High Dose of Simvastatin Normalizes Postprandial Remnant-Like Particle Response in Patients With Heterozygous Familial Hypercholesterolemia


Abstract—Familial hypercholesterolemia (FH) and disturbances in postprandial lipoprotein metabolism are both associated with premature atherosclerosis. The effect of β-hydroxy-β-methylglutaryl coenzyme A reductase inhibitors on plasma cholesterol levels in patients with FH is well established; however, it is not known whether postprandial lipoproteins are also influenced. In this case-controlled intervention study, we investigated the effects of high-dose simvastatin on postprandial lipoproteins. We used a new method to analyze remnant lipoproteins based on the immunoseparation principle (remnant-like particle cholesterol [RLP-C] assay) and the well-established measurement of retinyl ester (RE) analysis in plasma and in the Svedberg flotation unit (Sf)<1000 fraction. Seven heterozygous FH patients and 7 control subjects matched for sex, age, body mass index, triglycerides, and apolipoprotein E genotype were enrolled in the study. An oral vitamin A (RE) fat-loading test was performed at baseline in both groups and after 3 months of high-dose simvastatin (80 mg/d) treatment in FH patients. Before treatment, FH patients had significantly higher fasting and postprandial concentrations of lipoprotein remnants (plasma RLP-C 42±19 mg/dL and area under the RLP-C curve 415±82 mg·L⁻¹·h⁻¹, respectively) than did control subjects (7±3 mg/dL and 101±35 mg·L⁻¹·h⁻¹, respectively; P<0.05), suggesting a delayed clearance of chylomicron remnant particles in the FH patients. Treatment with simvastatin significantly reduced fasting and postprandial remnant lipoprotein cholesterol concentrations (13±3 mg/dL and 136±53 mg·L⁻¹·h⁻¹, respectively; P<0.05 for both). Postprandial RE in the Sf<1000 fraction, not total RE in plasma, was also significantly higher in FH patients than in control subjects (24±10 versus 6.3±5.9 mg·L⁻¹·h⁻¹, P<0.05), but treatment with simvastatin did not result in improvement of the postprandial RE response, either in the Sf<1000 fraction or in plasma. It is concluded that heterozygous FH patients have increased fasting and postprandial remnant lipoprotein concentrations. Treatment with simvastatin significantly reduced the fasting and postprandial RLP-C concentrations but did not result in improved postprandial RE response. (Arterioscler Thromb Vasc Biol. 2000;20:2422-2427.)

Key Words: heterozygous familial hypercholesterolemia • remnant lipoproteins • simvastatin

Growing evidence exists that a disturbed plasma triglyceride (TG) metabolism plays an important role in the development of premature atherosclerosis.1,2 Disturbances in TG metabolism are characterized by postprandial accumulation of lipoprotein remnants and have been observed in various populations with elevated cardiovascular risk.3–7 Patients with familial hypercholesterolemia (FH) and disturbances in postprandial lipoprotein metabolism have higher risks for coronary artery disease.8 Animal studies have shown that a deficiency in the LDL receptor is associated with a delayed chylomicron remnant removal.9–11 Castro Cabezas et al12 have demonstrated a small but significant postprandial increase in retinyl palmitate concentration in the chylomicron remnant fraction (Svedberg flotation unit [Sf]<1000) in heterozygous FH patients compared with normolipidemic control subjects. However, Rubinsztein et al13 did not observe any accumulation of vitamin A–labeled chylomicrons, IDL-sized remnants, or chylomicron remnants in homozygous FH patients. Additionally, Kowal et al14 reported that only 5% of the binding capacity of the LDL receptor was needed to remove chylomicron particles, suggesting only a minor role for the LDL receptor in the removal process of lipoprotein remnants.

Several laborious methods, ie, incorporation of vitamin A as core label and high-performance liquid chromatographic analyses of retinyl esters (REs) in isolated lipoprotein fractions or measurements of apoB-48 and apoB-100 concentrations in the different lipoprotein fractions with the use of SDS-PAGE, have been used to study postprandial lipoprotein remnant metabolism. The suitability of vitamin A as a marker

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for chylomicrons and its remnants has been criticized.\textsuperscript{15,16} Incorporation of vitamin A by the enterocyte occurs mostly in the larger sized chylomicron particles in the late postprandial period,\textsuperscript{17} as reflected by the delayed postprandial RE response compared with apoB-48 in the VLDL/chylomicron density fraction. A new remnant lipoprotein method based on the immunosenparation principle (remnant-like particle [RLP] cholesterol [RLP-C assay]) offers the possibility of separating lipoprotein remnant particles with the use of an immunoaffinity gel with coupled monoclonal antibodies against apoB-100 and apoA-I.\textsuperscript{18,19} In the unbound fraction, cholesterol and TG concentrations in apoB-48 particles (chylomicron remnants) and apoE-enriched apoB-100 (\(\beta\)-VLDL and IDL) particles are detected.\textsuperscript{20}

\(\beta\)-Hydroxy-\(\beta\)-methylglutaryl coenzyme A reductase inhibitors (statins) upregulate the LDL receptor and partly inhibit hepatic apoB secretion,\textsuperscript{21} resulting in an increased removal of LDL particles from the circulation, thereby improving the atherogenic lipid profile. Their effects on postprandial remnant metabolism have not been completely established. Most studies showed a tendency to decrease postprandial TG concentrations that appear to correlate with the degree of fasting plasma TG reduction.\textsuperscript{22} Therefore, we investigated postprandial lipoprotein remnant metabolism in heterozygous FH patients and the effect of simvastatin treatment by use of RE analysis and the RLP-C assay for remnant characterization.

Methods

Subjects

The present study is a substudy of the Express multicenter study, in which the safety and efficacy of high-dose simvastatin (80 mg once a day) were assessed in heterozygous patients with FH. Patients were recruited from the lipid clinic of the University Hospital Utrecht. Diagnosis of heterozygous FH was made on the basis of existing hypercholesterolemia (>6.50 mmol/L), the presence of tendon xanthomas, and hypercholesterolemia in at least 1 first-degree relative.\textsuperscript{23} These patients were asked to stop all lipid-lowering drugs at least 8 weeks before the study (washout period). At the end of the washout period, patients were given intravenous injections of heparin, and then blood samples were collected for lipoprotein lipase (LPL) and hepatic lipase (HL) activity measurements. On a separate day, patients were given an oral fat-loading test followed by a 4-month treatment with simvastatin at a dose of 80 mg a day. The oral fat-loading test was repeated at the end of the treatment period. Healthy normolipidemic subjects with fasting plasma cholesterol <6.0 mmol/L and TGs <2.0 mmol/L were recruited by advertisement to match the FH patients in age, sex, body mass index, apoE genotype, and fasting plasma TG concentration. Exclusion criteria included the following: diabetes; hepatic, renal, or thyroid diseases; and a positive family history for cardiovascular diseases and type 2 diabetes mellitus. Control subjects also had an oral fat-loading test. The human investigation review committee from University Hospital Utrecht and a national committee representing the Express multicenter study approved this study protocol, and written informed consent was obtained from all participants.

Oral Fat-Loading Test

After an overnight fast (12 hours), participants were admitted to the metabolic ward at 7:30 AM. Cream (consisting of 40% fat [wt/vol] with a polyunsaturated to saturated fatty acids ratio of 0.06, 0.001% cholesterol [wt/vol], and 2.8% carbohydrates [wt/vol]) was given as a single fat load at a dose of 50 g fat per square meter body surface area. After ingestion of the cream supplemented with 120,000 IU aqueous vitamin A, 10 hourly venous blood samples were collected from an indwelling catheter in the antecubital vein into EDTA-containing tubes. All blood samples, protected from light, were immediately put on ice, centrifuged, and analyzed. During the postprandial period, the subjects were allowed to drink only water or tea without sugar. None of the subjects experienced gastrointestinal complaints after drinking the cream.

Laboratory Measurements

Plasma was obtained by centrifugation at 3000 rpm for 15 minutes at 4°C. Plasma TGs and cholesterol were analyzed in duplicate and measured with an enzymatic colorimetric assay (Monotest cholesterol kit No. 237574 and GPO-PAP No. 701912, Boehringer-Mannheim). LDL cholesterol was calculated with the Friedewald formula\textsuperscript{24} in the control subjects and was determined with ultracentrifugation as described by Redgrave et al\textsuperscript{25} in FH patients. Cholesterol was analyzed in the HDL fraction isolated by the heparin-MnCl\textsubscript{2} dextran-sulfate precipitation method.\textsuperscript{26} Plasma apoE concentrations (in milligrams per liter) and plasma apoC-III concentrations (in milligrams per liter) were determined by a commercial test kit with use of the electroimmunodiffusion technique (coefficient of variation <7.5%, Hydragel LP E, Reference No. 4058, and LP CHI, Sebia Inc). ApoE genotype was determined as described by Dallinga-Thie et al\textsuperscript{27} Plasma for LPL and HL was obtained 20 minutes after intravenous injection of 50 IU/kg heparin. Postheparin LPL activity and HL activity were assayed as described previously.\textsuperscript{28,29} Nonesterified fatty acids (expressed as nanomoles free fatty acids per minute [millimoles per millilitter]) were measured with an enzymatic assay (Wako Chemicals).

Assessment of Lipoprotein Remnants

Lipoproteins were separated in a single ultracentrifugation step by flotation in an Si\textsubscript{1} >1000 fraction (containing chylomicrons, large chylomicron remnants, and large hepatic TG-rich lipoproteins) and a remaining infranatant fraction (Si\textsubscript{1}<1000, containing small chylomicron remnants and all the other lipoproteins).\textsuperscript{30,31} In both fractions, RE concentrations were determined by use of high-performance liquid chromatography as described by Ruotolo et al.\textsuperscript{32} Briefly, 5 \(\mu\)L of serum was added to 300 \(\mu\)L of mixed immunoaffinity gel suspension containing monoclonal anti-human apoA-I (H-12) and anti-human apoB-100 (JH-1) antibodies (Japan Immunoresearch Laboratories). The reaction mixture was gently shaken for 120 minutes at room temperature. After the supernatant was left standing for 15 minutes, 200 \(\mu\)L was withdrawn for the assay of RLP-C. Cholesterol in the RLP fraction (coefficient of variation <3%) was measured by an enzymatic assay with use of an automatic chemistry analyzer (Cobas Mira autoanalyzer, ABX).

Statistical Analysis

Data are presented as mean±SD, unless stated otherwise. Area under the integrated curve (AUC) was calculated by use of data from the first 8 hours after start of the oral fat-loading test for postprandial TG, RE, and RLP-C with GraphPad Prism software (version 3.1). Normality was tested with the Kolmogorov-Smirnov test. If non-normality occurred, data were normalized by logarithmic transformation. The effects of simvastatin treatment were tested by paired Student t test. Comparisons between FH patients and controls were tested by 2-tailed unpaired Student t test. Pearson correlation or Spearman rank correlation was applied to evaluate relationships between parameters. A 2-sided value of \(P<0.05\) was considered to be significant. Statistical analysis was performed with Graphpad InStat version 3.00 for Windows 95 (Graphpad software).

Results

Study Population

Characteristics of the subjects are summarized in Table 1. FH patients and control subjects were normotriglyceridemic. FH patients had significantly increased fasting plasma cholesterol, LDL cholesterol, apoB, and apoE concentrations and decreased HDL cholesterol concentrations compared with control subjects. After simvastatin treatment, plasma chole-
TABLE 1. Characteristics of Patients With FH and Control Subjects

<table>
<thead>
<tr>
<th></th>
<th>FH Simva (−)</th>
<th>FH Simva (+)</th>
<th>Controls</th>
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<tr>
<td>No. of subjects</td>
<td>7</td>
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<td>Male, n</td>
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<td>Age, y</td>
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<td>47.7±6.9</td>
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<td>Body mass index</td>
<td>25.5±1.5</td>
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<td>Cholesterol, mmol/L</td>
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<td>6.34±1.11‡</td>
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<td>TGs, mmol/L</td>
<td>1.39±0.4</td>
<td>1.36±0.4</td>
<td>0.91±0.3</td>
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<tr>
<td>HDL cholesterol, mmol/L</td>
<td>0.94±0.22‖</td>
<td>1.03±0.32</td>
<td>1.51±0.47</td>
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<td>LDL cholesterol, mmol/L</td>
<td>10.28±1.6*†</td>
<td>4.78±0.91*</td>
<td>3.18±0.64</td>
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<tr>
<td>ApoB, mg/L</td>
<td>210±21*†</td>
<td>140±15*‡</td>
<td>92±14</td>
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<td>ApoC-III, mg/L</td>
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<td>21.36±7.44</td>
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<td>ApoE, mg/L</td>
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<td>60.30±12.63‡</td>
<td>43.48±10.10</td>
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<td>LPL activity, mU/mL</td>
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<td>164±48</td>
<td>158±33</td>
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<tr>
<td>HL activity, mU/mL</td>
<td>543±269</td>
<td>398±146</td>
<td>343±174</td>
</tr>
</tbody>
</table>

Values are mean±SD. Simva (−) indicates without simvastatin treatment; Simva (+), with simvastatin treatment.

Postprandial Responses

Postprandial TG Responses

After the fat load, maximal postprandial plasma TG concentrations were reached at 4 hours and were higher in FH patients than in matched control subjects (2.61±0.50 versus 1.66±0.53 mmol/L, P=0.02; Figure 1). The area under the TG curve (AUC-TG) was also significantly higher in FH patients; after correction for baseline plasma TG concentrations, the ∆AUC-TG in FH patients was not significantly different from that in control subjects (Table 2). Simvastatin treatment did not result in the improvement of postprandial plasma TG concentration, AUC-TG, and ∆AUC-TG.

Figure 1. Postprandial TG responses in patients with FH without simvastatin (○) and with simvastatin (●) and the matched control subjects (●). Values are expressed as mean±SEM.

Postprandial RLP-C Responses

Fasting plasma RLP-C concentrations were significantly higher in FH patients than in control subjects (P<0.05, Figure 2). After simvastatin treatment, fasting plasma RLP-C concentrations normalized and were similar to those of control subjects. Fasting RLP-C was correlated positively with baseline plasma cholesterol (r=0.80, P<0.01), TG (r=0.52, P<0.05), LDL cholesterol (r=0.79, P<0.01), apoB (r=0.84, P<0.01), and apoE (r=0.52, P<0.05) concentrations. The maximal postprandial plasma RLP-C concentration was reached between 2 and 4 hours and was significantly higher than that in control subjects (72.15±8.77 versus 18.00±2.63 mmol/L, P=0.004). The area under the RLP-C curve (AUC–RLP-C) and ∆AUC–RLP-C were also higher in treated patients than in control subjects (Table 2). Simvastatin treatment resulted in a significant decrease in maximum

Figure 2. Postprandial RLP-C responses in FH patients without simvastatin (○) and with simvastatin (●) and the matched control subjects (●). Total plasma RLP-C (A) and incremental plasma RLP-C (B) are presented. Values are expressed as mean±SEM.
creased postprandial lipoprotein remnant concentration. The with high-dose simvastatin resulted in a significantly de-
creased postprandial RLP-C response in heterozygous FH patients compared with matched control subjects. Treatment
observed higher fasting RLP-C concentrations and an in-
creased postprandial RLP-C concentration, AUC-RLP-C (P<0.01), and ΔAUC–RLP-C (P<0.05) in the FH patients.

Postprandial RE Response
Maximal postprandial plasma RE concentrations were reached at 4 hours and were higher, albeit not statistically
significant, in FH patients than in control subjects (10.97±2.22 versus 6.18±1.86 mmol/L, Figure 3A). There
was no statistical difference in the area under the RE curve (AUC-RE) between the FH and control subjects (Table 2). Treatment with simvastatin did not decrease the maximal postprandial plasma RE concentrations nor AUC-RE in the FH patients. AUC-RE in the Sf<1000 fraction was correlated,

Figure 3. Postprandial RE response of patients with FH without simvastatin (○) and with simvastatin (●) and the matched control subjects (□). ΔAUC-RE (A) and total plasma RE (B) in the Sf<1000 fraction are presented. Data are expressed as mean±SEM.

**Postprandial RE Response**
Maximal postprandial plasma RE concentrations were reached at 4 hours and were higher, albeit not statistically
significant, in FH patients than in control subjects (10.97±2.22 versus 6.18±1.86 mmol/L, Figure 3A). There
was no statistical difference in the area under the RE curve (AUC-RE) between the FH and control subjects (Table 2). Treatment with simvastatin did not decrease the maximal postprandial plasma RE concentrations nor AUC-RE in the FH patients. AUC-RE in the Sf<1000 fraction was correlated with baseline plasma TG (r=0.61, P<0.05), apoB (r=0.49, P<0.05), and apoE (r=0.53, P<0.05) concentrations. Negative correlation between AUC-RE in the Sf<1000 fraction and plasma HDL cholesterol concentrations was found (r=-0.54, P<0.05). Unlike total REs, maximum postprandial plasma RE concentrations and AUC-RE in the Sf<1000 fraction were significantly higher in FH patients than in control subjects (Figure 3B). However, like total REs, sim-
vastatin treatment did not result in improvement of the postprandial RE in the Sf<1000 fraction.

**Discussion**
In the present study, specific monoclonal antibodies for apoB-100 and for apoA-I have been used to isolate RLPs. We observed higher fasting RLP-C concentrations and an in-
creased postprandial RLP-C response in heterozygous FH patients compared with matched control subjects. Treatment
with high-dose simvastatin resulted in a significantly de-
creased postprandial lipoprotein remnant concentration. The
importance of detecting a disturbed postprandial lipoprotein metabolism in FH patients was recently stressed because these patients had significantly increased risks for coronary artery disease.8,33–37

Hitherto, contradictory results about possible abnormalities in postprandial lipoprotein remnant metabolism in FH pa-
tients have been reported. Different methodologies were used to isolate lipoprotein remnants. Most studies of postprandial lipoprotein remnant metabolism in FH used vitamin A (REs) as a core label for chylomicron particles. Studies involving postprandial lipoprotein remnants with REs as a marker in homozgyous or heterozygous FH patients revealed either abnormalities12,38 or a normal removal of chylomicron rem-
ants.13,31 We confirmed an earlier report by Castro Cabezas et al12 that postprandial chylomicron remnants reflected by REs in the Sf<1000 fraction were elevated in heterozygous FH patients compared with matched control subjects. In the fasting state and the early postprandial period (first 3 hours after a meal), smaller sized chylomicrons (considered to be atherogenic) are secreted. Later in the postprandial period, de novo–formed larger chylomicrons are secreted. It has been shown in vivo that conversion of larger chylomicron particles into smaller-sized remnant particles is a phenomenon that is not occurring very frequently.39 REs are mostly incorporated in larger-sized chylomicron particles. This could be observed in the present study by the delay of RE appearance in the blood. Similar observations were reported for apoB-48 and RE.40 We hypothesize that apoB-48 and RLP-C reflect particles with identical behavior, whereas RE marks the properties of intestinal postprandial lipoprotein particles with a different metabolic behavior.

The results suggest that secretion of larger particles con-
tinued to be abnormal in FH patients even after simvastatin treatment. An in vivo study in rats41 supported the concept that larger chylomicron particles were removed by the liver via alternative pathways involving the LDL receptor–related protein and proteoglycans. This process was not influenced by LDL receptor modulation.42 Therefore, even after high-
dose simvastatin treatment (with its positive effects on plasma cholesterol homeostasis), the peripheral pathway through which Sf<1000 REs were removed was still satu-
rated. In contrast, the removal of the smaller postprandial plasma RLP-C levels decreased after high-dose simvastatin therapy.

Our results showed for the first time increased fasting and postprandial RLP-C concentrations in heterozygous FH pa-
tients despite normal TG concentrations in these patients. It has been recognized that there is a strong correlation between RLP-C and TG concentrations. Therefore, it has been argued that TG measurements are sufficient to estimate remnant concentrations. However, several clinical studies43,44 have demonstrated that in addition to TGs, RLP-C offers indepen-
dent assessment for the risk of coronary heart disease. In the present study, we show that RLP-C and TGs clearly had different postprandial responses to simvastatin treatment; therefore, they are not interchangeable (Figures 1 and 2). Secretion of VLDL apoB-100 was increased, and hepatic removal of VLDL/IDL particles by the liver was decreased in untreated FH patients. As a result, plasma IDL and LDL concentrations are increased in untreated FH. Therefore, the increase in fasting RLP-C levels reflects increasing levels of
circulating IDL-like apoB-100/apoE remnant particles. Because removal pathways are shared by RLP and IDL, accumulation of IDL could be expected when the influx of RLP increased after a fatty meal. Our observed postprandial RLP-C peak is a reflection of this process and is the result of an accumulation of apoB-48 remnant particles and apoB-100/apoE–enriched remnants. The strong association of AUC–RLP-C with baseline plasma apoB is suggestive of this concept. After treatment with simvastatin, postprandial RLP-C concentrations in FH patients were comparable to those observed in control subjects. As a result of simvastatin intervention, hepatic secretion of precursor lipoproteins, which eventually will become IDL/LDL-like particles, decreased, whereas catabolism of apoB-containing particles increased, leading to less accumulation. The role of apoE in this process is less clear. Elevation of apoE levels in patients with FH has been reported previously.

More extensive studies are required to analyze the effect of simvastatin treatment on apoE in FH.

In conclusion, heterozygous FH patients have a disturbed postprandial lipoprotein metabolism. After simvastatin treatment, the postprandial RLP-C response was decreased toward that of matched control subjects. No differences were observed in the postprandial plasma response after treatment. This observation stresses the importance of the different approaches for analysis of postprandial lipoprotein remnant metabolism. Additionally, the response of lipoprotein remnants that dominate the early postprandial period could be modulated by simvastatin treatment, whereas the “later” and larger plasma chylomicron particle concentrations continued to be elevated. Improvement of the RLP-C response after simvastatin treatment in FH reduces the postprandial atherogenicity of plasma in addition to lowering LDL cholesterol.

Acknowledgments

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References


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