Magnitude of HDL Cholesterol Variation After High-Dose Atorvastatin Is Genetically Determined at the LDL Receptor Locus in Patients With Homozygous Familial Hypercholesterolemia

Andrei C. Sposito, Sophie Gonbert, Eric Bruckert, Marielle Atassi, Isabel Beucler, Sabine Amsellem, Oumayma Khallouf, Pascale Benlian, Gerard Turpin

Objective—The combination of LDL apheresis with high doses of a potent hepatic hydroxymethylglutaryl coenzyme A reductase inhibitor, such as atorvastatin, has been the best therapy available for the prevention of cardiovascular disease in patients with homozygous familial hypercholesterolemia (HFH). However, some concerns have been made about the effect of atorvastatin on HDL cholesterol levels in these patients.

Methods and Results—HDL cholesterol levels were determined bimonthly over the course of 2 years of treatment with high-dose atorvastatin in genotypically defined HFH patients either receptor-defective (n=6) or receptor-negative (n=6) under long-term treatment with LDL apheresis. We additionally stratified the atorvastatin effect on HDL cholesterol according to the genotype as an indicator of residual in vivo LDL receptor activity. Our findings indicate that (1) an early and transitory reduction of plasma HDL cholesterol levels occurs during the first 4 weeks of atorvastatin treatment; (2) the degree of the transient HDL reduction is higher in receptor-negative than in receptor-defective patients (−21±11 versus −10±4%; P=0.01); and (3) after long-term treatment, HDL cholesterol concentration remains higher in receptor-defective than receptor-negative patients (P=0.026).

Conclusions—The present study reveals that HDL cholesterol reduction after high-dose atorvastatin is an early and transient event in HFH patients which magnitude depends on the presence of a residual LDL-R activity. (Arterioscler Thromb Vasc Biol. 2003;23:2078-2082.)

Key Words: hypercholesterolemia ¡ prevention ¡ follow-up studies

Familial hypercholesterolemia is a genetic disorder featuring a functional defect in the cellular uptake of apolipoprotein (apo) B-containing lipoproteins by LDL receptors (LDLR-R), which is caused by dominantly inherited mutations at the LDL-R locus.1 Patients with homozygous familial hypercholesterolemia (HFH) display extremely high levels of circulating, cholesterol-rich, apoB-containing lipoproteins and, as a consequence, manifest premature cardiovascular disease and mortality in childhood or early adulthood.1 The combination of LDL apheresis with high doses of a potent hepatic hydroxymethylglutaryl coenzyme A reductase inhibitor, such as atorvastatin, is therefore critical for the prevention of cardiovascular disease in HFH patients. However, there has been concern about the effect of atorvastatin at high doses on plasma HDL cholesterol levels in HFH patients. Indeed, contradictory reports exist in terms of HDL cholesterol variation after high-dose atorvastatin, which may result from differences in the duration of the statin treatment and as well from distinct FH phenotypes.2–6 A long-term assessment of HDL cholesterol variation in well-defined receptor-negative and receptor-defective HFH patients treated with high-dose atorvastatin is a unique opportunity to shed light on this issue. With such a study design, we show that plasma HDL cholesterol reduction after high-dose atorvastatin in FH patients is a transient phenomenon and that the type of allele at the LDLR locus and its ability to preserve a residual LDL-R activity influences the magnitude of this effect.

Methods

Twelve patients (3 male and 9 female, age 10 to 35 years) with molecular diagnosis of HFH in whom LDL apheresis was regularly performed once every 2 weeks were enrolled in this study. All patients were included for the study when they were proven carriers of deleterious mutations on both their LDLR alleles after genomic DNA analysis.7 In addition, LDL-R phenotype was assessed in 9
patients on the functional ability of their cultured skin fibroblasts to take up LDL in vitro. All patients were on the NCEP Step One diet and regular LDL-apheresis therapy for more than 1 year before inclusion in the study. Atorvastatin was given at 80 mg/d for all adult patients and at 40 mg/d for 2 receptor-defective patients (10 and 12 years old) and for 2 receptor-negative patients (10 and 12 years old) in an open, single-group trial of 2 years. No other lipid-lowering therapy was administered. Once every 2 weeks, before LDL apheresis, the patients were examined and their plasma levels of total cholesterol and triglycerides were determined enzymatically (KoneLab), as described elsewhere. Plasma HDL cholesterol concentration was determined by the same method as for total cholesterol, after LDL and VLDL precipitation with magnesium phosphotungstate. LDL cholesterol was estimated by the Friedewald formula. Because we followed 4 juveniles (10 to 12 years old), a 12-hour fast was not regularly obtained during the follow-up. LDL apheresis was carried out using either the affinity chromatography with a dextran sulfate–cellulose column technique (Liposorber System MA-01; Kaneka Corporation) or by the direct adsorption of lipoproteins (Fresenius Corporation). Routine safety parameters were regularly determined in all patients.

Data are expressed as mean ± SD. Mean values and percentage variation of lipid values during the treatment were analyzed using the Friedman test for repeated measures. The comparison between the baseline and specific time points was performed by Wilcoxon signed-ranks test. Patients were defined as receptor-negative if they were carriers on both LDLR alleles of a nonsense or frameshift mutation, whereas receptor-defective patients were defined by the presence of missense mutations on both alleles. The comparison of continuous variables between receptor-defective and receptor-negative groups was performed using Mann-Whitney test. The juveniles and adults enrolled in the current study were analyzed as 1 group, as there was no significant difference in the variation of lipid levels after treatment between the 4 juveniles (10 to 12 years of age) on apheresis at 40 mg/d and the 8 adults on 80 mg/d. All P values were 2-tailed, and P < 0.05 was considered significant. SPSS statistical software (Chicago), version 8.0, was used for statistical analysis.

Results
There was no significant heterogeneity between receptor-negative and receptor-defective patients with respect to age (16 ± 6 versus 20 ± 10 years; P = 0.6, respectively), male/female sex distribution (1/5 versus 2/4; P = 0.3, respectively), or mean baseline values for plasma HDL cholesterol (38 ± 12 versus 39 ± 7 mg/dl; P = 0.7, respectively) or triglycerides (123 ± 44 versus 82 ± 38 mg/dl; P = 0.2, respectively) during the period of LDL-apheresis therapy (Table 1). As expected, baseline LDL cholesterol tended to be higher in receptor-negative patients, but the difference did not reach statistical significance (474 ± 110 versus 351 ± 87 mg/dl; P = 0.11). Most studied patients were in LDL apheresis by the direct adsorption of lipoprotein technique (n = 10); the dextran sulfate–cellulose column technique was used in only 1 receptor-negative patient and 1 receptor-defective patient (P = 1.0). During treatment, no patient had clinical or biochemical signs of liver or muscular toxicity. The Figure depicts the variation of plasma HDL cholesterol over a 2-year period of treatment with high-dose atorvastatin. There was a transitory fall in plasma HDL cholesterol levels in the first 4 weeks of treatment (−16 ± 10%; 95% CI, −1 to −22; P for trend <0.0001). After 3 months of treatment, there was a subsequent increase in plasma HDL that achieved a mean value similar to baseline (−3 ± 13%; 95% CI, −3 to 14; P = 0.13). Interestingly, when looking at individual variation, the same pattern of fluctuation in HDL cholesterol was observed for almost all patients, ie, fall at week 4 (11 patients, 92%) and rise by month 3 (10 patients, 83%) of treatment.

No such pattern of fluctuation was observed for plasma LDL cholesterol or triglyceride levels. In fact, when compared with baseline values, there was a significant reduction in LDL cholesterol after 4 weeks of treatment (−9 ± 6%; 95% CI, −13 to −5; P = 0.005), which was maintained after 12 weeks (−14 ± 8%; 95% CI, −18.9 to −9.2; P = 0.002) and after 2 years (−14 ± 8; 95% CI, −20 to −10; P = 0.002). No significant variation in triglyceride levels was observed after 4 weeks (−2 ± 24%; 95% CI, −17 to 13; P = 0.93), 12 weeks (−10 ± 28; 95% CI, −8 ± 28; P = 0.21), or 2 years (−11 ± 25; 95% CI, −26 to 5; P = 0.19).

Because we found a higher LDL-R activity in receptor-defective patients compared with receptor-negative patients (12.6 ± 8% versus <2%; P = 0.04), we compared both groups, aiming to investigate the influence of a residual in vivo LDL-R activity on plasma HDL cholesterol variation. Both receptor-negative (P for trend = 0.001) and receptor-defective (P for trend = 0.06) groups had a similar pattern of HDL cholesterol fluctuation during the follow-up. However, as indicated in the Figure, the reduction of HDL cholesterol levels at week 4 was 2 times greater in receptor-negative patients than in receptor-defective counterparts (−21 ± 11 versus −10 ± 4%; P = 0.009). This difference was maintained at 3 months (−11 ± 10 versus 2 ± 14%; P = 0.13) and at 2 years (−10 ± 3 versus 5 ± 12%; P = 0.02). No significant change was observed in the comparison between HDL cholesterol levels at baseline and those at 2 years in both groups. The reduction in LDL cholesterol was 2 times greater in receptor-defective than in receptor-negative patients at the third month (−20 ± 6 versus −10 ± 5%; 95% CI, −16 to −1; P = 0.045), and this difference was maintained at the same

### TABLE 1. Clinical and Biological Characteristics in FH Patients

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Sex</th>
<th>Before LDL Apheresis</th>
<th>LDLR Mutation</th>
<th>LDL-R Allele†</th>
<th>Maximal TC 4 weeks after LDL Apheresis</th>
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Plasma lipid and lipoprotein levels are expressed in mg/dL (for values in mmol/L, multiply by 0.02586).

*As defined by genotype.
†Cases in whom 2 mutations were found in cis (ie, E256K + I402T and G524R + T705I) on the same chromosome by segregation analysis.
degree until the end of the 2-year follow-up (−21±7 versus −11±5%; 95% CI, −17 to −2; P=0.015). There was no short- or long-term difference in triglyceride level variation between the 2 groups.

Discussion
The present study shows, for the first time, the time-dependent and LDL-R phenotype-dependent effect of high-dose atorvastatin on HDL cholesterol levels in patients with

Bars = Standard Error
Time course of the mean percentage variation of plasma HDL cholesterol under high-dose atorvastatin in homozygous FH patients with defective (●) or negative (★) LDLR alleles.

### TABLE 2. Lipid and Lipoproteins Plasma Levels Before and 24 Months After High-Dose Atorvastatin Under LDL Apheresis

<table>
<thead>
<tr>
<th>Case</th>
<th>LDL Cholesterol</th>
<th>Triglycerides</th>
<th>HDL Cholesterol</th>
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Plasma lipid and lipoprotein levels are expressed in mg/dL (for values in mmol/L, multiply by 0.02586).
HFH. Our major findings are that (1) an early and transitory reduction of HDL cholesterol levels occurs during the first weeks of atorvastatin treatment in HFH patients; (2) the degree of the transitory reduction is greater in receptor-negative patients than in receptor-defective patients; and (3) after long-term treatment, HDL cholesterol concentration remains higher in receptor-defective than in receptor-negative patients as a function of residual LDL-R activity in receptor-defective HFH patients.

Consistent with previous reports,5–6 we did not find a significant variation in mean HDL cholesterol levels after long-term treatment of HFH patients with high-dose atorvastatin. However, we confirmed in those patients a short-term reduction in HDL cholesterol levels that was reported in heterozygous FH patients,3 with the nadir after the fourth week of treatment. This initial reduction was followed by a gradual raise in HDL cholesterol levels, and by the third month of treatment, the levels were no longer significantly different from baseline. Thus, our findings explain the apparent divergence between short- and long-term studies in terms of the effect of high-dose atorvastatin on HDL cholesterol in FH patients.2–6

The mechanisms underlying such a transitory effect on HDL cholesterol levels are unknown but could be partially related to an upregulation of liver cholesterol transporters, which may compensate for statin-induced cellular cholesterol deprivation. In fact, studies in human cells and in animal models have shown that decreases in cellular cholesterol levels, whether resulting from inhibition of hepatic hydroxymethylglutaryl coenzyme A reductase or increase in cholesterol consumption, stimulate the synthesis and expression of cholesterol transporters, such as scavenger receptor class B, type I.9,10 By mediating selective cholesterol uptake from HDL, these transporters might contribute to liver cholesterol replenishment while reducing plasma HDL cholesterol.11 In the present study, the initial reduction in HDL cholesterol levels was 2 times greater in receptor-negative than in receptor-defective HFH patients, indicating that such an effect on HDL cholesterol is proportional to the degree of LDL-R deficiency. Moreover, a significant difference in LDL and HDL cholesterol levels was observed at the end of the follow-up between patients with a receptor-negative and those with a receptor-defective phenotype. Thus, a combination of a residual LDL-R activity and activity of other cellular cholesterol transporters should influence the degree of the transitory fall in HDL cholesterol and steady-state levels of HDL and LDL cholesterol during the treatment with high-dose atorvastatin in HFH patients. This mechanistic hypothesis, however, requires additional studies to be confirmed.

Intravascular remodeling of HDL particles may also be a mechanism potentially involved in the atorvastatin effect on HDL cholesterol levels in HFH patients. Metabolic steps such as triglyceride hydrolysis by hepatic lipase (HL) and interchange of triglyceride and cholesterol ester between HDL and other lipoproteins by cholesteryl ester transfer protein (CETP) are globally implicated in the multistep process of HDL intravascular remodeling. In this context, it was recently demonstrated in diabetic subjects that atorvastatin induces a dose-dependent decrease in HL activity, favoring the increase in HDL cholesterol levels. Atorvastatin therapy also induces a dose-dependent decrease in both CETP mass and activity, which also favors the increase in HDL cholesterol levels. Moreover, it was recently shown in normolipidemic male New Zealand White rabbits that atorvastatin induces a small increase in apoAI production, but such an effect is offset by a greater effect of accelerating the clearance of HDL apoAI. Thus, these recent evidences suggest that the short-term effect of atorvastatin at higher doses is likely to be caused by enhanced HDL catabolism, which is not entirely compensated by a concomitant increase in apoAI synthesis and inhibition of HL and CETP. Despite the uniqueness of the HFH patients enrolled in the current study, our findings are in line with this assumption.

In contrast with HFH patients, long-term studies with high-dose atorvastatin have demonstrated a significant increase in HDL cholesterol levels in patients with polygenic hypercholesterolemia. In short-term studies, however, the increase in HDL cholesterol levels after high-dose atorvastatin is blunted or absent in these subjects. Interestingly, in subjects with enhanced cellular cholesterol turnover, such as those with acute coronary syndromes, treatment with high doses of atorvastatin also promotes a short-term reduction in HDL cholesterol followed by a slow normalization. Potentially, the effect of high-dose atorvastatin on HDL cholesterol levels of these patients could also be related to the degree of cellular cholesterol deprivation that occurs on the initiation of statin therapy. However, the exceptionality of HFH patients precludes generalization of the present findings, and additional studies are required to verify this hypothesis.

The present study has some limitations. First, the wide range of HFH genotypes precluded examining different responses to high-dose atorvastatin as a function of specific LDLR gene mutations. Second, because we enrolled 4 juvenile (10 to 12 years old), it was not possible to administrate atorvastatin 80 mg/d for all enrolled patients. However, these young patients displayed a pattern of HDL cholesterol fluctuation similar to that observed in the adult patients using the higher dosage of atorvastatin.

In conclusion, the present study demonstrates that HDL cholesterol reduction after high-dose atorvastatin is a transitory event in HFH patients and that the degree of this reduction is a function of the in vivo LDL-R activity.

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References


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