Elevated Remnant-Like Particles in Heterozygous Familial Hypercholesterolemia and Response to Statin Therapy

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Background—Remnant lipoproteins (RLP-C) are considered important in atherogenesis. Hence, this study was designed to assess RLP-C levels and the effect of statin therapy in patients with familial hypercholesterolemia (FH). Elevated RLP-C levels have been associated with the presence and progression of atherosclerotic disease, and their presence in FH patients has been proposed but never established in a large cohort, nor has their response to statin therapy been confirmed.

Methods and Results—FH patients were recruited from 36 lipid clinics. After a washout period of 6 weeks, all patients were started on monotherapy with 80 mg of simvastatin for 2 years. RLP-C levels were assessed by an immune-separation assay. In 327 FH patients, RLP-C measurements could be performed before and after treatment. Mean total cholesterol (10.55 ± 2.17 mmol/L), mean LDL cholesterol (8.40 ± 2.13 mmol/L), and median RLP-C (0.47 mmol/L) levels were all severely elevated at baseline. After treatment, RLP-C levels were reduced by 49% (0.24 mmol/L; P < 0.0001). Even patients with normal triglyceride levels had elevated RLP-C levels at baseline, and those with high RLP-C levels were generally characterized by a very atherogenic lipoprotein profile.

Conclusions—Baseline RLP-C levels are severely elevated in FH patients and are reduced by simvastatin but do not return to normal. These elevated RLP-C levels could be the consequence of impaired function of the LDL receptor in FH. RLP-C levels in FH contribute to an atherogenic lipoprotein profile and could identify patients who require additional treatment. (Circulation. 2002;106:788-792.)

Key Words: hypercholesterolemia ■ lipids ■ lipoproteins ■ atherosclerosis

Familial hypercholesterolemia (FH) is an autosomal dominant disorder of lipoprotein metabolism.1 Mutations in the LDL receptor gene are the cause of this disease and lead to a reduction in the clearance of LDL cholesterol (LDL-C), which causes a rise in LDL-C levels and predisposes to the development of atherosclerosis.2 Therefore, FH patients are at great risk of developing premature coronary artery disease. However, there is a wide variation of coronary risk among FH patients.3

Increasing experimental and clinical evidence suggests that triglyceride (TG)-rich lipoproteins, and in particular remnant-like particles (RLPs), contribute to atherogenesis and consequently to cardiovascular disease progression. High levels of remnant lipoproteins of both hepatic (VLDL) and intestinal (chylomicron) origin are associated with the progression of coronary atherosclerosis.4–7 In a study by Phillips et al.,7 it was found that neither LDL-C nor TG levels correlate well with lesion progression or clinical events. TG-rich lipoprotein remnant levels, however, did correlate with both lesion progression and cardiac events. Recently, Nakajima et al.8–10 developed a simple technique to analyze RLP cholesterol (RLP-C) using an immune-affinity gel containing anti-apolipoprotein (apo) A-I and anti-apoB100 monoclonal antibodies. This unique anti-apoB100 monoclonal antibody was shown to recognize apoB100 in LDL and most VLDL but not in apoE-enriched VLDL, whereas anti-apoA-I recognizes and binds all HDL particles. This technique isolates apoE-rich VLDL particles containing apoB100 together with chylomicron remnants containing apoB48, neither of which binds to the immunoaffinity gel. Increased levels of these remnant particles have been associated with the presence and progress of cardiovascular disease and with endothelial dysfunction.11–18 However, these previous results were examined in non-FH patients. Evidently, these patients have a different lipid profile from the extremely elevated LDL-C levels seen in FH patients. Therefore, this association between elevated levels of RLP-C and cardiovascular disease cannot be extrapolated to FH patients as such.
Levels of remnant particles could be relevant for the understanding of the heterogeneity in coronary risk among FH patients. RLP-C levels have thus far not been assessed in a large FH population. To date, only 2 small studies (in 15 and 7 FH patients, respectively) reported RLP-C levels of FH patients and did indeed show increased RLP-C levels.\textsuperscript{19,20} In the present study, we aimed to assess accumulation of RLP-C and also to evaluate whether RLP-C levels would be lowered by statin therapy in a large, well-defined cohort of FH patients.

**Methods**

**FH Subjects**

The present study is a substudy of the ExPRESS FH (Examination of Probands and Relatives in Statin Studies with Familial Hypercholesterolemia) study, in which efficacy, safety, and pharmacogenomics of simvastatin 80 mg were assessed in 526 heterozygous FH patients. For this open-label, multicenter study, FH patients were recruited from 36 lipid clinics in the Netherlands. Patients were included if they met the following criteria: all patients had to have either a molecular diagnosis for FH or were diagnosed with definite FH and had to have 6 or more points, according to an algorithm (to allow standardization of the diagnosis of FH based on clinical findings, personal and familial clinical history, and biochemical parameters);\textsuperscript{21} all patients were at least 18 years of age; and patients had a history of myocardial infarction, CABG, or PTCA could be included if the physician thought it was medically allowed for the patient to have a washout period. Patients were excluded if they were pregnant or nursing women or premenopausal women not using adequate contraceptives; had acute liver disease, hepatic dysfunction, or persistent elevations of serum transaminases; had hypersensitivity or intolerance to simvastatin or any of its components; had hyperlipidemia type I, III, IV, or V or homozygous FH; had a recent history of alcohol or drug abuse; had secondary hypercholesterolemia due to any cause; had inadequately controlled diabetes, unstable angina, or intermediate coronary syndrome, or clinically significant ventricular arrhythmia at study entry, or myocardial infarction within the past 3 months; were concurrently using erythromycin and similar drugs that affect the cytochrome P450 enzyme; or had a history of cancer.

**Controls**

Controls for the 327 FH patients in whom RLP-C levels were measured were recruited post hoc from their families and matched for age and sex. From these 77 individuals, we obtained demographic characteristics, lipid levels, and lipoprotein levels.

**Study Design**

After a washout period of 6 weeks, patients were started on monotherapy with simvastatin 80 mg. No other lipid-lowering medication was allowed. Medical history, physical examination, and additional risk factors for cardiovascular disease, as well as laboratory analyses of lipid and lipoprotein levels and routine safety parameters, were obtained from all patients. The biochemical analyses of lipid levels and safety parameters were performed in the hospitals themselves and were standardized by a virtual central laboratory. The apo determinations were performed in the Academic Medical Center in Amsterdam and the RLP determinations in the University Medical Center in Utrecht. The ethics committees of all 36 centers approved the protocol, and written informed consent was obtained from all participants.

**Biochemical Analysis**

Blood samples were taken in the morning after an overnight fast. Total cholesterol, HDL cholesterol (HDL-C), and TG levels were routinely determined in the different laboratories and standardized by a virtual central laboratory. LDL-C was calculated with the Friedewald formula.\textsuperscript{22} ApoA-I and apoB were determined by an immuno-logic rate-nephelometric procedure with a polyclonal goat anti-human antibody (Array protein system, Beckman Coulter).\textsuperscript{23} The RLP fraction was prepared by use of an immune-separation technique described by Campos et al\textsuperscript{8} and Nakajima and colleagues.\textsuperscript{9} Briefly, 5 μL of serum was added to 300 μL of mixed immunoaffinity gel suspension containing monoclonal anti-human apoA-I (H-12) and anti-human apoB100 (JH-H) antibodies (Immuresearch Laboratories). The reaction mixture was gently shaken for 120 minutes at room temperature. After the supernatant was left standing for 15 minutes, 200 μL was withdrawn for the assay of RLP-C. Cholesterol in the RLP fraction (coefficient of variation <3%) was measured by an enzymatic assay on a Cobas Mira S autoanalyzer (ABX Diagnostics). ApoE genotyping was performed as described by Reymer et al.\textsuperscript{24}
TABLE 1. Lipid, Lipoprotein, and RLP-C Levels in Controls and in FH Patients at Baseline and After 1 Year of Therapy

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline (n=327)</th>
<th>Simvastatin 80 mg (n=327)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC, mmol/L</td>
<td>10.55±2.17</td>
<td>6.36±1.37</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>8.40±2.13</td>
<td>4.32±1.30</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.25±0.35</td>
<td>1.39±0.39</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>1.80 (1.20–2.40)</td>
<td>1.20 (0.90–1.73)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ApoA-I, g/L</td>
<td>1.24±0.22</td>
<td>1.36±0.24</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ApoB, g/L</td>
<td>1.97±0.45</td>
<td>1.19±0.31</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>RLP-C, mmol/L</td>
<td>0.47 (0.34–0.80)</td>
<td>0.24 (0.20–0.31)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

TC indicates total cholesterol.

All values are given as mean with SD except that TG and RLP-C are given as median with interquartile range.

(P<0.0001), which is consistent with a 49% median reduction. At baseline, only 5 FH patients (1.5%) had normal RLP-C levels (<0.20 mmol/L); in contrast, after simvastatin therapy, 84 patients (25.7%) were below this RLP-C level.

In Table 2, baseline RLP-C levels are divided into 3 equal groups by the 33rd and 66th percentiles. FH patients in the highest third were older, were more often male, had a higher BMI, and more often had diabetes. To address whether FH patients with type 2 diabetes were outliers in terms of remnant accumulation and caused significant shifts of the medians in the different groups, we calculated median RLP-C levels with these individuals included or excluded. However, results indicated no significant changes in the medians of these 3 groups (data not shown). In addition, the statistically significant increase in total cholesterol, LDL-C, and TG levels and the decrease in HDL-C illustrate the association between plasma RLP-C and the presence of a severe atherogenic lipoprotein profile. RLP-C levels were also correlated with HDL-C levels; the Spearman rank correlation coefficient was r = −0.37 at P<0.0001. In the lowest third of baseline RLP-C levels, median TG levels were normal (1.10 mmol/L), whereas in that third, median RLP-C levels were still above normal (0.32 mmol/L). Moreover, significantly more patients in the highest RLP-C tertile had an apoE2 allele compared with the 2 lower tertiles (P=0.001). Finally, RLP-C levels were more reduced in the highest tertile than in the lower tertiles. Because pretreatment and posttreatment measurements of the variable of interest were, in general, not perfectly correlated, the evaluation of treatment effects must be adjusted for regression to the mean. Chen et al25 proposed 4 models, which included either or both additive and multiplicative effects. We applied this model to evaluate RLP-C changes. The model that included both additive and multiplicative effects fit better than that with only additive effects (regression to the mean) for RLP-C change (P<0.0001). Therefore, changes in RLP-C could not be attributed to regression to the mean only but indeed exhibited a relationship with baseline levels. Of FH patients in the highest versus the lowest third of RLP-C levels, 53 (48.6%) had cardiovascular disease versus 30 (27.5%) of the cases (χ²=10.5, P=0.005). However, on multiple logistic regression analysis with age, sex, BMI, and major lipids in the model, this relation was no longer statistically significant (P=0.32).

Table 3 shows the data stratified according to baseline TG levels in quartiles. Patients in the lowest quartile had completely normal median baseline TG levels (0.90 mmol/L); however, the corresponding RLP-C levels were already strongly elevated (0.32 mmol/L), whereas plasma LDL-C levels were similar in all groups (P=0.09).

**Discussion**

**RLP-C Increase at Baseline**

In the present study, we observed that median RLP-C levels in FH patients were severely elevated compared with their siblings (0.47 versus 0.20 mmol/L; P<0.0001). Elevated

TABLE 2. Lipids, RLP, ApoE2, and Clinical Features in FH Patients With Baseline RLP-C Levels Divided in 3 Equal Groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>RLP-C&lt;0.39 mmol/L (n=109)</th>
<th>0.39&lt;RLP-C&lt;0.65 mmol/L (n=109)</th>
<th>RLP-C≥0.65 mmol/L (n=109)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>44.0±14.2</td>
<td>46.6±13.2</td>
<td>51.5±11.0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>41 (45.0)</td>
<td>47 (51.4)</td>
<td>60 (65.1)</td>
<td>0.009</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.6±3.7</td>
<td>25.6±3.2</td>
<td>27.3±3.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>0 (0)</td>
<td>1 (0.9)</td>
<td>5 (4.6)</td>
<td>0.029</td>
</tr>
<tr>
<td>TC, mmol/L</td>
<td>9.50±1.70</td>
<td>10.68±2.08</td>
<td>11.47±2.23</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>7.58±1.63</td>
<td>8.64±2.13</td>
<td>8.99±2.32</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.37±0.36</td>
<td>1.29±0.35</td>
<td>1.09±0.27</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>1.10 (0.90–1.50)</td>
<td>1.80 (1.30–2.05)</td>
<td>2.80 (2.10–3.60)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ApoA-I, g/L</td>
<td>1.28±0.21</td>
<td>1.25±0.24</td>
<td>1.18±0.19</td>
<td>0.004</td>
</tr>
<tr>
<td>ApoB, g/L</td>
<td>1.74±0.35</td>
<td>2.00±0.39</td>
<td>2.17±0.49</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>RLP-C, mmol/L</td>
<td>0.32 (0.26–0.34)</td>
<td>0.47 (0.43–0.53)</td>
<td>1.06 (0.79–1.62)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>RLP reduction, %</td>
<td>29.0 (14.9–41.0)</td>
<td>50.5 (39.8–57.7)</td>
<td>67.5 (57.2–76.8)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ApoE2 allele, n (%)</td>
<td>5 (4.6)</td>
<td>4 (3.7)</td>
<td>17 (15.6)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

TC indicates total cholesterol.

All values are given as mean with SD or n (%), except that TG, RLP-C, and RLP-C reduction are given as median with interquartile range.
RLP-C levels were reported before in FH patients, albeit in very small cohorts. Twickler et al. measured RLP-C levels in 7 FH patients and found significantly elevated mean RLP-C levels compared with 7 controls (42.1 mg/dL [1.09 mmol/L] versus 7.49 mg/dL [0.19 mmol/L]; \( P<0.01 \)). Dane-Stewart et al. also found elevated median RLP-C levels in 15 FH patients compared with 15 controls (16.2 mg/dL [0.42 mmol/L] versus 8.5 mg/dL [0.22 mmol/L]; \( P=0.003 \)). In the present cohort, mean age and BMI increased from the lowest to the highest RLP-C tertiles. Why RLP levels rise with age is unknown, but older people have higher BMIs, often combined with higher TG levels. This association points to increased synthesis of VLDL, which could lead to higher RLP levels.26 We also found a higher prevalence of apoE2 allele carriers in the highest RLP-C tertile. ApoE is the major ligand for hepatic lipoprotein receptors and mediates chylomicron and VLDL remnant uptake.27 ApoE2 is 1 of the 3 E isoforms and exhibits a very low affinity to the LDL receptor.28 It is therefore not unexpected to find more apoE2 alleles in the highest third of RLP-C levels. The apoE allele distribution was in Hardy-Weinberg equilibrium (\( \chi^2=3.59, P>0.1 \)) in our FH sample, but the frequency of the e-2 allele was lower than previously reported in the general Dutch population (0.047 and 0.082, respectively).29 It is therefore unlikely that an overrepresentation of the e-2 allele of the apoE gene contributed to our findings.

These elevated RLP-C levels might play an important role, in addition to elevated LDL-C levels, in the acceleration of atherosclerosis in FH. This idea is supported by the findings of Karpe et al., who did not find an association between LDL-C and new lesions in vein grafts in 395 patients with coronary artery disease but did find a strong trend for higher RLP-C concentrations.

**RLP-C Reduction After Statin Intervention**

RLP-C levels are significantly decreased by simvastatin. Median RLP-C levels almost returned to normal but remained slightly elevated. This observation was reported previously in 7 FH patients.20 Recently, 2 studies in patients with combined dyslipidemia showed similar results.30,31 Stein et al. found that median elevated RLP-C levels of 0.34 mmol/L were reduced by simvastatin 20 mg (6.0%) and by atorvastatin 10 mg (25.9%) but not by pravastatin 40 mg in 22 patients in a crossover study. In addition, Sasaki et al.32 found that atorvastatin 10 to 20 mg reduced RLP-C levels from 0.31 to 0.16 mmol/L. These studies also illustrate a treatment effect of statins on the RLP-C fraction.

**Accumulation as the Proposed Mechanism**

The central abnormality in heterozygous FH is an impaired function of the LDL receptor. As a consequence, plasma levels of LDL-C are severely elevated, as are levels of RLP-C. Moreover, these elevated RLP-C levels are significantly reduced by simvastatin treatment. Statins likely improve RLP clearance by upregulating LDL receptors and by decreasing hepatic VLDL synthesis.32,33 Both mechanisms lead to less competition for the clearance mechanisms shared by chylomicrons and VLDL.34 The reductions in apoB and LDL-C were very consistent with those of RLP-C, as is anticipated, because by upregulating the LDL (apoB, E) receptor, all apoB (LDL-) and apoE (RLP-C)-containing lipoproteins will be reduced in essentially similar proportions.

The raised TG levels in the higher RLP-C tertiles support the accumulation of TG-rich lipoproteins. The conclusion could therefore be drawn that TG levels measured in FH patients primarily reflect atherogenic remnant lipoproteins. However, even patients with normal TG levels had elevated RLP-C levels. This observation was also made previously.20 All these data indicate the presence of remnant particle accumulation in FH patients, irrespective of concomitant TG elevation. Likewise, stratification of the data according to baseline TG into quartiles resulted in an association between TG and RLP-C levels, but LDL-C levels were equally elevated in all quartiles. This suggests that despite the fact that plasma LDL-C levels are strongly elevated in FH patients, RLP-C levels could further contribute to the atherogenic lipoprotein profile over and above LDL-C measurements. We therefore hypothesize that remnant accumulation in FH might be explained by a combination of factors, such as the LDL-receptor mutation; VLDL production associated with advancing age, central obesity, and glucose intolerance; carrierness of an e-2 allele of the apoE gene; and possibly defective LDL-receptor related protein function. These findings may raise the need to prescribe combination therapy with simvastatin 80 mg and either nicotinic acid or fenofibrate or to prescribe more powerful statins to lower the risk associated with the residual remnant increase.

In conclusion, baseline RLP-C levels are severely elevated in FH patients. Treatment with high-dose simvastatin resulted in a strong reduction of RLP-C, but in the majority of patients, RLP-C levels remained elevated. RLP-C levels in FH contribute to an atherogenic lipoprotein profile and could identify patients who require additional treatment.

**Acknowledgment**

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**References**


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