Complete Deficiency of the Low-Density Lipoprotein Receptor Is Associated With Increased Apolipoprotein B-100 Production

John S. Millar, Cyrille Maugeais, Katsunori Ikewaki, Daniel M. Kolansky, P. Hugh R. Barrett, Elaine C. Budreck, Raymond C. Boston, Norio Tada, Seibu Mochizuki, Joep C. Defesche, James M. Wilson, Daniel J. Rader

Objective—We addressed the role of the low-density lipoprotein (LDL) receptor in determining clearance rates and production rate (PR) of apolipoprotein B (apoB) in humans.

Methods and Results—Kinetic studies using endogenous labeling of apoB with deuterated leucine were performed in 7 genetically defined patients with homozygous familial hypercholesterolemia (FH) and compared with 4 controls. The fractional catabolic rates (FCR) and PRs for apoB were determined by multicompartmental modeling. The FCRs of very-low-density lipoprotein 1 (VLDL1), VLDL2, intermediate-density lipoprotein (IDL), and LDL apoB were lower in FH than in controls, with the LDL apoB FCR being significantly lower (0.148±0.049 versus 0.499±0.099 pools·d⁻¹; P=0.008). Whereas receptor-defective FH patients had a total apoB PR similar to controls, receptor-null FH patients had a significantly greater total apoB PR than controls (35.97±10.51 versus 21.32±4.21 mg·kg⁻¹·d⁻¹, respectively; P=0.02).

Conclusions—This first study of apoB metabolism in homozygous FH using endogenous labeling with stable isotopes demonstrates that the LDL receptor contributes significantly to the clearance of LDL from plasma but plays a lesser role in the clearance of larger apoB-containing lipoproteins. Furthermore, these data also indicate that absence of a LDL receptor in humans substantially influences the apoB PR in vivo. (Arterioscler Thromb Vasc Biol. 2005;25:560-565.)

Key Words: cholesterol ■ familial hypercholesterolemia ■ VLDL ■ LDL ■ apoB

The low-density lipoprotein (LDL) receptor is best known for its role in mediating the hepatic uptake of LDL from plasma.¹ Mutations in the LDL receptor frequently result in familial hypercholesterolemia (FH), a disorder associated with hypercholesterolemia and premature atherosclerosis.¹ Whereas patients with FH have elevations in LDL cholesterol levels, plasma triglyceride levels are generally normal,¹ suggesting that other mechanisms clear the majority of triglyceride-rich very-low-density lipoprotein (VLDL) and VLDL remnants. Previous studies conducted using lipoproteins exogenously labeled with radiotracers have demonstrated a slower fractional catabolic rate (FCR) of intermediate-density lipoprotein (IDL) and LDL apolipoprotein (apoB), but not VLDL apoB, in homozygous FH patients.²,³

In addition to the role of mediating clearance of apoB-containing lipoproteins, a role for the LDL receptor in regulating the hepatic production rate (PR) of VLDL apoB has been proposed.⁴,⁵ Williams et al⁴ observed that blocking LDL receptor on the surface of the HepG2 cells affected apoB secretion. This led them to conclude that there can be reuptake of newly secreted apoB from hepatocytes by the LDL receptor. Twisk et al⁵ studied apoB secretion in vitro using primary hepatocyte cultures from Ldlr⁻/⁻ and wild-type mice. They found that hepatocytes from Ldlr⁻/⁻ mice had reduced intracellular degradation resulting in an increased PR of apoB, which led to their hypothesis that the LDL receptor targets apoB for degradation within the secretory pathway.⁵

In vivo studies using the Watanabe heritable hyperlipidemic (WHHL) rabbit showed no effect of LDL receptor
deficiency on VLDL production. However, the WHHL rabbit has defective LDL receptors that can potentially influence apoB production. We measured apoB production in Ldlr−/− mice and found that despite having a complete absence of LDL receptors, the apoB PR in these animals was similar to wild-type mice. Kinetic studies of apoB in homozygous FH patients conducted using radiolabeled lipoproteins have not conclusively established whether deficiency of LDL receptors influences apoB production in humans.

In the current study, we used an endogenous labeling approach with a stable isotope tracer to study the metabolism of apoB in vivo in homozygous FH subjects. The results indicate that in patients lacking a functional LDL receptor, the PR of apoB is significantly increased.

Methods

Subjects

Seven homozygous FH patients were recruited from a cohort followed at the University of Pennsylvania. Four healthy individuals were recruited to act as control subjects. All patients and subjects were nonsmokers with normal hepatic, renal, and thyroid function. Control subjects were not using any medications known to affect plasma lipid levels. FH patients stopped all lipid-lowering treatments 4 weeks before the kinetic studies. The protocol was approved by the Human Investigational Review Board of the University of Pennsylvania. All subjects gave informed consent.

DNA Analysis

Genomic DNA was prepared from peripheral blood leukocytes. For denaturing gradient gel electrophoresis analysis, the promoter region and all 18 exons of the LDL receptor, including the exon–intron boundaries, were individually amplified from genomic DNA by polymerase chain reaction using Taq DNA polymerase (Qiagen, Germany).

Fragments showing an aberrant pattern of bands, suggestive of a sequence variation, were separately amplified by polymerase chain reaction using the same oligonucleotide primer pairs as used for denaturing gradient gel electrophoresis. The sequence reactions were performed using fluorescently labeled dideoxy chain terminations with the Big Dye Terminator ABI Prism Kit (Applied Biosystems) and analyzed on the Applied Biosystems Model 310 automated DNA sequencer. For confirmation of the sequence variant in other patients, a polymerase chain reaction protocol followed by restriction endonuclease digestion was designed.

Infusion Protocol

Subjects were admitted to the General Clinical Research Center of the Hospital of the University of Pennsylvania. After an overnight fast, subjects consumed the first of 10 bi-hourly solid meals followed at the University of Pennsylvania. Four healthy individuals were recruited to act as control subjects. All patients and subjects were nonsmokers with normal hepatic, renal, and thyroid function. Control subjects were not using any medications known to affect plasma lipid levels. FH patients stopped all lipid-lowering treatments 4 weeks before the kinetic studies. The protocol was approved by the Human Investigational Review Board of the University of Pennsylvania. All subjects gave informed consent.

Isolation and Quantification of Apolipoproteins

ApoB was isolated from the lipoprotein fractions by isopropanol precipitation, norleucine added as an internal standard, followed by hydrolysis with 6 mol/L HCl at 100°C for 24 hours. Under the study dietary conditions there was a negligible contribution of apoB48 (<4%) to the precipitated apoB in the VLDL1 and VLDL2 fractions. ApoE phenotypes were determined on neuraminidase-treated plasma using the method of Havekes et al.

Determination of Isotopic Enrichment

Free amino acids were isolated from apoB protein hydrolysates by cation exchange chromatography followed by derivatization and determination of tracer/tracee ratio using gas chromatography/mass spectrometry as described. Plasma apoB concentrations were measured using an immunoturbidimetric assay (Wako Chemicals USA). The apoB concentration within each lipoprotein fraction was calculated from the apoB leucine content of that fraction, relative to the norleucine internal standard, and adjusted to reflect the proportion of total apoB.

Kinetic Analysis

ApoB metabolic parameters (PR and FCR) were calculated from apoB tracer/tracee ratios using 2 independent compartmental models. The first analysis used a previously described model with a total VLDL tracer/tracee data set created by taking the weighted mean of the VLDL1 and VLDL2 tracer/tracee data. A confirmatory analysis was conducted using the model shown in Figure 1. Both analyses provided similar results. The data included in the manuscript are from the latter analysis. Model calculated pool sizes were within 5% of measured pool size (average of 6 apoB measurements taken over the course of the 96-hour study period).

Statistical Analysis

Statistical comparisons between study groups were made with a Wilcoxon rank sum test. Probability values <0.05 were considered statistically significant.

Results

The subject characteristics at the time of the kinetic study are shown in Table 1. The FH patient group contained 4 males and 3 females and the control group consisted of 2 males and 2 females. All patients and controls displayed an apoE3/3

Figure 1. The multicompartmental model used in the analysis of the deuterated leucine tracer kinetic data.
phenotype. At the time of the kinetic study, the FH patients had significantly higher total cholesterol levels (511±100 versus 128±14 mg·dL⁻¹; \(P<0.008\)) and significantly lower high-density lipoprotein cholesterol levels (26±9 versus 42±3 mg·dL⁻¹; \(P<0.023\)) than controls, whereas plasma triglyceride levels were similar between the 2 groups (114±74 versus 73±22 mg·dL⁻¹; \(P=0.26\)). Patient FH6 was hypertriglyceridemic whereas the remaining FH patients were normotriglyceridemic.

Representative tracer/tracee ratio curves for the FH and control groups are shown in Figures 2 and 3. The calculated pool sizes within each fraction are shown in Table 2. The VLDL1 and VLDL2 apoB pool sizes in FH patients were similar to controls (1.55±1.95 versus 0.68±0.42 mg·kg⁻¹; \(P=0.45\); 1.80±2.26 versus 0.68±0.22 mg·kg⁻¹; \(P=0.19\), respectively), whereas the IDL and LDL apoB pool sizes were increased in the FH patients (3.85±4.21 versus 1.15±0.25 mg·kg⁻¹; \(P=0.02\); 98.12±16.12 versus 24.79±5.44 mg·kg⁻¹; \(P<0.001\)). When the hypertriglyceridemic patient, FH6, was excluded, the VLDL1 apoB pool size was similar to controls (0.83±0.46 versus 0.68±0.42 mg·kg⁻¹; \(P=0.25\)), whereas VLDL2, IDL, and LDL apoB pool sizes were significantly larger than those of the controls (0.96±0.31 versus 0.68±0.22 mg·kg⁻¹; \(P=0.02\); 2.27±0.62 versus 1.15±0.25 mg·kg⁻¹; \(P=0.02\); 98.94±17.5 versus 24.79±5.44 mg·kg⁻¹; \(P=0.02\), respectively). Please see http://atvb.ahajournals.org for the apoB concentrations of each fraction.

The FCRs for apoB within each lipoprotein fraction are shown in Table 2. As expected, the FCR of LDL apoB was markedly slower in the FH patients compared with the controls (0.148±0.049 versus 0.499±0.099 pools·d⁻¹; \(P=0.008\)). Although not significant, the FH patients also had slower FCRs for VLDL1 (7.02±2.78 versus 10.29±4.92 pools·d⁻¹; \(P=0.19\)), VLDL2 (12.64±7.19 versus 16.94±5.98 pools·d⁻¹; \(P=0.57\)), and IDL (9.56±9.10 versus 12.17±1.92 pools·d⁻¹; \(P=0.26\)) apoB.

There were no significant differences between FH patients and controls in the direct PRs of VLDL1, VLDL2, IDL, or LDL apoB (Table 3). There were also no significant differences in the total PRs (direct hepatic production plus conversion from other fractions) of apoB in any lipoprotein fraction or conversion rates of apoB between lipoprotein fractions. There was a nonsignificant trend toward an increased total apoB PR in FH patients compared with controls (32.11±10.44 versus 21.32±4.21 mg·kg⁻¹·d⁻¹; \(P=0.06\)).

### Table 1. Subject Characteristics at the Time of the Kinetic Study

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age/Sex</th>
<th>Weight, kg</th>
<th>TC, mg·dL⁻¹</th>
<th>TG, mg·dL⁻¹</th>
<th>HDL, mg·dL⁻¹</th>
<th>LDL Receptor Mutation(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FH1</td>
<td>15/F</td>
<td>68.7</td>
<td>520</td>
<td>59</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>FH2</td>
<td>34/F</td>
<td>75.0</td>
<td>471</td>
<td>86</td>
<td>21</td>
<td>Homozygote: FH Puerto Rico (S156L)</td>
</tr>
<tr>
<td>FH3</td>
<td>46/F</td>
<td>73.4</td>
<td>329</td>
<td>95</td>
<td>26</td>
<td>Compound heterozygote: FH Miami-2 (C249Y); FH Sassari-3 (C255W)</td>
</tr>
<tr>
<td>FH4*</td>
<td>14/M</td>
<td>77.9</td>
<td>522</td>
<td>71</td>
<td>17</td>
<td>Compound heterozygote: FH Albuquerque (C&lt;--&gt;T at -137); FH Algeri (C387K)</td>
</tr>
<tr>
<td>FH5</td>
<td>15/M</td>
<td>58.0</td>
<td>630</td>
<td>118</td>
<td>19</td>
<td>Homozygote: FH Lebanese (C660X)</td>
</tr>
<tr>
<td>FH6</td>
<td>16/M</td>
<td>55.0</td>
<td>615</td>
<td>276</td>
<td>31</td>
<td>Homozygote: FH Lebanese (C660X)</td>
</tr>
<tr>
<td>FH7*</td>
<td>18/M</td>
<td>59.4</td>
<td>492</td>
<td>91</td>
<td>22</td>
<td>Homozygote: FH Lebanese (C660X)</td>
</tr>
<tr>
<td>C1</td>
<td>20/F</td>
<td>63.5</td>
<td>118</td>
<td>60</td>
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<td></td>
</tr>
<tr>
<td>C2</td>
<td>19/F</td>
<td>59.0</td>
<td>146</td>
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<tr>
<td>C3</td>
<td>20/M</td>
<td>75.0</td>
<td>132</td>
<td>49</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>C4</td>
<td>21/M</td>
<td>88.0</td>
<td>117</td>
<td>84</td>
<td>44</td>
<td></td>
</tr>
</tbody>
</table>

*FH4 and FH7 are siblings.

Figure 2. Representative tracer kinetic data for the VLDL1 and VLDL2 lipoprotein fractions in control subject C3 and FH patient FH7. The model-derived fit to the data (solid triangles) is shown as a solid line.

Figure 3. Representative tracer kinetic data for the IDL and LDL lipoprotein fractions in control subject C3 and FH patient FH7. The model-derived fit to the data (solid triangles) is shown as a solid line.
Three of the FH patients were receptor-defective and therefore expressed partially functional LDL receptor. A subgroup analysis was conducted in the 4 LDL receptor-null patients with the FH Lebanese mutation (C660X). This mutation produces a truncated receptor that is not fully processed and is degraded before reaching the cell surface, resulting in a LDL receptor-null phenotype. A subanalysis comparing receptor-null FH patients to controls showed that these patients had significantly increased total VLDL2 and LDL apoB PRs than control subjects (20.42±4.44 versus 12.10±1.56 mg·kg⁻¹·d⁻¹; P=0.02; 21.44±8.21 versus 13.69±1.51 mg·kg⁻¹·d⁻¹; P=0.04, respectively) and greater total apoB PRs than controls (35.97±10.51 versus 21.32±4.21 mg·kg⁻¹·d⁻¹; P=0.02). FH patients that have expression of partially functional LDL receptors or low-level expression of fully functional LDL receptors had total apoB PRs that were similar to controls (25.85±7.72 versus 21.32±4.21 mg·kg⁻¹·d⁻¹, receptor-defective FH versus control, respectively, P=0.48).

**Discussion**

This report of apoB kinetics using endogenous labeling with stable isotopes confirms that LDL apoB clearance is markedly decreased in homozygous FH patients. It also establishes...
that homozygous FH patients with mutations resulting in a complete absence of the LDL receptor have an increased apoB PR, supporting predictions based on in vitro studies.

An important aspect of this study is that all FH patients who were characterized for their LDL receptor mutations at the molecular level. Three of the 7 FH patients (FH1 to FH3) had receptor-defective mutations that either resulted in reduced levels of functional receptor (FH1) or affected ligand binding (FH2, FH3). Four patients (FH4-FH7) were homozygous for the FH Lebanese mutation. This mutation results in transcription of a truncated receptor that does not fully processed and is retained in the endoplasmic reticulum (ER), resulting in a receptor-null phenotype.

Although FH patients have elevated total cholesterol levels, plasma triglyceride levels are frequently normal suggesting less of a role for the LDL receptor in the clearance of triglyceride-rich lipoproteins. Although VLDL clearance can be enhanced in Ldlr mice through overexpression of the LDL receptor, the extent to which VLDL and IDL apoB are cleared from plasma by LDL receptors in vivo in humans is not established. Packard et al found that modification of the LDL receptor binding region of apoB had no effect on VLDL clearance but reduced clearance of IDL in normal subjects. Using radiolabeled lipoproteins, James et al found VLDL apoB clearance but reduced clearance of IDL apoB in normal subjects whereas the FCR of IDL apoB was significantly slower than controls. Soutar et al observed slower clearance of LDL apoB in homozygous FH. In the current study, using endogenous labeling, we observed slower, but not significant, clearance of IDL apoB in FH patients. The FCRs of VLDL1 and VLDL2 apoB were not significantly different between FH patients and controls indicating a minor role of the LDL receptor in the clearance of these lipoproteins.

The role of the LDL receptor in regulating apoB production was first highlighted in in vitro studies by Williams et al. They noted an increased secretion of apoB from hepatocytes in which the LDL receptor was blocked to prevent lipoprotein uptake. Twisk et al found increased secretion of apoB by primary hepatocytes from Ldlr mice when compared with wild-type mice. Analysis of the tracer data suggested that this was caused by reduced presecretory degradation of apoB and reduced capture of newly secreted VLDL. This led to their hypothesis that the LDL receptor may bind apoB intracellularly and target it for degradation. In vivo studies in the WHHL rabbit and Ldlr mice showed no differences in the PR of VLDL triglyceride or apoB when compared with Ldlr control animals. Whereas the WHHL rabbit is considered receptor-defective and may be capable of targeting newly synthesized apoB for degradation, the same cannot be said for Ldlr mice that are receptor-null. The discrepancy observed between in vivo and in vitro studies in mice may be caused by differential changes in expression of genes involved in VLDL secretion by wild-type and Ldlr primary hepatocytes after several hours in culture, although this remains to be tested.

Human kinetic studies using exogenously radiolabeled lipoproteins in patients with homozygous FH have not shown consistent differences with control subjects in the apoB PR. A report by Soutar et al found that VLDL apoB PRs among uncharacterized FH homozygotes, heterozygotes, and normal control subjects were similar. Subsequently, James et al reported that homozygous FH patients had significantly greater (>60%) apoB PRs as compared with control subjects. Despite this increased PR in the total group of FH homozygotes, those patients with <10% LDL receptor activity had total apoB PRs that were similar to those for control subjects. In the current study, we used endogenous labeling of apoB and also characterized the precise molecular defects in the LDL receptor gene. We found that the total apoB PR in the homozygous FH patients trended higher than control subjects but was not statistically significant. A subgroup analysis comparing patients with an LDL receptor-null phenotype and control subjects demonstrated that the apoB PR was significantly elevated in the receptor-null FH patients. One receptor-null patient (FH6) had elevated plasma triglyceride levels that could potentially result from increased VLDL production. The kinetic analysis revealed that the raised plasma triglyceride level was primarily caused by reduced clearance of VLDL apoB. Despite the metabolic heterogeneity of this receptor-null subgroup, the overall elevated PR measured is consistent with the increased PR measured by Tremblay et al in a single receptor-null homozygote. This result supports a role for the LDL receptor in regulating the presecretory degradation of apoB in vivo in humans.

An alternative explanation for increased apoB production in receptor-null FH patients is unregulated hepatic lipid synthesis resulting from the lack of exogenous cholesterol uptake. Homozygous FH patients with a portacaval shunt have a relatively low total apoB PR. Portacaval shunting has been reported to reduce hepatic lipid synthesis and therefore support this alternative explanation. Unregulated hepatic lipid synthesis could also explain the increased apoB production observed in some studies in heterozygous FH. A recent report indicates that heterozygous FH patients with a receptor-null allele have been shown to have increased apoB production. Further studies in FH patients with other receptor-null mutations would be required to determine whether this is a generalized or mutation-specific effect.

In summary, this first study of apoB metabolism in a group of genetically defined homozygous FH patients confirmed a major role for the LDL receptor in the clearance of LDL, but not VLDL or IDL. More importantly, the total apoB PR was significantly increased in receptor-null FH homozygotes. These studies indicate that the LDL receptor plays a major role in the clearance of cholesterol-rich apoB-containing lipoproteins and may also function in modulating the PR of apoB in vivo in humans.

Acknowledgments

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thank the patients whose participation and cooperation made these studies possible.

References


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Table I. ApoB concentrations within each lipoprotein fraction (4 weeks off lipid-lowering treatment).

<table>
<thead>
<tr>
<th>Subject</th>
<th>VLDL1 (mg·dl⁻¹)</th>
<th>VLDL2 (mg·dl⁻¹)</th>
<th>IDL (mg·dl⁻¹)</th>
<th>LDL (mg·dl⁻¹)</th>
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<tr>
<td>FH1</td>
<td>0.29</td>
<td>1.04</td>
<td>2.40</td>
<td>215.84</td>
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<td>2.58</td>
<td>6.13</td>
<td>202.64</td>
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<td>FH3</td>
<td>2.69</td>
<td>1.49</td>
<td>4.98</td>
<td>157.84</td>
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<tr>
<td>FH4</td>
<td>0.93</td>
<td>2.58</td>
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<tr>
<td>FH5</td>
<td>2.18</td>
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<td>5.73</td>
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<td>FH6</td>
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<td>15.29</td>
<td>29.58</td>
<td>230.22</td>
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<td>FH7</td>
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<td>2.53</td>
<td>6.02</td>
<td>238.38</td>
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<tr>
<td>FH mean</td>
<td>3.43 ± 4.34</td>
<td>4.01 ± 5.01</td>
<td>8.55† ± 9.36</td>
<td>218.05† ± 35.82</td>
</tr>
<tr>
<td>FH Null* mean</td>
<td>4.76 ± 5.58</td>
<td>5.74† ± 6.37</td>
<td>11.58† ± 12.00</td>
<td>237.50† ± 35.82</td>
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<tr>
<td>C1</td>
<td>0.82</td>
<td>1.82</td>
<td>3.36</td>
<td>48.38</td>
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<td>C2</td>
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<td>0.71</td>
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<td>1.49</td>
<td>1.51</td>
<td>2.44</td>
<td>50.53</td>
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<tr>
<td>C4</td>
<td>2.84</td>
<td>2.02</td>
<td>2.22</td>
<td>48.27</td>
</tr>
<tr>
<td>Control mean</td>
<td>1.52 ± 0.93</td>
<td>1.52 ± 0.58</td>
<td>2.55 ± 0.55</td>
<td>55.08 ± 12.09</td>
</tr>
</tbody>
</table>

* Subjects FH4-FH7 † p<.05 vs. Control