Fluvastatin Lowers Atherogenic Dense Low-Density Lipoproteins in Postmenopausal Women With the Atherogenic Lipoprotein Phenotype

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Background—Although HMG-CoA reductase inhibitors (HMGRIs) are effective lipid-lowering agents, it remains controversial whether these agents also lower dense LDL (dLDL), a predominance of which is considered to contribute to the atherogenicity of the metabolic syndrome.

Methods and Results—In a multicenter, double-blind, randomized, placebo-controlled study, we determined the effect of the HMGRI fluvastatin on lipids, apolipoproteins, and LDL subfractions (by equilibrium density gradient ultracentrifugation). A total of 52 postmenopausal women with combined hyperlipidemia and increased dLDL were treated with either fluvastatin 40 mg/d (n = 35) or placebo (n = 17). After 12 weeks’ treatment, significant reductions (P < 0.001) in total cholesterol (−19%), IDL cholesterol (−35%), LDL cholesterol (−23%), apolipoprotein B (−21%), and apolipoprotein B in dLDL (−42%) were apparent among fluvastatin recipients. No significant changes in triglycerides or HDL cholesterol were observed. The effect of fluvastatin on dLDL was correlated with baseline values. There was no consistent relationship, however, between the effect of fluvastatin on triglycerides and the decrease in dLDL.

Conclusions—Fluvastatin lowers total and LDL cholesterol and the concentration of dLDL. This profile may contribute to an antiatherogenic effect for fluvastatin that is greater than expected on the basis of changes in lipids and apolipoproteins. (Circulation. 2001;103:1942-1948.)

Key Words: fluvastatin ■ coronary disease ■ lipoproteins

Low-density lipoprotein (LDL) comprises a heterogeneous group of particles, of which the dense subfraction (dLDL) is considered most atherogenic.1–3 A link between dLDL and increased risk of coronary heart disease (CHD) was first proposed by Austin and coworkers.4 Subsequent case-control and prospective studies have shown that a predominance of dLDL increases the risk of CHD by up to 7-fold.5–12 Evidence for a role of dLDL in CHD also comes from angiographic trials. In the St Thomas’ Atherosclerosis Regression Study, patients showing regression of coronary atherosclerosis had low concentrations of dLDL during treatment.13 Dense LDLs are associated with other components of the metabolic syndrome, particularly elevated triglycerides and low HDL cholesterol (HDL-C). The independent contribution of dLDL to the risk of CHD has therefore been difficult to determine. Thus, LDL subclass profile remained predictive of CHD after adjustment for triglycerides or HDL in some9,11 but not all studies.6–8,10,12 Very recently,14 dLDLs were shown to cause endothelial dysfunction independent of LDL cholesterol (LDL-C), triglycerides, and HDL-C. Clearly, any therapeutic principle that lowers LDL-C and triglycerides and raises HDL-C might therefore be enhanced by a reduction in dLDL.

The effects of HMG-CoA reductase inhibitors (HMGRIs) on LDL subfractions have been studied by gradient gel electrophoresis (GGE) and ultracentrifugation.15–24 Providing a coarse estimate of the size distribution of LDL rather than the concentration of dLDL, GGE did not reveal increases of the average LDL diameter during HMGRI treatment.16,19,20,25 Some of the ultracentrifugation-based studies showed a decrease of the concentration of dLDL.15,19 on HMGRIs; others did not.18,19,22–24 It is thus controversial whether these agents lower dLDL. The HMGRI fluvastatin offers a wide spectrum of clinical benefits in various patient subgroups, including the elderly.26 We therefore examined the effect of short-term therapy with fluvastatin on dLDL levels in patients at increased risk of CHD, namely, postmenopausal women with an atherogenic lipoprotein profile.


Methods

Study Design
This was a double-blind, randomized, placebo-controlled, 20-week study comprising an 8-week run-in phase followed by an active treatment period of 12 weeks, conducted at 10 sites in Germany. The study protocol was approved by the Ethics Committee of the University of Freiburg and the institutional review boards at each study site. All patients gave informed, written consent.

Patients
A total of 52 postmenopausal women (≥12 months since last menstrual period; levels of follicle-stimulating hormone >28 IU/L), 44 to 75 years old, with LDL-C $150 mg/dL, triglycerides >120 mg/dL, and apolipoprotein (apo) B in dLDL (LDL-5+LDL-6) >25 mg/dL, participated. Major exclusion criteria were LDL-C ≥300 mg/dL; triglycerides ≥500 mg/dL; acute myocardial infarction within 3 months of study commencement; insulin-dependent diabetes mellitus or poorly controlled non–insulin-dependent diabetes mellitus (glucose >150 mg/dL or HbA1c >8%); severe obesity; overt liver disease; chronic renal failure; myopathy; alcohol or drug abuse; several other significant diseases; known hypersensitivity to HMGCRIs; or use of other lipid-lowering therapy, immunosuppressants, erythromycin and/or neomycin, ketoconazole, and hormone-replacement therapy.

Patients commenced an 8-week run-in period, during which previous lipid-lowering therapy was discontinued. Dietary advice was provided according to the American Heart Association Step I diet, and patients were requested to maintain smoking habits, physical activity, and alcohol consumption. After the run-in phase, patients were randomized to receive either fluvastatin 40 mg every evening (n=35) or placebo (n=17) for 12 weeks. A randomization ratio of 2:1 was selected to reduce the number of patients receiving the potentially inferior treatment. At week 6, concomitant diseases, adverse events, and compliance (capsule counting) were recorded, and at week 12, laboratory assessments and physical examinations were repeated.

Laboratory Assessments
Two weeks before randomization (baseline) and after 12 weeks' active treatment, fasting venous blood samples were drawn. Serum samples were stored up to 1 week at 4°C before lipoprotein subfractionation. Previous experiments indicated that the lipid and lipoprotein measurements were not affected by storage at 4°C for 1 week. All laboratory assessments were performed centrally at the Department of Medicine, University of Freiburg, Germany.

Lipids and Apolipoproteins
Cholesterol, triglycerides, and phospholipids were measured with enzymatic methods, calibrated with secondary standards from Roche Diagnostics. ApoB was determined by kinetic nephelometry (Behring), standardized by reference to the Centers for Disease Control standard. Lipid and apoB measurements had coefficients of variation of <5%.

Lipoproteins and Lipoprotein Subfractions
Quantitative lipoprotein electrophoresis was used to estimate LDL-C before subfractionation at week -2. VLDL (d<1.0063 kg/L), IDL (1.0063<d<1.019 kg/L), LDL (1.019<d<1.065 kg/L), and HDL (1.065<d<1.21 kg/L) were isolated by ultracentrifugation. LDL was subsequently fractionated into 6 density classes by equilibrium density gradient centrifugation. LDL subfractions were quantified by measurement of lipids and apoB. The coefficients of variation for the analysis of cholesterol, free cholesterol, phospholipids, and apoB in LDL subfractions were <7% (and <10% in the case of triglycerides). Radii of LDL subfractions were calculated as described.

Statistical Methods
Contingency tables were analyzed by Fisher’s exact test. Intraindividual changes between baseline and week 12 were calculated and compared between treatment groups by Student’s t test. Bivariate correlations were analyzed by Pearson’s correlation coefficients. A value of P<0.05 was considered significant.

Results
A total of 162 women were included in the dietary run-in period, of whom 52 (32%) met the inclusion criterion regarding apoB in dLDL (≥25 mg/dL) and were randomized. All randomized patients completed the study according to protocol. Overall, the 2 treatment groups were comparable in terms of clinical characteristics and baseline lipid and lipoprotein concentrations (Table 1). There was no significant difference in the changes of body mass index between groups (P=0.382).

Patients were recruited from 10 centers, 1 of which was overrepresented (providing 11 and 6 patients in the fluvastatin and placebo groups, respectively). Exclusion of patients at this center from the statistical analyses did not affect the study findings.

Changes in Lipids, Lipoproteins, and Apolipoproteins
After 12 weeks’ treatment, significant reductions in total cholesterol, LDL-C, and total apoB were observed among fluvastatin recipients compared with placebo (Table 2). Triglycerides and HDL-C did not change significantly.

Major ApoB-Containing Lipoproteins
No significant changes in VLDL constituents were observed (Table 3). More marked changes were apparent for IDL. With the exception of triglycerides (decrease by 9%), all constituents of IDL decreased by ≥35%, with apoB in IDL decreasing by almost 30% (Table 3). With regard to LDL, total

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Fluvastatin (n=35)</th>
<th>Placebo (n=17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y (mean±SD)</td>
<td>66±7 66±6</td>
<td>66±6 66±6</td>
</tr>
<tr>
<td>Body mass index, baseline, kg/m² (mean±SD)</td>
<td>27.1±2.9 26.7±3.1</td>
<td>26.7±2.9 26.6±3.2</td>
</tr>
<tr>
<td>Coronary heart disease, n (%)</td>
<td>10 (29) 4 (24)</td>
<td>0 0</td>
</tr>
<tr>
<td>Peripheral vascular disease, n (%)</td>
<td>1 (3) 0</td>
<td>0 0</td>
</tr>
<tr>
<td>Cerebrovascular disease, n (%)</td>
<td>0 1 (6)</td>
<td>0 0</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>3 (9) 3 (18)</td>
<td>3 (9) 3 (18)</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>3 (9) 2 (12)</td>
<td>2 (12) 2 (12)</td>
</tr>
<tr>
<td>Current smoker, n (%)</td>
<td>2 (6) 2 (12)</td>
<td>2 (12) 2 (12)</td>
</tr>
</tbody>
</table>

*LDL-5+LDL-6 fraction.
cholesterol decreased by 23%, and apoB in LDL decreased by 24%; similar changes were observed for esterified and non-esterified cholesterol and phospholipid content (Table 3). Relative to placebo, the effect of fluvastatin on LDL triglycerides (−14%) was not statistically significant. No significant changes in particle radius were apparent for VLDL, IDL, or LDL during fluvastatin therapy (Table 3).

**LDL Subfractions**

Among LDL subfractions, the most marked changes were observed in LDL-5 and LDL-6 (Figure 1). Fluvastatin lowered lipids and apoB in LDL-5 by 40% to 45% (Table 4). In LDL-6, the respective reductions ranged from 36% to 42%, with the exception of triglycerides, which decreased by 25% (Table 4). For dLDL (dLDL-5 + dLDL-6), relative changes were 39% to 44% for apoB, cholesterol, and phospholipids and 33% for triglycerides (Table 4). Less marked (but statistically significant) changes in the concentration of LDL-4 were also observed, whereas the lightest subfractions (LDL-1 and LDL-2) remained unaffected (Figure 1).

**Correlation Analysis**

The decrease in LDL-6 apoB was most closely related to the levels of LDL-6 apoB before treatment ($r = -0.878, P < 0.001$), ie, the higher the baseline LDL-6 apoB level, the greater the reduction evoked by fluvastatin (Figure 2). Total

**TABLE 2. Effect of Fluvastatin (40 mg/d) on Mean (± SD) Lipids and ApoB Levels**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Fluvastatin (n=35)</th>
<th>Placebo (n=17)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Study End</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>331±43</td>
<td>267±44</td>
</tr>
<tr>
<td>LDL-C</td>
<td>174±28</td>
<td>134±30</td>
</tr>
<tr>
<td>HDL-C</td>
<td>47±9</td>
<td>50±9</td>
</tr>
<tr>
<td>Total triglycerides</td>
<td>227±67</td>
<td>217±104</td>
</tr>
<tr>
<td>Total apoB</td>
<td>142±23</td>
<td>111±21</td>
</tr>
</tbody>
</table>

Values are mg/dL.

**TABLE 3. Effect of Fluvastatin (40 mg/d) on Mean (± SD) Composition and Particle Radius of ApoB-Containing Lipoproteins**

<table>
<thead>
<tr>
<th>Lipoprotein/Constituent</th>
<th>Fluvastatin (n=35)</th>
<th>Placebo (n=17)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Study End</td>
</tr>
<tr>
<td>VLDL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>45.4±17.9</td>
<td>39.6±22.6</td>
</tr>
<tr>
<td>Nonesterified cholesterol</td>
<td>16.6±5.4</td>
<td>13.9±6.8</td>
</tr>
<tr>
<td>Esterified cholesterol</td>
<td>28.3±13.2</td>
<td>25.9±16.7</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>133.9±47.1</td>
<td>127.0±69.8</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>44.7±16.6</td>
<td>38.5±21.4</td>
</tr>
<tr>
<td>ApoB</td>
<td>12.0±3.6</td>
<td>11.3±5.7</td>
</tr>
<tr>
<td>Particle radius</td>
<td>16.4±0.9</td>
<td>16.3±1.1</td>
</tr>
<tr>
<td>IDL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>25.1±7.7</td>
<td>16.2±6.7</td>
</tr>
<tr>
<td>Nonesterified cholesterol</td>
<td>7.0±2.3</td>
<td>4.4±1.8</td>
</tr>
<tr>
<td>Esterified cholesterol</td>
<td>18.1±5.5</td>
<td>11.9±5.1</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>20.1±6.3</td>
<td>18.3±10.2</td>
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<tr>
<td>Phospholipids</td>
<td>17.8±4.7</td>
<td>12.9±4.7</td>
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<tr>
<td>ApoB</td>
<td>9.4±2.8</td>
<td>6.6±2.4</td>
</tr>
<tr>
<td>Particle radius</td>
<td>12.3±0.5</td>
<td>12.4±0.7</td>
</tr>
<tr>
<td>LDL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>174.0±27.7</td>
<td>134.2±30.2</td>
</tr>
<tr>
<td>Nonesterified cholesterol</td>
<td>39.6±8.9</td>
<td>29.7±8.5</td>
</tr>
<tr>
<td>Esterified cholesterol</td>
<td>135.3±20.0</td>
<td>105.3±22.8</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>37.6±12.2</td>
<td>32.4±14.0</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>18.2±106.8</td>
<td>16.6±81.2</td>
</tr>
<tr>
<td>ApoB</td>
<td>106.8±16.0</td>
<td>81.2±19.1</td>
</tr>
<tr>
<td>Particle radius</td>
<td>9.7±0.2</td>
<td>9.9±0.4</td>
</tr>
</tbody>
</table>

Values are mg/dL except for particle radius (nm).
An inverse correlation between triglycerides and dLDL was described previously. We therefore examined whether changes in triglycerides and LDL subfractions during fluvastatin therapy (n=23; mean reduction 27%) did changes in LDL-6 apoB correlate significantly with changes in triglycerides (r=−0.403, P=0.016) and VLDL apoB (r=−0.589, P<0.001) were weaker, albeit significant, predictors of the change in LDL-6 apoB. Interestingly, positive correlations were obtained between apoB in LDL-2 to LDL-4 and the change in LDL-6 apoB, ie, the higher the apoB level in these fractions, the smaller the change in LDL-6 apoB during fluvastatin therapy (Figure 2).

Changes in Triglycerides and LDL Subfractions

An inverse correlation between triglycerides and dLDL was described previously. We therefore examined whether the decrease in dLDL was associated with changes in triglycerides. The overall correlation between change in triglycerides and change in LDL-6 apoB was weak (r=−0.101). Only in those patients showing triglyceride reductions during fluvastatin therapy (n=23; mean reduction 27%) did changes in LDL-6 apoB correlate significantly with changes in triglycerides (r=0.655, P=0.001). The remaining patients (n=12) showed a mean increase in triglycerides of 50%, but experienced a substantial decrease in LDL-6 apoB as well (average of −19.6 mg/dL, compared with −5.7 mg/dL in those patients showing a decrease in triglycerides).

**Safety and Tolerability**

Fluvastatin was well tolerated, and there were no safety concerns. Mean serum levels of total bilirubin, transaminases, alkaline phosphatase, γ-glutamyl transferase, and creatine phosphokinase did not change.

**Discussion**

Of available lipid-lowering agents, it is generally believed that only fibrates and niacin have the potential to alter the distribution of LDL subclasses. Indeed, most reports using GGE or ultracentrifugation to study the effects of...
HMGRIs on the distribution of LDL subfractions have been negative. Two ultracentrifugation-based reports indicated that HMGRIs lowered dLDL. For fluvastatin, 2 negative studies have been published. Given the inconsistency of these investigations, we examined the effect of fluvastatin on dLDL in patients at increased risk of CHD with an atherogenic lipoprotein profile. Because we were aware that an atherogenic lipoprotein profile appears to have a greater impact on cardiovascular risk in women than in men, the study was performed in postmenopausal women.

Our major finding was that fluvastatin decreased dLDL by ~40%, an effect that was almost twice the change in total LDL-C (~23%). Buoyant LDL subfractions did not change, whereas IDL (which includes atherogenic remnants of triglyceride-rich lipoproteins) decreased by ~30%. In designing the study, we expected that substantial reductions in dLDL would be seen in individuals exhibiting high concentrations of dLDL at baseline. Hence, we selectively recruited women with apoB in dLDL >25 mg/dL, which approximately corresponds to the median in postmenopausal women (unpublished observations). This assumption was confirmed in the present study.

Our findings contrast with the general literature pertaining to the effect of HMGRIs on LDL subfractions. There are subtle differences, however, between the present and previous studies. For example, most studies derived LDL subfraction distribution from measurements of LDL peak particle size by GGE. Similarly, we were unable to detect a significant increase in the mean size of whole LDL when particle radius was calculated according to a previously published algorithm. This is surprising, given the substantial reduction in dLDL, but consistent with theoretical considerations. Using the concentrations and particle radii of all LDL subfractions, we calculated the expected radii for whole LDL at baseline and at week 12 as weighted means of the individual LDL subfractions. This yielded particle radii of 9.8 and 9.9 nm, respectively, at these time points, values close to those determined empirically (9.7 and 9.9 nm, respectively; Table 3). Provided that there are no substantial changes in the sizes of individual subfractions, it cannot therefore be ex-
pected that the changes of LDL-5 and LDL-6 in the present study would translate into more than a small change of the average size of the whole LDL fraction. Other studies that used ultracentrifugation for LDL subfractionation did not specifically select patients on the basis of an atherogenic lipoprotein profile. In this context, it is noteworthy that 3 GGE-based studies that included such patients were small (≤12 patients) and may therefore have been underpowered. The difference in findings between the present investigation and other ultracentrifugation-based studies may have methodical reasons. If ultracentrifugation is not performed until complete equilibrium is reached (as in the present study), separation may be driven not only by density but also by particle size. Other explanations may relate to the study design. For example, most studies that used ultracentrifugation failed to specifically select for individuals with elevated dLDL at baseline, and some lacked placebo groups.

The mechanisms by which fluvastatin selectively decreased the most atherogenic lipoprotein fractions, IDL, and dLDL, remain elusive. One potential mechanism relates to the effect of HMGRIs on the release of triglyceride-rich particles in the liver, because an inverse correlation exists between dLDL and triglycerides. Surprisingly, however, there was no consistent relationship between changes in triglycerides and dLDL for the overall patient population, ruling out the possibility that alterations in VLDL metabolism (eg, reduced secretion of VLDL or high lipoprotein lipase activity) fully accounted for the changes in dLDL. The latter finding contrasts with those of Tilly-Kiesi, who reported decreases in dLDL on lovastatin only in those individuals who responded with decreases in triglycerides.

Treatment with fluvastatin stimulates the expression of hepatic LDL receptors, which also catabolize IDL. The most obvious explanation for the decrease in IDL in the present study is therefore that these lipoproteins are taken up by LDL receptors at an enhanced rate during fluvastatin therapy. The change in dLDL is less easily explained, because these particles are poor ligands of LDL receptors and other, less evident, mechanisms might be active. One possible mechanism, an altered rate of transfer of cholesteryl esters from HDL to apoB-containing lipoproteins, was ruled out by the finding that activity of cholesteryl ester transfer protein was unchanged during fluvastatin therapy (not shown). Another potential mechanism focuses on hepatic lipase, which has been implicated in the generation of dLDL. In individuals showing high concentrations of triglyceride-rich lipoproteins, transfer of triglycerides (in exchange with cholesteryl esters) into LDL and HDL may occur; these triglycerides may then be hydrolyzed by hepatic lipase, leading to the formation of smaller, lipid-depleted particles. In the present study, significant correlations were found between the decreases in LDL-5 and LDL-6 and an increase of HDL-2b, the most buoyant HDL subfraction (not shown). Because buoyant HDL particles are the preferred substrate of hepatic lipase, this would accord with the suggestion of decreased hepatic lipase during fluvastatin therapy. This is also supported by Hoogerbrugge and Jansen, who found that atorvastatin lowers hepatic lipase.

The possibility that our results were confounded by lifestyle changes and/or concomitant cardiovascular therapy (eg, antihypertensive drugs) warrants discussion. For example, other authors have shown that β-adrenergic receptor antagonists raise both IDL and dLDL. In total, 7 patients (fluvastatin, n=5; placebo, n=2) received β-adrenergic receptor antagonists, and no patient received α-adrenergic receptor–blocking agents. Thus, the proportion of patients receiving β-adrenergic receptor antagonists was small and comparable for each treatment group, and no dosage adjustments were made during the study. Exclusion of these patients from the statistical analyses had no effect on the statistical significance of the findings, indicating that concomitant β-blocker therapy did not affect our results to a relevant extent. Exclusion of diabetic individuals also did not affect the statistical significance of our results (P<0.001 for the change in dLDL for all patients, P<0.001 for the non-diabetic patients). A similar conclusion can be drawn with regard to the potential confounding effect of lifestyle changes. Thus, although patients were not requested to formally record their dietary habits and degree of exercise during the study (rather, patients were advised to adhere to a lipid-modified diet and not change usual exercise habits), there is no reason to assume that minor changes occurred more frequently in the fluvastatin group than in those treated with placebo. Moreover, there was no significant difference between the changes in body mass index for either treatment group. ANOVA using the change of dLDL as the dependent variable, treatment as the independent variable, and the variation of body mass index as a covariate says that changes in LDL subfractions remain significant after adjustment for body mass index (data not shown).

In conclusion, fluvastatin produces a shift in LDL subfractions toward more buoyant, less atherogenic LDL particles in patients at increased risk of CHD. This profile may contribute to an antiatherogenic effect for fluvastatin that is greater than expected on the basis of changes in lipids and apolipoproteins.

Acknowledgments
The authors thank Novartis Pharma AG, Nürnberg, Germany, for financial support and Sabiene Jotterand, Dagmar Reduth, Gisela Zöllner, and Rita Gläsper for technical assistance.

References
1948 Circulation April 17, 2001


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Circulation. 2001;103:1942-1948
doi: 10.1161/01.CIR.103.15.1942

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