Increased Production of HDL ApoA-I in Homozygous Familial Defective ApoB-100


Abstract—Familial defective apolipoprotein (apo) B-100 (FDB) is a frequent cause of hypercholesterolemia. Hypercholesterolemia in homozygous FDB is less severe than in homozygotes for familial hypercholesterolemia. Recently, we showed decreased low density lipoprotein (LDL) apoB-100 fractional catabolism and decreased production of LDL due to an enhanced removal of apoE-containing precursors in a patient with homozygous FDB. The effects of defective apoB-100 on high density lipoprotein (HDL) metabolism are unknown. We studied HDL apoA-I metabolism in this FDB patient and in 6 control subjects by using $^2$H$_4$-L-leucine as a tracer. ApoA-I levels were normal in all study subjects. However, the fractional catabolic rate and the production rate of apoA-I were increased, by 79% and 70%, respectively, in FDB; the fractional catabolic rate of apoA-I in FDB was 0.34 day$^{-1}$ compared with 0.19±0.03 day$^{-1}$ in normal controls. The production rate of apoA-I in FDB was 18.4 mg · kg$^{-1}$ · d$^{-1}$ compared with 10.8±2.3 mg · kg$^{-1}$ · d$^{-1}$ in controls. Thus, we have shown for the first time that defective apoB-100 may influence HDL kinetics. The increase in total HDL turnover might enhance reverse cholesterol transport and could contribute to the seemingly benign clinical course of FDB compared with that of familial hypercholesterolemia. (Arterioscler Thromb Vasc Biol. 2000;20:1796-1799.)

Key Words: hypercholesterolemia ■ reverse cholesterol transport ■ tracer kinetics ■ stable isotopes

Familial defective apoB-100 (FDB) is one of the most common monogenetic abnormalities of lipoprotein metabolism. This disorder results from a glutamine-for-arginine substitution at position 3500 of apoB-100, which leads to defective binding of apoB-100 to the LDL receptor and the accumulation of LDL in the plasma (reviewed in References 1 and 2).

Recently, we identified a homozygous FDB patient$^{3,4}$ and studied the in vivo kinetics of apoB-100–containing lipoproteins in this subject by using a stable isotope tracer technique.$^5$ Our investigation revealed an increased removal of VLDL apoE, whereas the VLDL apoB-100 residence time was prolonged. The LDL apoB-100 production was reduced (7.4 versus 15 mg · kg$^{-1}$ · d$^{-1}$ in normals), and the residence time of LDL was increased (8.3 versus 2.3 days in normals).$^5$ These findings are in agreement with data from heterozygous FDB subjects.$^6$ They indicate that the metabolism of apoB-100–containing lipoproteins is distinct in FDB compared with that in familial hypercholesterolemia (FH) due to LDL receptor deficiency.

In FH, HDL metabolism has also been found to be abnormal. Schaefer et al$^6$ demonstrated decreased production and increased fractional catabolism of apoA-I in individuals with FH, suggesting a “cross-talk” between LDL and HDL metabolism. On the background of these observations, we decided to examine whether HDL metabolism might be altered in FDB as well. We therefore investigated the kinetics of HDL apoA-I in a homozygous FDB subject by using the stable-isotope tracer technique.

Methods

Study Subjects

We studied the homozygous FDB patient described previously.$^{3,4}$ The patient is a 54-year-old white male without any symptoms of disease. He had multiple xanthelasmas and a discrete arcus corneae. His apoE phenotype was 3/3. Routine blood biochemistry was normal except for a slight increase in $\gamma$-glutamyl transferase (36 U/L; upper limit of normal, 27 U/L). The patient did not take any lipid-lowering drugs in the 6 weeks before and during the study. Six healthy, normolipidemic male subjects served as controls. All but one (No. 6 was apoE 2/4) had an apoE 3/3 phenotype and normal body-weight.

Sequencing of ApoA-I

DNA of the homozygous FDB patient was extracted from white blood cells by using blood polymerase chain reaction (PCR) DNA isolation cartridges (Diagen GmbH). Oligonucleotide primers were synthesized (BIG) to allow amplification of part of the promoter and of individual exons of the apoA-I gene: apoA-I promoter $5'$: $5'$-AGA GCT GAT CCT TGA ACT CTT AAG-3'

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bands were excised from the gels and hydrolyzed in 6 mol/L HCl at 110°C for 24 hours under N₂. The protein hydrolysates were lyophilized in a Speed-Vac evaporator (Savant Instruments). Free amino acids were purified from plasma or protein hydrolysates by cation exchange chromatography (AG-50W-X6, Bio-Rad Laboratories) and then derivatized to n-heptafluorobutylisobutyl esters and analyzed by GC-MS in the chemical ionization and selected ion monitoring mode as reported recently. The ions monitored were 363.1 m/z (mass-to-charge ratio) for unlabeled L-leucine and 366.1 m/z for radiolabeled [3H]-L-leucine as parent ions (first MS) and 280.1 m/z for the daughter ions of both types of leucine (second MS). Tracer enrichment was calculated as the tracer-to-tracer ratio according to Cobelli et al.

A monoexponential function was fitted to the tracer-to-tracer curves of VLDL apoB-100 and HDL apoA-I with the use of SAAM 30 software as previously described. The function was defined as

\[ E(t) = E_0 \left(1 - e^{-kt}\right) \]

where \( E(t) \) is the tracer-to-tracer ratio at time \( t \) of HDL apoA-I and \( E_0 \) is the tracer-to-tracer ratio at plateau of the precursor pool of interest (VLDL apoB-100)\( ^{16} \), \( d \) is the delay time and \( k \) is the fractional catabolic rate.

### Results and Discussion

Lipids and lipoprotein profiles in the FDB and in 6 normal control subjects are shown in Table 1. In the FDB patient, total cholesterol was higher compared with that in controls, but his total apoA-I was normal. Although HDL cholesterol, HDL phospholipids, and HDL apoA-II were significantly lower in FDB compared with controls, there was no significant difference in the composition of HDL (Table 2). In addition, sequencing revealed no mutation either in the promoter or in the coding sequence of the apoA-I gene of the homozygous FDB patient. The kinetic parameters of HDL apoA-I metabolism in the FDB homozygote and in the 6 control subjects are shown in Table 3. The FDB subject revealed a clearly higher fractional catabolic rate (+79%, \( P<0.0001 \)) and production rate (+70%, \( P<0.0005 \)) of apoA-I compared with those values in normal subjects.

Defective apoB-100 leads to significant changes in the in vivo kinetics of apoB-containing lipoproteins. We and others have shown that both the production rate of LDL apoB and its fractional catabolic rate are reduced compared with normal,\(^{2,5,6} \) but the metabolic situation in FDB is completely different from that in FH. In FH, delayed fractional catabolism of LDL apoB occurs together with an increased production of LDL apoB.\(^{11-13} \) Moreover, in homozygous FH, in an earlier report we found dramatic changes in HDL apoA-I kinetics, characterized by a decrease in apoA-I production and an increased fractional catabolic rate of apoA-I.\(^{7} \) In FH,
coordinated regulation of apoB and apoA-I kinetics was thus suggested, and we were interested to search for a similar effect in FDB.

This is the first study to examine the metabolism of HDL apoA-I in FDB. The single homozygous individual presented in this study may not be completely representative of the entire disorder. However, owing to the potential influence of normal apoB-100 in heterozygous FDB, the study of a homozygous individual may provide more valid information about the metabolism in FDB than would study of several individuals heterozygous for the disease. Despite normal apoA-I levels, we found an increased fractional catabolic rate of apoA-I in FDB and a marked increase in the total production rate of apoA-I. These changes may indicate that reverse cholesterol transport is upregulated in FDB. Increased production of apoA-I is found in subjects with the longevity syndrome. In addition, an increased fractional catabolic rate of apoA-I might be beneficial, since it is known that hypoalphalipoproteinemia due to increased fractional catabolism of apoA-I appears to be less atherogenic compared with the inborn abnormalities of apoA-I metabolism associated with decreased apoA-I production. The increased production of apoA-I may thus protect our FDB homozygote from atherosclerosis.

The metabolic basis for the increase in apoA-I production and fractional catabolism in FDB is currently unclear. Alterations of the apoA-I gene in our homozygous FDB patient and major alterations of the composition of HDL have been excluded as possible reasons for our findings. A speculative possibility is that the increase in the fractional catabolism of apoA-I in FDB is due to an increase in the catabolic rate of apoE-containing HDL. ApoE-containing HDLs are known to be catabolized by the LDL receptor, and LDL receptor activity appears increased in FDB due to the low influx of LDL cholesterol into the liver.

Brown et al demonstrated an increase in apoA-I levels when they transferred human LDL receptors by using a recombinant adenovirus into LDL receptor–deficient rabbits, a finding that, at first sight, appears inconsistent with an increased apoA-I fractional catabolic rate in FDB due to the upregulation of LDL receptors. However, in the study by Brown et al, the greatest increase of apoA-I was found in preβ-HDL, a fraction considered to contain mainly nascent HDL. This suggests that the increase in HDL was due to an increase in apoA-I production rather than decreased catabolism of apoA-I. Such interpretation of their data would then completely align with our findings in FDB, wherein we

### TABLE 2. Composition of HDLs From a Patient With Homozygous FDB and 6 Healthy, Normolipidemic Individuals

<table>
<thead>
<tr>
<th>Subject</th>
<th>HDL-Ch, mg/dL</th>
<th>HDL-C/ ApoA-I</th>
<th>HDL-TG, mg/dL</th>
<th>HDL-TG/ ApoA-I</th>
<th>HDL-PL, mg/dL</th>
<th>HDL-PL/ ApoA-I</th>
<th>HDL-ApoA-II, mg/dL</th>
<th>HDL–ApoA-II/ ApoA-I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>34</td>
<td>0.362</td>
<td>5.7</td>
<td>0.061</td>
<td>55.5</td>
<td>0.592</td>
<td>38.7</td>
<td>93.8</td>
</tr>
<tr>
<td>2</td>
<td>44</td>
<td>0.578</td>
<td>12.8</td>
<td>0.168</td>
<td>72.9</td>
<td>0.958</td>
<td>35.1</td>
<td>76.1</td>
</tr>
<tr>
<td>3</td>
<td>47</td>
<td>0.422</td>
<td>10.2</td>
<td>0.092</td>
<td>68.5</td>
<td>0.615</td>
<td>37.7</td>
<td>111.4</td>
</tr>
<tr>
<td>4</td>
<td>50</td>
<td>0.333</td>
<td>4.5</td>
<td>0.03</td>
<td>47.5</td>
<td>0.317</td>
<td>32.1</td>
<td>150</td>
</tr>
<tr>
<td>5</td>
<td>37</td>
<td>0.474</td>
<td>14.2</td>
<td>0.182</td>
<td>93.7</td>
<td>1.20</td>
<td>38.1</td>
<td>78</td>
</tr>
<tr>
<td>6</td>
<td>35</td>
<td>0.398</td>
<td>5.9</td>
<td>0.067</td>
<td>62.3</td>
<td>0.708</td>
<td>40</td>
<td>88</td>
</tr>
<tr>
<td>Mean ± SD in controls</td>
<td>41.2 ± 6.7</td>
<td>0.43 ± 0.09</td>
<td>8.9 ± 4.09</td>
<td>0.09 ± 0.06</td>
<td>66.7 ± 16</td>
<td>0.73 ± 0.31</td>
<td>37.0 ± 2.87</td>
<td>99.6 ± 27.8</td>
</tr>
<tr>
<td>FDB</td>
<td>30</td>
<td>0.41</td>
<td>4.6</td>
<td>0.06</td>
<td>42.8</td>
<td>0.59</td>
<td>32</td>
<td>72.7</td>
</tr>
<tr>
<td>P</td>
<td>0.01</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.01</td>
<td>NS</td>
<td>0.01</td>
<td>NS</td>
</tr>
</tbody>
</table>

-C indicates cholesterol; TG, triglycerides; and PL, phospholipids. Ratios do not have units of measure because they can be canceled out. 
P values were calculated by the 2-tailed, 1-sample t test.

### TABLE 3. Kinetics of HDL ApoA-I From a Patient With Homozygous FDB and 6 Healthy, Normolipidemic Individuals

<table>
<thead>
<tr>
<th>Subject</th>
<th>FCR, Days⁻¹</th>
<th>RT, Days</th>
<th>ApoA-I, mg/dL</th>
<th>PR, mg · kg⁻¹ · d⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.19</td>
<td>5.1</td>
<td>108</td>
<td>8.5</td>
</tr>
<tr>
<td>2</td>
<td>0.17</td>
<td>5.9</td>
<td>143</td>
<td>9.8</td>
</tr>
<tr>
<td>3</td>
<td>0.19</td>
<td>5.3</td>
<td>142</td>
<td>10.7</td>
</tr>
<tr>
<td>4</td>
<td>0.24</td>
<td>4.1</td>
<td>157</td>
<td>15.2</td>
</tr>
<tr>
<td>5</td>
<td>0.17</td>
<td>5.7</td>
<td>137</td>
<td>9.5</td>
</tr>
<tr>
<td>6</td>
<td>0.19</td>
<td>5.3</td>
<td>142</td>
<td>10.8</td>
</tr>
<tr>
<td>Mean ± SD in controls</td>
<td>0.19 ± 0.03</td>
<td>5.2 ± 0.6</td>
<td>138.2 ± 16.2</td>
<td>10.8 ± 2.3</td>
</tr>
<tr>
<td>FDB</td>
<td>0.34</td>
<td>2.9</td>
<td>135</td>
<td>18.4</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.0001</td>
<td>&lt;0.0003</td>
<td>NS</td>
<td>&lt;0.0005</td>
</tr>
</tbody>
</table>

FCR indicates fractional catabolic rate; RT, residence time; and PR, production rate. 
P values were calculated by the 2-tailed, 1-sample t test.
observed an increased apoA-I production together with an increased fractional catabolic rate. A mechanism linking the upregulation of LDL receptors and stimulation of apoA-I production is also not readily apparent at this time. The existence of such a mechanism, however, is well in agreement with studies by Mitchell et al.\(^{18}\) as well as with the studies of our group. In rats treated with drugs that upregulate the LDL receptor, Mitchell et al.\(^{18}\) demonstrated an increase in apoA-I mRNA and a decrease in hepatic apoB mRNA. Consistently in subjects treated with the 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor pravastatin, we have found an increased production rate of apoA-I.\(^{19}\)

Finally, we recently obtained evidence of increased production of apoA-I during administration of lifibrol to individuals with moderate hyperlipidemia.\(^{20}\) Lifibrol is a novel hypolipidemic compound that stimulates LDL receptors without substantially affecting cholesterol biosynthesis.\(^{21,22}\)

In conclusion, the finding of a significantly increased HDL metabolism in FDB is in clear contrast to the situation in FH. In FH there is an increased fractional catabolic rate of apoA-I combined with a decrease in apoA-I production. With this combination, there are major metabolic differences between FDB and FH, with respect to not only the kinetics of apoB-containing lipoproteins but also in the kinetics of apoA-I. These differences might contribute to the rather benign clinical course of FDB compared with homozygous FH.

References


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