Effects of Lovastatin on ApoA- and ApoB-Containing Lipoproteins
Families in a Subpopulation of Patients Participating in the Monitored Atherosclerosis Regression Study (MARS)


Abstract To establish whether lovastatin, an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase, exhibits a specific effect on apolipoprotein (apo) A- and apoB-containing lipoproteins, 63 subjects, a subset of the 270 Monitored Atherosclerosis Regression Study (MARS) patients with hypercholesterolemia (190 to 295 mg/dL) and documented coronary artery disease, were randomized into either lovastatin 40 mg twice daily or matching placebo tablets twice daily. Both groups consumed a diet containing 27% calories as fat (polyunsaturated fat/saturated fat ratio, 2.85) and a daily cholesterol intake of less than 250 mg. The plasma lipid and apolipoprotein profiles were determined at the time of randomization and after 2 years of treatment, and the levels of apoA-I and apoB-containing lipoprotein families were measured after 2 years of treatment. After this treatment period, the drug group was characterized in comparison with the placebo group by significantly reduced levels of total cholesterol (33%), triglycerides (30%), very-low-density lipoprotein cholesterol (36%), low-density lipoprotein cholesterol (43%), apoB (36%), apoC-III (18%), and apoE (17%) and slightly but insignificantly increased levels of high-density lipoprotein cholesterol (6%) and apoA-I (1%). The 2-year levels of lipoprotein containing apoA-I but no apoA-II (LpA-I) and lipoprotein containing both apoA-I and apoA-II (LpA-I/A-II) particles separated by immunoaffinity chromatography on an anti-apoA-I immunosorbent did not differ between the two treatment groups. However, the apoB-containing lipoprotein (Lp) families defined by apolipoprotein composition and separated by immunoaffinity chromatography on anti-apoA-II and anti-apoC-III immunosorbents were affected in a selective manner. Compared with the placebo group, lovastatin subjects had significantly lower levels of cholesterol-rich LpB particles (40%), but there was no difference in the levels of intact and/or partially delipidized triglyceride-rich LpB/C+LpB/C/E and LpA-II/B/C/D/E families. We conclude that the reducing effect of lovastatin on apoB-containing lipoproteins is mediated through its selective decrease of cholesterol-rich LpB particles. Lovastatin is a potent agent for lowering the levels of potential atherogenic LpB particles but a less effective drug for reducing other forms of apoB-containing lipoproteins or for increasing the levels of putative nonatherogenic apoA-containing lipoproteins. (Arterioscler Thromb. 1994;14:1906-1914.)

Key Words • apolipoproteins • lipoprotein families • immunosorbents • immunoaffinity chromatography • lovastatin

Lovastatin (Mevacor) is an effective competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase, the rate-limiting enzyme in cholesterol biosynthesis1 and a potent hypocholesterolemic drug in patients with heterozygous familial2,3 and other types of primary, nonfamilial hypercholesterolemia.4 It has been suggested that the hypocholesterolemic effect of lovastatin is mainly due to increased activity of hepatic low-density lipoprotein (LDL) receptors, with subsequent increase in the uptake and degradation of cholesterol-rich LDL particles.2 It appears that this cholesterol-lowering mechanism operates primarily in patients heterozygous for familial hypercholesterolemia. However, in patients with either moderate nonfamilial hypercholesterolemia4 or combined hyperlipoproteinemia5 the lovastatin-induced reduction in total and LDL cholesterol (LDL-C) levels may be more closely related to the decreased production or secretion rates than increased fractional catabolic rates of apolipoprotein (apo) B-containing lipoproteins.

When administered to patients with either familial hypercholesterolemia2,3,5,8,9 or nonfamilial hypercholesterolemia4,6,9–12 at a dosage of 20 mg twice daily or 40 mg once daily in short-term (4 to 18 weeks) clinical trials, lovastatin reduced the levels of total cholesterol (TC) by 19% to 39%, LDL-C by 28% to 48%, and apoB by 23% to 43%. In these studies, the lovastatin-induced lowering of triglyceride (TG) levels varied between 11% and 35%. Modestly increased levels of high-density lipoprotein cholesterol (HDL-C) (2% to 13%) were accompanied by equally modest increases in the levels of apoA-I (1% to 9%) and apoA-II (1% to 8%). In long-term studies similar changes induced by lovastatin in the levels of lipids and lipoprotein cholesterol were found to be sustained for 48 weeks4 to 3 years.13 These studies have also shown that lovastatin is a generally well-tolerated and effective therapy for patients with familial and nonfamilial hypercholesterolemia.
Studies from our laboratory as well as others have demonstrated a marked physical-chemical, immunochemical, and kinetic heterogeneity of major lipoprotein density classes. This heterogeneity is ascribed to the occurrence of discrete lipoprotein particles of similar density properties but distinct apolipoprotein composition. Classification of lipoproteins on the basis of apo-lipoproteins as unique chemical markers recognizes apoA- and apoB-containing lipoproteins as two major lipoprotein classes. Fractionation of apoA-containing lipoproteins by sequential immunoaffinity chromatography has shown that lipoprotein containing apoA-I but no apoA-II (LpA-I) and lipoprotein containing both apoA-I and apoA-II (LpA-II/A-II) are the characteristic lipoprotein families of the HDL class thought to be nonatherogenic. A similar fractionation of apoB-containing lipoproteins, which occur mainly in very-low-density lipoprotein (VLDL) and LDL density ranges and are considered to possess atherogenic potential, reveals the existence of four major lipoprotein species. These include cholesterol ester-rich LpB and TG-rich LpB/C-I/C-II/C-III (LpB/C), LpB/C-I/C-II/C-III/E (LpB/C/E), and LpA-II/B/C-I/C-II/C-III/D/E (LpA-II/B/C/D/E) particles. All these chemically distinct, polydisperse lipoprotein families seem to possess distinct metabolic and functional properties and, possibly, different atherogenic and nonatherogenic potentials. Moreover, hypolipidemic drugs seem to have a rather specific effect on individual apoA- and apoB-containing lipoproteins. For example, nicotinic acid raises and probucol reduces LpA-I levels. Fenofibrate decreases levels of LpA-I and increases levels of LpA-I/A-II particles, whereas cholestyramine, simvastatin, and pravastatin seem to raise to varying degrees the levels of one or both of these lipoprotein families. A preliminary study has shown that nicotinic acid in combination with colestipol specifically reduces the levels of cholesterol ester-rich LpB particles. On the other hand, gemfibrozil and fenofibrate selectively decrease the levels of TG-rich LpB/C/E and LpB/C particles. To our knowledge, there are no published reports on the effect of lovastatin on the concentrations of apoA- and apoB-containing lipoprotein families.

To establish the long-term effect of lovastatin on the concentrations of apoA- and apoB-containing lipoprotein families, we determined these concentrations in a randomly selected subset of patients with moderate hypercholesterolemia and coronary artery disease from the Monitored Atherosclerosis Regression Study (MARS), a coronary angiographic trial evaluating the effect of monotherapy with lovastatin on the progression and regression of coronary atherosclerosis.

Methods

Patients

The MARS study design has been described in detail. In brief, the study cohort consisted of 270 men and women aged 21 through 67 years with coronary artery disease in ≥2 coronary artery segments with ≥1 segment narrowed by ≥50% (but <100%) stenosis and unaltered by percutaneous transluminal coronary angioplasty. TC levels ranged from 190 to 295 mg/dL. The subjects were recruited at two centers (the University of Southern California/Kaiser Permanente Medical Center, Los Angeles, and the University of Wisconsin). Study patients were randomized, within blocks defined by gender, smoking status, and plasma TC (<240 mg/dL or ≥240 mg/dL), to either lovastatin 40 mg twice daily or matching placebo tablets twice daily. Both experimental groups received dietary counseling with identical target goals for cholesterol and fat intake. Dietary goals included <27% of calories as fat, with saturated fat constituting <7% of total calories, and monounsaturated and polyunsaturated fats <10% of calories each. Target daily cholesterol intake was <250 mg.

To determine the concentrations of individual apoA- and apoB-containing lipoprotein families, a subset of 63 subjects was randomly selected from the total 270 randomized MARS patients. Lipid and apolipoprotein profiles were determined at the time of randomization (baseline levels) and after 2 years of treatment. Because the methodology for measuring the concentrations of apoA- and apoB-containing lipoprotein families was not available at the beginning of the study, these lipoprotein families were analyzed only after 2 years of treatment. The apoA-containing lipoprotein families were determined in 15 placebo and 13 lovastatin subjects, whereas apoB-containing lipoprotein families were estimated in 31 placebo and 32 lovastatin subjects. The reason for measuring apoA-containing lipoprotein families in only 28 of 63 participants was a methodological one in that an anti-apoA-II immunosorbent of high capacity was not available at the beginning of this study, and only the last 28 blood samples drawn were analyzed.

Blood samples were drawn after an overnight (8 hours or more) fast into EDTA-containing Vacutainer tubes, and plasma samples were recovered by low-speed centrifugation (1000g) for 10 minutes at 4°C. After measurement of lipid levels, plasma samples were stored at −20°C and transported on dry ice to the Oklahoma Medical Research Foundation for apolipoprotein and lipoprotein analyses. A preservative solution (0.13% e-aminocaproic acid, 0.1% EDTA, and 0.1% thiomersal) was added (10 µL/mL) to all plasma samples prior to storage.

Determination of ApoA- and ApoB-Containing Lipoprotein Families

Approximately 20% to 25% of the two major apoA-containing lipoprotein families (LpA-I and LpA-I/A-II) contain, in addition to their main apolipoproteins, varying amounts of minor apolipoproteins. These two lipoprotein families were separated by immunofinity chromatography of whole plasma on an anti-apoA-II immunosorbent. The preparation of the anti-apoA-II immunosorbent and the fractionation of LpA-I and LpA-I/A-II have been described. Plasma samples (0.3 to 0.5 mL) were applied to the anti-apoA-II immunosorbent and incubated overnight at 4°C. The retained fraction (LpA-I/A-II) was desorbed and eluted with 3 mol/L sodium thiocyanate at a flow rate of 30 mL/h. The retained and unretained fractions were tested immunochemically for the presence of apoA-I and apoA-II. Unretained fractions containing immunochemically detectable apoA-II were rechromatographed on an anti-apoA-II immunosorbent, and only fractions free of apoA-II were used for quantitative measurement of apoA-I distributed between LpA-I (unretained fraction) and LpA-I/A-II (retained fraction). The recoveries of apoA-I ranged between 80% to 92% of applied apoA-I. The concentrations of apoA-I in LpA-I and LpA-I/A-II particles were calculated on the basis of plasma apoA-I values and the percent distribution of apoA-I in retained and unretained fractions from anti-apoA-II immunosorbents. Isolated LpA-I and LpA-I/A-II preparations with known concentrations of apoA-I served as primary standards, and reference plasma samples of known concentrations of LpA-I and LpA-I/A-II served as the secondary standards. The interassay coefficients of variation were 4.0% for the LpA-I assay and 1.5% for the LpA-I/A-II assay.
There are two groups of apoB-containing lipoprotein families, one of which is represented by cholesterol ester–rich LpB, the major lipoprotein of LDL, and the other by intact or partially delipidized TG-rich LpB/C, LpB/C/E, and LpA-II/B/C/D/E particles, the characteristic lipoproteins of the VLDL and intermediate density lipoprotein (IDL) ranges. However, as polydisperse systems of lipoprotein particles, LpB particles may also be found in varying concentrations in VLDL and IDL, whereas LpB/C, LpB/C/E, and LpA-II/B/C/D/E particles may also occur in LDL. To avoid the potentially damaging effect of ultracentrifugation on the structural integrity of lipoprotein families, especially those containing apoC polypeptides and/or apoE, the measurement of apoB-containing lipoproteins was performed with whole plasma as the starting material.

The determination of apoB-containing lipoproteins was performed by immunooaffinity chromatography by using anti-apoA-II and anti-apoC-III immunosorbs. Monoclonal antibodies to human apoA-II and monospecific, affinity-puriﬁed polyclonal antibodies to human apoC-III were coupled to Affi-gel 10 (Bio-Rad Laboratories). Aliquots of whole plasma were chromatographed separately on anti-apoA-II and anti-apoC-III immunosorbs. Plasma aliquots (0.2 to 0.4 mL) were applied to the immunosorbs and incubated for 12 hours at room temperature (20°C). The unretained fractions were eluted with 0.05 mol/L Tris-HCl buffer, pH 7.4, containing 0.5 mol/L NaCl and 1.5 mg/mL EDTA. After the columns were washed with this buffer, the retained fractions were eluted with 3 mol/L NaSCN, pH 7.4. The retained fraction from the anti-apoA-II immunosorbent contained the LpA-II/B/C/D/E particles, and the unretained fraction contained all other apoB-containing lipoprotein families. If the retained fraction reacted positively with anti-apoA-II serum, it was chromatographed on the anti-apoA-II immunosorbent until free of apoA-II. The retained fraction from the anti-apoC-III immunosorbent contained TG-rich apoB-containing lipoproteins (LpB), including LpB/C, LpB/C/E, and LpA-II/B/C/D/E particles, while the unretained fraction consisted of LpB. If the unretained fraction tested positively for apoC-III, it was rechromatographed on the anti-apoC-III immunosorbent until free of apoC-III. All unretained and retained fractions as well as the starting whole plasma were analyzed for apoB, and the concentrations of apoB-containing lipoproteins were expressed in terms of their apoB content in milligrams per deciliter. The apoB content of the anti-apoA-II retained fraction corresponded to LpB particles and that of the anti-apoC-III retained fraction corresponded to LpB particles. The apoB content of the anti-apoA-II retained fraction represented the LpA-II/B/C/D/E particles. The apoB content of the anti-apoC-III retained fraction was calculated by subtracting the apoB content of the LpA-II/B/C/D/E particles (anti-apoA-II retained fraction) from that of the LpB particles (anti-apoC-III retained fraction). The concentrations of apoB in apoB-containing lipoprotein families were calculated on the basis of plasma apoA-I, apoB, apoC-III, and apoE concentrations in the retained and unretained fractions from both immunosorbs. The recoveries of apoB from anti-apoA-II and anti-apoC-III immunosorbs ranged between 79% to 90% of applied apoB. The interassay coefficients of variation were 6.2% for the measurement of LpB, 8.7% for the assay of LpB, and 15.8% for the analysis of LpA-II/B/C/D/E particles.

A separate experiment revealed no statistically significant differences in the percentage distribution and concentration of LpB and LpB particles measured in fresh and frozen plasma samples from the same subjects.

**Determination of Lipids and Apolipoproteins**

Fasting blood TC and TG levels were determined by using enzymic methods standardized against reference materials supplied by the Standardization Program of the National Centers for Disease Control and Prevention in the VPSS analyzer (Abbott). HDL-C was measured after the precipitation of apoB-containing VLDL, IDL, and LDL in whole plasma by heparin–manganese chloride. VLDL was assumed to equal one ﬁfth of the serum TG concentration, and LDL was calculated by difference according to the method of Friedewald et al. Apolipoproteins were quantiﬁed by electrophoresis of apoA-I, apoB, apoC-III, and apoE. ApoC-III was measured in heparin–manganese chloride–precipitated (apoC-III–HP) and heparin–manganese chloride–nonprecipitated (apoC-III–HS) lipoproteins. ApoC-III–HP corresponds to the apoC-III content of VLDL+IDL+LDL, and apoC-III–HS corresponds to the apoC-III content of HDL+very-high-density lipoprotein. The apoC-III–HS/apoC-III–HP ratio was used as a means of assessing the efﬁciency of processes responsible for the catabolism of TG-rich lipoproteins. All measurements were performed in duplicate and repeated if they disagreed by more than 5%.

**Statistical Analysis**

To ensure that the subset of 63 subjects selected for this study was comparable to the remaining 207 randomized MARS subjects, the two groups were tested for differences in baseline lipids, apolipoproteins, and clinical variables. These same baseline variables were compared by treatment group within the subset of 63 subjects selected for this study to ensure baseline comparability. All baseline comparisons used either a t test for independent samples or a nonparametric Wilcoxon rank sum test for continuous variables (depending on the distribution of each variable) and a χ² test for categorical variables. Treatment group differences of on-trial apoA- and apoB-containing lipoprotein families were tested by using a nonparametric Wilcoxon rank sum test since these variables were not normally distributed. Correlation coefﬁcients were calculated by Pearson’s method.

**Results**

**Lipids and Apolipoproteins**

A comparison of clinical characteristics and plasma lipid and apolipoprotein proﬁles showed that the subset of 63 patients randomly selected from the total randomized 270 MARS subjects was representative of the total MARS study group except for lower baseline apoB levels (Table 1). The 31 placebo and 32 lovastatin subjects were well matched with respect to age (58.7±1.2 versus 58.5±1.1 years), weight (184.5±4.6 versus 176.3±4.4 lb), systolic (124.8±2.6 versus 125.3±2.3 mm Hg) and diastolic (79.8±1.6 versus 81.6±1.4 mm Hg) blood pressure, sex (90% men and 10% women in both groups), and smoking habits (90% nonsmokers and 10% smokers in both groups). There were no signiﬁcant differences between these two treatment groups in their baseline values of plasma lipids, lipoprotein cholesterol, apolipoproteins A-I, B, C-III, E, and apoC-III–HS and apoC-III–HP (Table 1).

Two years of lovastatin treatment resulted in a highly signiﬁcant reduction of plasma TC (33%, P<.0001) that was paralleled by an equally signiﬁcant reduction of LDL-C (43%, P<.0001) in comparison with the placebo group (Table 2). Compared with baseline values, TC levels decreased in all subjects treated with lovastatin by 14% to 46%. TG levels decreased by an average 31% (P<.02) in the drug group compared with the placebo group (Table 2), but the TG-lowering effect of lovastatin varied considerably among individual subjects: although TG levels decreased by 2% to 14% in 25% of subjects, by 18% to 28% in 22% of subjects, and by 35% to 60% in 28% of subjects, they increased by 2% to 66% in 25% of subjects. In the lovastatin-treated subjects,
the decrease in VLDL cholesterol levels was similar to that in TG levels (Table 2). The on-trial concentration of HDL-C was slightly (6%) but insignificantly higher in the drug group than in the placebo group.

Commensurate with the significantly reduced levels of TC and LDL-C, the mean apoB concentration was also significantly lower (36%, P<.0001) in the drug group than in the placebo group (Table 2). In contrast to the uniform reduction in the concentrations of TC, however, the changes in apoB levels differed considerably among lovastatin-treated subjects. In approximately two thirds of these subjects apoB levels decreased from baseline values by 1% to 51%, but in one third the concentrations of apoB increased by 4% to 33%. The mean concentrations of apoC-III and apoE were significantly lower in the drug group than in the
placebo group by 18% and 17% ($P<.008$ and $P<.02$, respectively). However, as in the case of apoB, the response to drug treatment varied: in 68% of lovastatin-treated subjects, the levels of these minor apolipoproteins decreased by 1% to 51%, but in 32% of subjects they actually increased by 10% to 75%. The mean levels of both apoC-III-HS (10%, NS) and apoC-III-HP (25%, $P<.02$) were lower in the drug group than in the placebo group, reflecting a slight reduction in plasma apoC-III. Due to a larger decrease in the levels of apoC-III-HP than apoC-III-HS, there was an insignificant increase in the apoC-III-HS/apoC-III-HP ratio (Table 2).

Although the effect of lovastatin on the concentrations of lipids, lipoprotein cholesterol, and apolipoproteins was evaluated by percentage differences between the on-trial values of the lovastatin and placebo groups, the changes between baseline and on-trial values of the lovastatin-treated subjects were of the same magnitude (Tables 1 and 2). As a result of differences in the levels of lipids and apolipoproteins between the drug and placebo groups, there were also significant differences in some of the frequently calculated lipid and apolipoprotein ratios. Thus, the drug group was characterized by significantly higher HDL-C/LDL-C (0.55 ± 0.21 versus 0.29 ± 0.08, $P<.0001$) and apoA-I/apoB (1.68 ± 0.55 versus 1.06 ± 0.29, $P<.0001$) ratios and a significantly lower TC/HDL-C (3.60 ± 0.77 versus 5.71 ± 0.35, $P<.0001$) ratio than the placebo group.

### ApoA- and ApoB-Containing Lipoprotein Families

Although the measurement of HDL-C and apoA-I indicated minimal changes in levels of these HDL constituents in subjects treated with lovastatin (Tables 1 and 2), it has been of considerable interest to establish the distribution of apoA-I between LpA-I and LpA-I/A-II, the two major apoA-containing lipoprotein families of high-density characteristics. There was no significant difference either in the percentage distribution or concentration of on-trial LpA-I and LpA-I/A-II particles between the drug and placebo groups (Table 3).

In contrast to the negligible effect of lovastatin on the levels of LpA-I and LpA-I/A-II particles, there was a significant and differential effect of this drug on the concentrations of major apoB-containing lipoprotein families (Table 4). As expected from the results of well-established lovastatin-lowering effects on TC, LDL-C, and apoB levels, the mean concentration of on-trial cholesterol ester–rich LpB particles was significantly lower (40%, $P<.0001$) in the drug than in the placebo group. All subjects treated with lovastatin had LpB levels lower than the mean LpB value of the

### Table 2. Effect of Lovastatin on Plasma Lipid, Lipoprotein Cholesterol, and Apolipoprotein Levels In a Subpopulation of MARS Patients

<table>
<thead>
<tr>
<th>Lipids and Apolipoproteins</th>
<th>Placebo (n=31)</th>
<th>Lovastatin (n=32)</th>
<th>Difference, %</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>227.2 (6.0)</td>
<td>151.4 (4.1)</td>
<td>−33</td>
<td>.0001</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>190.6 (19.7)</td>
<td>130.7 (10.1)</td>
<td>−31</td>
<td>.02</td>
</tr>
<tr>
<td>VLDL cholesterol</td>
<td>39.6 (3.8)</td>
<td>25.3 (2.0)</td>
<td>−36</td>
<td>.002</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>147.0 (6.2)</td>
<td>82.6 (3.3)</td>
<td>−43</td>
<td>.0001</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>41.2 (1.4)</td>
<td>43.5 (1.3)</td>
<td>6</td>
<td>NS</td>
</tr>
<tr>
<td>ApoA-I</td>
<td>124.0 (3.7)</td>
<td>124.1 (3.9)</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>ApoB</td>
<td>121.0 (3.7)</td>
<td>77.4 (3.0)</td>
<td>−36</td>
<td>.0001</td>
</tr>
<tr>
<td>ApoC-III</td>
<td>13.9 (0.7)</td>
<td>11.3 (0.6)</td>
<td>−18</td>
<td>.008</td>
</tr>
<tr>
<td>ApoE</td>
<td>14.1 (0.8)</td>
<td>11.7 (0.6)</td>
<td>−17</td>
<td>.02</td>
</tr>
<tr>
<td>ApoC-III-HS</td>
<td>6.9 (0.4)</td>
<td>6.2 (0.4)</td>
<td>−10</td>
<td>NS</td>
</tr>
<tr>
<td>ApoC-III-HP</td>
<td>6.0 (0.5)</td>
<td>4.5 (0.4)</td>
<td>−25</td>
<td>.02</td>
</tr>
<tr>
<td>ApoC-III ratio</td>
<td>1.4 (0.2)</td>
<td>1.6 (0.1)</td>
<td>14</td>
<td>NS</td>
</tr>
</tbody>
</table>

MARS indicates Monitored Atherosclerosis Regression Study; VLDL, very-low-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; Apo, apolipoprotein; HS, heparin–manganese chloride supernate; HP, heparin–manganese chloride precipitate; and ApoC-III ratio, apoC-III-HS/apoC-III-HP. Values are mean (SEM) and are expressed in milligrams per deciliter unless otherwise indicated. Significance was calculated by a Wilcoxon rank sum test.

### Table 3. Effect of Lovastatin on Plasma Levels of ApoA-Containing Lipoprotein Families In a Subpopulation of MARS Patients

<table>
<thead>
<tr>
<th>Lipoprotein Families</th>
<th>Placebo (n=15)</th>
<th>Lovastatin (n=13)</th>
<th>Difference, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>LpA-I, mg/dL*</td>
<td>24.4 (3.6)</td>
<td>25.5 (4.1)</td>
<td>4</td>
</tr>
<tr>
<td>LpA-I/A-II, mg/dL*</td>
<td>87.8 (5.5)</td>
<td>94.5 (6.5)</td>
<td>8</td>
</tr>
<tr>
<td>LpA-I, %†</td>
<td>21.7 (3.0)</td>
<td>20.8 (2.8)</td>
<td>−4</td>
</tr>
<tr>
<td>LpA-I/A-II, %†</td>
<td>78.3 (3.0)</td>
<td>79.2 (2.8)</td>
<td>1</td>
</tr>
</tbody>
</table>

Apo indicates apolipoprotein; MARS, Monitored Atherosclerosis Regression Study; LpA-I, lipoprotein containing apoA-I but no apoA-II; and LpA-I/A-II, lipoprotein containing apoA-I and apoA-II. Values are mean (SEM) unless otherwise indicated. There were no significant differences by Wilcoxon rank sum test.

†The concentrations and percentages of LpA-I and LpA-I/A-II families are expressed in terms of apoA-I values.

†Percentages of total apoA-I present in LpA-I and LpA-I/A-II particles.
placebo group (112.6±3.6 mg/dL); none of the placebo subjects had LpB levels equal to or lower than the mean LpB of the lovastatin group (67.7±2.5 mg/dL) (Fig 1). In contrast, the mean concentration of LpB particles, which represented the sum of all major TG-rich apoB-containing lipoprotein families, was slightly higher (20%, NS) in the lovastatin- than in the placebo-treated group (Table 4). Fig 2 illustrates the major overlap in LpB, levels between lovastatin- and placebo-treated subjects; more lovastatin-treated subjects had levels in the highest quartile, and more placebo-treated subjects had levels in the lowest quartile. The lovastatin-treated subjects had nonsignificantly higher mean levels of LpB/C+LpB/C/E particles than placebo-treated subjects, but there was no difference in the mean levels of LpA-II/B/C/D/E particles between the two groups (Table 4).

As a result of the selective effect of lovastatin on apoB-containing lipoprotein families, the percentage content of LpB particles was significantly lower (P<.0004) and the percentage content of LpA-II/B/C/D/E particles significantly higher (P<.002) in the lovastatin group than in the placebo group, with little or no difference in the percentage of LpB/C+LpB/C/E particles (Table 4).

The rather selective effect of lovastatin on the levels of cholesterol ester-rich LpB particles was also reflected in different correlations between on-trial levels of lipids, apolipoproteins, and lipoprotein families in the placebo and drug groups. In the placebo group, TC correlated significantly with LDL-C (r=.86, P<.001), apoB (r=.68, P<.001), and LpB particles (r=.69, P<.001), whereas TG correlated significantly with VLDL cholesterol (r=.93, P<.001) and apoC-III (r=.74, P<.001). The large disproportion in the levels of cholesterol ester-rich and TG-rich lipoproteins in the placebo group (Table 4) was clearly shown by a low correlation (r=.21, NS) between TC and TG. However, the marked lovastatin-induced reduction in the levels of LpB particles also lowered the high ratio of cholesterol ester-rich to TG-rich lipoproteins and revealed previously masked correlations between lipid and apolipoprotein constituents common to both types of lipoprotein particles. Thus, in the lovastatin group, TC correlated significantly with TG (r=.58, P<.001), VLDL cholesterol (r=.56, P<.001), and both apoC-III (r=.37, P<.05) and LpB, (r=.44, P<.05) in addition to LDL-C (r=.85, P<.001), apoB (r=.81, P<.001), and LpB (r=.74, P<.001). On the other hand, TG correlated in the lovastatin group with apoB (r=.50, P<.01) and LpB, (r=.67, P<.001) in addition to VLDL cholesterol (r=.95, P<.001) and apoC-III (r=.71, P<.001). The

TABLE 4. Effect of Lovastatin on Plasma Levels of ApoB-Containing Lipoprotein Families In a Subpopulation of MARS Patients

<table>
<thead>
<tr>
<th>Lipoprotein Families</th>
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<th>Lovastatin (n=32)</th>
<th>Difference, %</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>LpB, mg/dL*</td>
<td>112.6 (3.6)</td>
<td>67.7 (2.5)</td>
<td>-40</td>
<td>.0001</td>
</tr>
<tr>
<td>LpB, mg/dL</td>
<td>8.4 (0.7)</td>
<td>10.1 (1.3)</td>
<td>20</td>
<td>NS</td>
</tr>
<tr>
<td>LpB/C+LpB/C/E, mg/dL*</td>
<td>2.1 (0.4)</td>
<td>3.2 (0.8)</td>
<td>52</td>
<td>NS</td>
</tr>
<tr>
<td>LpA-II/B/C/D/E (LpA-II/B complex), mg/dL*</td>
<td>6.3 (0.7)</td>
<td>6.5 (0.8)</td>
<td>3</td>
<td>NS</td>
</tr>
<tr>
<td>LpB, %†</td>
<td>93.0 (0.6)</td>
<td>88.0 (1.1)</td>
<td></td>
<td>.0004</td>
</tr>
<tr>
<td>LpB/C+LpB/C/E, %†</td>
<td>1.9 (0.4)</td>
<td>3.8 (0.9)</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>LpA-II/B/C/D/E (LpA-II/B complex), %†</td>
<td>5.2 (0.6)</td>
<td>8.3 (0.8)</td>
<td>&lt;.002</td>
<td></td>
</tr>
</tbody>
</table>

Apo indicates apolipoprotein; MARS, Monitored Atherosclerosis Regression Study; Lp, lipoprotein; and LpB, triglyceride-rich Lp Values are mean (SEM) unless otherwise indicated. Significance was calculated by Wilcoxon rank sum test.

*Percentages of total apoB present in LpB, LpB/C+LpB/C/E, and LpA-II/B/C/D/E families are expressed in terms of apoB values.

correlations between the levels of LpB and any of the other apoB-containing lipoproteins were insignificant.

Discussion

The results of this long-term study agree with the well-established qualitative and quantitative effects of lovastatin on individual lipoprotein constituents that have been observed both in short- and long-term clinical trials. These effects include highly significant reductions in the concentrations of TC, LDL-C, and apoB, moderate and varied lowering effects on the levels of TG and VLDL cholesterol, and a negligible effect on the concentrations of HDL-C and apoA-I. Our findings clearly show that lovastatin affects mainly, if not exclusively, the apoB-containing lipoproteins. It has been generally assumed and accepted, on the basis of TC, TG, and lipoprotein cholesterol measurements, that lovastatin and other HMG CoA inhibitors primarily affect LDL and, to a lesser degree, VLDL particles. This assumption has not taken into account the marked chemical and metabolic heterogeneity of these lipoprotein density classes that is caused mainly by the presence of several distinct apoB-containing lipoproteins of similar density properties but specifically different apolipoprotein compositions. Although cholesterol ester–rich LpB particles constitute the main apoB-containing lipoprotein family of IDL and LDL, they also occur in measurable amounts in the VLDL of normolipidemic and dyslipoproteinemic subjects.

Similarly, the polydisperse TG-rich apoB-containing lipoproteins such as LpB/C, LpB/C/E, and LpA-II/B/C/D/E are not confined to VLDL but are detectable, in partially delipidized forms, in IDL and lower density ranges of LDL in several dyslipoproteinemic states. Thus, drug effects on lipoprotein transport should be evaluated in terms of discrete lipoprotein families. Our analyses have shown that lovastatin affects apoB-containing lipoprotein families in a selective manner.

There is a considerable variability in the concentration profiles of apoB-containing lipoprotein families and their response to lovastatin treatment. This is shown by substantial individual variation in on-trial values for lipids and apolipoproteins, with the possible exception of TC and LDL-C, which are the only constituents found to be reduced in all drug-treated subjects in this MARS subpopulation study. Although the lovastatin treatment of subjects with primary hypercholesterolemia has been shown by others to consistently and significantly reduce the levels of plasma TC and LDL-C, its effect on plasma TG and VLDL cholesterol varies from a moderate reduction to either no change or an actual increase in the concentrations of these lipoprotein constituents. These differences in the lovastatin effect on VLDL levels may possibly be explained by differences in the levels of LpB particles in the VLDL of individual subjects and their responses to drug treatment.

In contrast to its marked potential to lower the levels of cholesterol ester–rich LpB particles, lovastatin had little effect on the concentrations of TG-rich LpBc particles. Data on preliminary separations of LpB/C from LpB/C/E particles by immunoaffinity chromatography on an anti-apoE immunosorber in 30 of the 63 MARS subjects suggested that lovastatin may decrease LpB/C and increase LpB/C/E particles. Because lovastatin has no effect on the levels of LpA-II/B/C/D/E particles, the overall drug effect on these complex apoB-containing lipoproteins is minimal in most cases. The negligible effect of lovastatin on the majority of LpB particles may also explain the finding that, despite decreased concentrations of LpB particles, one third of the drug-treated subjects had increased levels of plasma apoB; in these subjects the decrease in LpB particles was compensated for by an increase in LpB particles. The lack of lovastatin effect on certain LpB particles may account for our finding that the levels of apoC-III-HP were the best predictor for continued progression of coronary artery lesions (<50% stenosis) in lovastatin-treated subjects.

Several explanations exist for the average increase in the levels of LpB, particles in the presence of decreased levels of TG, VLDL cholesterol, and apolipoproteins C-III and E. In part, reduced levels of TG may be attributed to decreased concentrations of LpB particles, which contain TG as one of their neutral lipid constituents. However, the percentage of TG in LpB particles present in VLDL is twice as high as that of LpB particles present in LDL (40% versus 20% of total neutral lipids). Thus, the extent of TG decrease may be limited by levels of LpB particles remaining in the VLDL and IDL density ranges. Second, some TG-rich lipoprotein families may undergo a partial delipidization accompanied by corresponding quantitative but not qualitative changes in their lipid and apolipoprotein composition. In support of this hypothesis, we have shown that LpB/C, LpB/C/E, and LpA-II/B/C/D/E particles isolated from LDL have lower percent contents of TG, apoC polypeptides, and apoE than the corresponding particles isolated from VLDL. It should be emphasized that a partial loss of individual lipid and/or apolipoprotein constituents does not necessarily result in complete dissociation and degradation of these apoB-containing lipoprotein families. Third, a part of apoC-III and possibly apoE reduction in the whole plasma of lovastatin-treated subjects may also be due to decreased levels of these minor apolipoproteins in HDL (Table 2). Fourth, the possible lowering effect of lovastatin on the concentration of LpB/C particles may also contribute to the reduction of plasma TG, VLDL cholesterol, and apoC-III, although the concentration of these particles is generally low in hypercholesterolemic subjects.

We recognize that the lack of baseline values for lipoprotein particles may be a limitation of this study. This was unfortunately unavoidable because the methodology for isolation of lipoprotein particles was developed after trial onset. However, to evaluate the effect of lovastatin on apoA- and apoB-containing lipoprotein families based on changes between the baseline and on-trial values rather than differences in the on-trial values between the lovastatin and placebo groups, a separate group of moderately hypercholesterolemic patients (TC, 253 ± 8 mg/dL, n = 7) was treated with lovastatin (40 mg/d) for 4 weeks. Similar to the results based on on-trial differences between the 63 MARS lovastatin and placebo subjects, there was no significant change between the baseline and on-trial values in the concentrations of either LpA-I (34.5 ± 0.7 versus 34.9 ± 1.5 mg/dL, NS) or LpA-I/A-II (104 ± 3 versus 88 ± 5 mg/dL, NS). In addition, changes in the concentrations of LpB
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HL-49885, and the resources of the Oklahoma Medical Re-

In conclusion, this is the first study to demonstrate that the well-established lowering effect of lovastatin on apoB-containing lipoproteins in subjects with moderate hypercholesterolemia and coronary artery disease is mediated through its selective reduction of cholesterol ester–rich LPB particles. Lovastatin had little or no effect on the levels of TG-rich LPB/C+LPB/C/E and LPB- II/B/C/D/E particles and no effect on the concentrations of LPB-I and LPB-I/A-II particles. These results suggest that lovastatin is a potent agent for lowering the potentially atherogenic cholesterol ester–rich LPB particles but a less effective drug for lowering other forms of apoB-containing lipoproteins or for increasing the putative nonatherogenic apoA-containing lipoproteins.

Acknowledgments

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