceride levels were significantly higher in the LDLR−/− group compared with wild-type group (276±15 versus 109±20 mg/dL and 74±9 versus 52±5 mg/dL, for total cholesterol and triglyceride levels, respectively). There were no differences between LDLR−/− and wild-type mice in total or VLDL triglyceride production rates (Table 1, Figure 2). VLDL apoB production was assessed by quantifying the incorporation of \([35\text{S}]\)methionine into VLDL apoB-100 and apoB-48 after Triton WR1339 injection. There were no differences between LDLR−/− and wild-type mice in the production rates of VLDL apoB-100 or apoB-48 (Table 1, Figure 3).

Studies in Apobec1−/− Mice
ApoB-100 is capable of binding to the LDL receptor, whereas apoB-48 is not. We hypothesized that an effect of LDL receptor deficiency on VLDL apoB production would be more apparent in apobec1−/− mice because these animals produce only apoB-100 in liver. Therefore, we conducted similar experiments in LDLR−/− and LDLR+/+ mice on an apobec1−/− background. LDLR−/− mice had baseline total cholesterol and triglyceride concentrations that were significantly higher than those from the LDLR+/+ group (631±136 versus 195±20 mg/dL \([P<0.001]\) and 236±25 versus 95±13 mg/dL \([P<0.001]\) for cholesterol and triglyceride levels, respectively). The increase in total triglyceride concentrations after Triton WR1339 injection was linear between 30 minutes and 4 hours in apobec1−/− mice (Figure 1), a finding that is similar to what has been found for apobec1+/+ mice.18 There were no differences in the production rates of total or VLDL triglycerides from LDLR−/− and LDLR+/+ mice (Table 2, Figure 4). ApoB production was assessed by quantifying the incorporation of \([35\text{S}]\)methionine into VLDL apoB-100. This method showed a linear increase in \([35\text{S}]\) incorporation into apoB to 2 hours in apobec1−/− mice (Figure 1), a finding that is similar to that previously observed by us in apobec1+/+ mice.16 The production rate of VLDL apoB-100 was not different between the LDLR−/− and LDLR+/+ groups of apobec1−/− mice (Table 2, Figure 5). The incorporation of \([35\text{S}]\)methionine into IDL/LDL apoB was also assessed. The vast majority of newly synthesized apoB was isolated in the VLDL (density <1.006 g/mL) region (Figure 5), indicating very little direct production of apoB into the IDL/LDL fraction in either LDLR+/+ or LDLR−/− mice.

Discussion

The LDL receptor plays a well-known role in the clearance of lipoproteins from plasma.20 In vitro studies in primary hepatocytes have demonstrated an increase in triglyceride3,4 and apoB3 secretion in the absence of the LDL receptor, suggesting that the LDL receptor may also play a role in regulating the hepatic production rate of apoB-containing lipoproteins. These in vitro findings led Twisk et al3 to hypothesize that LDL receptors influence the posttranslational fate of apoB by promoting intracellular apoB degradation and capture of

![Figure 2](image_url)  
**Figure 2.** The changes in total and VLDL triglyceride concentrations in LDLR−/− and wild-type mice after Triton WR1339 injection.

![Figure 3](image_url)  
**Figure 3.** The incorporation of \([35\text{S}]\)methionine into apoB-100 and apoB-48 from LDLR−/− and wild-type mice after Triton WR1339 injection.
newly secreted apoB. We used \textit{LDLR}^{\text{-/-}} mice to test this hypothesis in vivo, and we used \textit{LDLR}^{+/--} and \textit{LDLR}^{+/+} mice as control animals. We also used \textit{apobec1} knock-out mice in some experiments because these are similar to humans in that they secrete only apoB-100 from their livers. We found no differences between LDL receptor–competent and LDL receptor–deficient mice in the production rates of VLDL triglyceride and apoB. This was true for mice on \textit{apobec1}+/+ and \textit{apobec1}^{/-/-} backgrounds.

The lipase inhibitor Triton WR1339 has been used extensively for 50 years for the study of triglyceride and apoB production in vivo because it was first noted that injection of animals with nonionic detergents can induce a sustained hyperlipidaemia.\textsuperscript{11} This detergent is thought to inhibit lipoprotein clearance by coating lipoprotein particles, thus preventing access of triglyceride lipases to their lipid core.\textsuperscript{14} There is also evidence that Triton WR1339 directly inhibits lipoprotein lipase.\textsuperscript{21} In addition to the inhibition of triglyceride hydrolysis, Triton WR1339 has been shown to inhibit the binding of LDL to lipoprotein receptors.\textsuperscript{22} The capture of newly secreted lipoproteins by receptors would not be expected to occur under these conditions. Therefore, newly secreted lipoproteins accumulate in the plasma after the injection of Triton WR1339, allowing the calculation of the production rate of triglyceride and apoB in mice overexpressing \textit{apobec1} and apoB.\textsuperscript{10}

Studies conducted with the use of Triton WR1339 in vivo in WHHL rabbits showed no differences in VLDL apoB or triglyceride production rates compared with the rates in New Zealand White rabbits.\textsuperscript{9} In this same study, there were also no differences in the production rate of apoB from perfused livers from WHHL and New Zealand White rabbits. However, because the WHHL rabbit produces LDL receptors that have partial ligand-binding properties, there could theoretically be enough receptor activity, particularly intracellular receptor activity, to reduce the apoB production rate in this animal model.\textsuperscript{10} In the present study, we used the Triton WR1339 method to measure triglyceride and apoB production in LDL receptor–competent and LDL receptor–deficient mice and found that absence of the LDL receptor had no effect on the apoB production rate.

A small number of human in vivo kinetic studies involving patients with homozygous FH in which total apoB production was measured have been conducted.\textsuperscript{5-7} Uauy et al\textsuperscript{8} studied LDL apoB production in homozygous FH subjects and concluded that there was no evidence of total apoB overproduction in FH. Soutar et al\textsuperscript{9} found that FH heterozygotes and homozygotes had similar VLDL apoB production rates, suggesting no effect of the LDL receptor on apoB production. This group later reported similar VLDL apoB production rates among FH homozygotes, heterozygotes, and normal control subjects.\textsuperscript{6} In contrast, James et al\textsuperscript{10} reported that homozygous FH subjects had significantly greater (>$60\%$) apoB production rates compared with rates in the control subjects. It is worth noting in this latter study that homozygous FH patients with LDL receptor activity that was 15\% to 30\% of normal had total apoB production rates that were 81\% higher than those of control subjects, whereas those patients with <10\% receptor activity had total apoB production rates that were only 4\% higher than those of control subjects. Although not definitive, these studies suggest that the absence of functional LDL receptors has no effect on the production rate of apoB in vivo in humans.

The possibility of the LDL receptor complexing with newly formed VLDL in the secretory pathway, thus targeting the newly synthesized apoB for degradation, has been proposed as a mechanism by which the LDL receptor could affect the production rate of apoB.\textsuperscript{3} Twisk et al\textsuperscript{3} used multicompartamental modeling of pulse-chase–labeled apoB from intracellular and extracellular pools of primary hepatocytes to demonstrate differences in intracellular degradation of apoB between \textit{LDLR}^{\text{-/-}} and \textit{LDLR}^{+/+} mice. Because

\begin{table}
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\begin{tabular}{llll}
\hline
& \textbf{Total Triglyceride, mg \cdot kg^{-1} \cdot h^{-1}} & \textbf{VLDL Triglyceride, mg \cdot kg^{-1} \cdot h^{-1}} & \textbf{VLDL ApoB-100, cpm \cdot \mu L^{-1} \cdot h^{-1}} \\
\hline
\textit{LDLR}^{+/--} & 91.1 \pm 20.4 (n=10) & 84.3 \pm 22.2 (n=10) & 353.7 \pm 104.7 (n=5) \\
\textit{LDLR}^{/-/-} & 95.5 \pm 42.6 (n=9) & 86.3 \pm 45.7 (n=9) & 386.5 \pm 114.8 (n=4) \\
\textbf{P} & 0.78 & 0.90 & 0.67 \\
\hline
\end{tabular}
\caption{Production Rates for Total and VLDL Triglyceride, and VLDL ApoB-100 in \textit{apobec1}^{/-/-} Mice}
\end{table}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure4.png}
\caption{The changes in total and VLDL triglyceride concentrations in \textit{LDLR}^{\text{-/-}} and \textit{LDLR}^{+/--} mice on an \textit{apobec1}^{/-/-} background after Triton WR1339 injection.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure5.png}
\caption{The production rate of apoB-100 in VLDL and IDL/LDL in \textit{LDLR}^{\text{-/-}} and \textit{LDLR}^{+/--} mice on an \textit{apobec1}^{/-/-} background.}
\end{figure}
the presecretory degradation pathways were not sampled, the structure and associated rate constants of these compartments were inferred from the experimentally determined kinetic data obtained from the endoplasmic reticulum and culture medium. Our finding that there was no difference in the VLDL triglyceride or apoB production rates between LDL receptor–competent and LDL receptor–deficient mice does not support the hypothesis that the LDL receptor targets nascent apoB for intracellular degradation in vivo.

A second mechanism that was proposed as a way by which the LDL receptor could affect the production rate of apoB is the capture of newly secreted apoB by LDL receptors on the cell surface. Twisk et al studied triglyceride and apoB secretion in vitro in the presence and absence of heparin with the capture of newly secreted apoB by LDL receptors on the nascent apoB for intracellular degradation in vivo.

receptor
VLDL triglyceride or apoB production rates between LDL medium. Our finding that there was no difference in the structure and associated rate constants of these compartments the presecretory degradation pathways were not sampled, the secretion-capture process in vivo, the triglyceride production rate suggests that triglyceride clearance from production of lipid-rich VLDL. J Clin Invest. 1999;103:1067–1076.


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