Lathosterol and Other Noncholesterol Sterols During Treatment of Hypercholesterolemia With Lovastatin Alone and With Cholestyramine or Guar Gum

Matti I.J. Uusitupa, Tatu A. Miettinen, Pertti Happonen, Tapani Ebeling, Hannu Turtola, Erkki Voutilainen, and Kalevi Pyörälä

Sixty-two patients aged 19–64 years with primary hypercholesterolemia (mean level of total cholesterol, 10.8 mmol/l) were treated with 80 mg/dayLovastatin (L) alone for 18 weeks and, after randomization to either L + 20 g/day guar gum (L+GG) or L + 16 g/day cholestyramine (L+C) treatments, for an additional 18 weeks. The total cholesterol level declined from baseline by 34% during L and by 44% and 48% during L+GG and L+C, respectively. In terms of micromoles per millimole of cholesterol, serum levels of the cholesterol synthesis precursors cholestenol, desmosterol, and lathosterol were decreased and those of the plant sterols campesterol and sitosterol were increased by treatment with L. The serum contents of cholesterol precursors were increased markedly after the combination of either GG or C with L, but the increase was greater after the addition of C (e.g., the lathosterol to cholesterol ratio was 51% versus 212% for L+GG and L+C, respectively; *p* < 0.001). Thus, a higher rate of removal of bile acids by C than by GG reduced more effectively the low density lipoprotein cholesterol level but simultaneously stimulated cholesterol synthesis compensatorily to a higher level even under concurrent treatment with L. The serum sitosterol to cholesterol ratio declined by 13% during L+GG but increased by 49% during L+C compared with the value under L alone, suggesting different effects of GG and C on the metabolism of plant sterols.


**KEY WORDS** • cholestyramine • guar gum • lovastatin • hypercholesterolemia • lathosterol

Inhibitors of the key enzyme in cholesterol synthesis, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, constitute the most potent drug therapy for patients with severe hypercholesterolemia.1–4 These drugs reduce the conversion of HMG-CoA to mevalonate.5,6 In previous studies urinary mevalonate excretion has been shown to be reduced by 30–40% during treatment with lovastatin.7,8 Furthermore, the serum levels of free and esterified cholesterol precursor sterols, including lathosterol, were reduced significantly by lovastatin and pravastatin.9,10 In contrast, the serum ubiquinone-10 level was not affected by compactin,11 but pravastatin has been recently reported to lower ubiquinone levels.12 Sterol balance studies did not show any marked reduction in whole-body cholesterol synthesis duringLovastatin therapy.13 The hypocholesterolemic effect of HMG-CoA reductase inhibitors is ultimately caused by a cholesterol synthesis inhibition–induced increase in the fractional catabolic rate of low density lipoprotein (LDL) through the activation of LDL receptors.14,15 In addition, the very low density lipoprotein apolipoprotein B production rate may be reduced byLovastatin.16 Cholestyramine, a bile acid–binding resin,17,18 and guar gum,19–24 a gel-forming dietary fiber, reduce serum total and LDL cholesterol levels by increasing the fecal elimination of cholesterol as bile acids.25,26 A reduced cholesterol content in hepatocytes in turn upregulates LDL receptor activity17,27 and stimulates cholesterol synthesis.25–28 In severe hypercholesterolemia, the combination of Lovastatin with a bile acid–binding agent is frequently necessary to obtain the desired serum cholesterol level.19–21 It has been observed that HMG-CoA reductase inhibitors reduce the increase in endogenous cholesterol synthesis observed during the ingestion of bile acid–binding agents or in ileal bypass patients,10 resulting in a profound additional reduction of the serum cholesterol level. Cholesterol precursor sterols in serum, on the other hand, reflect cholesterol synthesis in many clinical conditions.29–39 while serum plant sterols and to some extent cholestanol reflect absorption and biliary elimination of sterols.32,38–41 Serum levels of cholesterol precursor sterols are decreased during HMG-CoA reductase inhibitor treatment10 but increased during guar gum28 or cholestyramine34,35 treatment.
Therefore, the objective of this study was to examine the relation of different cholesterol synthesis precursors, cholestanol, and plant sterols in serum with cholesterol levels during the suppression of cholesterol synthesis by lovastatin alone and during subsequent possible increase of cholesterol synthesis by either cholestyramine or guar gum.

Methods

Patients

Altogether, 62 middle-aged patients (34 men, 28 women) with severe hypercholesterolemia treated initially with lovastatin alone for 18 weeks (80 mg/day for at least 4 weeks) and whose serum total cholesterol concentration remained >5.2 mmol/l were randomized into one of two combination therapies: lovastatin and guar gum (L+GG) or lovastatin and cholestyramine (L+C). The original study population consisted of 120 patients with familial hypercholesterolemia (FH) or nonfamilial hypercholesterolemia (non-FH), but those whose serum total cholesterol concentration declined below 5.2 mmol/l on lovastatin alone were not recruited for this study of the effects of combination therapies. In the original study the inclusion criteria were 1) total cholesterol concentration >6.5 mmol/l on a standard lipid-lowering diet and significant coronary atherosclerosis (pathological electrocardiographic finding during or after exercise, angina pectoris, previous myocardial infarction, coronary bypass surgery or coronary angioplasty, and occlusive coronary artery disease defined by coronary angiography), 2) total cholesterol concentration >7.0 mmol/l on diet and either a) current smoking history (≥10 cigarettes/day), b) positive family history of coronary heart disease (in parents or siblings <60 years old), or c) peripheral atherosclerotic vascular disease, and 3) total cholesterol concentration >7.8 mmol/l on diet alone.

The main exclusion criteria were age >65 years, premenopausal women without reliable contraception, triglyceride concentration >6 mmol/l, alcohol abuse, pathological liver function tests, gallstone disease, recent (<3 months) myocardial infarction or coronary bypass surgery, diabetes mellitus, renal diseases, hypothyroidism, poor mental function, drug abuse, and use of any of the following drugs: barbiturates, anticonvulsants, anticoagulants, quinidine, theophylline, cimetidine, corticosteroids, or antacids.

Former drug treatment for hyperlipidemia was discontinued 8 weeks before starting lovastatin. Diuretics, β-blockers, and sex hormones, if used, were allowed to be continued at the same dose throughout the study.

All subjects had received dietary advice aimed at reducing saturated fat and cholesterol and at increasing unsaturated fat in their diets, according to the American Heart Association Phase 1 diet. During the study the patients were asked not to change their dietary habits. During the study the patients were asked not to change their dietary habits.

On a clinical basis the patients were classified as having heterozygous FH fulfilling the following criteria: serum total cholesterol ≥7.5 mmol/l in the patient and in at least one first-degree relative and tendon xanthomas in the patient or a first- to second-degree relative or as having non-FH.

Study Design

The study was a randomized trial with two parallel groups. Both the patients and clinical investigators were masked with regard to treatment combination. Guar gum and cholestyramine could not, however, be prepared identically, but the packets in which they were distributed were similar.

After maintenance treatment with lovastatin alone for 18 weeks, the patients were randomly allocated to L+GG or L+C treatment. After randomization, no significant differences between groups were found in the mean age, sex distribution, number of subjects with FH and non-FH, body mass index, or serum lipids at baseline (Table 1).

The dose of lovastatin (80 mg/day) was kept unchanged throughout the combination phase of the study. The starting dose of guar gum was 2.5 g twice a day and that of cholestyramine was 2 g twice a day. After 1 week the doses were increased to 5 and 4 g twice a day, respectively. At 6 weeks the doses of guar gum and cholestyramine were increased to the final level, 10 and 8 g twice a day, respectively, if tolerated. The patients visited the outpatient clinic at 6-week intervals.

Lovastatin was taken in the evening before bedtime, whereas guar gum and cholestyramine were administered with breakfast and dinner. The treatment compliance of the patients was monitored at every visit by checking the number of unused packets.

The following measurements were performed before the beginning of lovastatin (week 0) and at 18, 24, 30, and 36 weeks: body weight and serum levels of total cholesterol, high density lipoprotein (HDL) cholesterol, and total triglycerides. Cholesterol synthesis precursors, cholestanol, and the plant sterols campesterol and sitosterol were measured in 56 subjects (28 from each group) before starting lovastatin (week 0) and at 18 and 36 weeks. Samples from three subjects of each group were not available. Liver enzymes and serum creatine kinase were monitored throughout the study, and before admission into the study the patients were checked for renal and thyroid diseases by measuring serum creatinine and thyroxine concentrations.

Measurements

Body weight was measured in light clothing with an electric weight scale. Body mass index was calculated as

<table>
<thead>
<tr>
<th>Table 1. Clinical Characteristics of Study Groups at Baseline</th>
<th>Group</th>
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<tbody>
<tr>
<td>Characteristic</td>
<td>Lovastatin+guar gum (n=31)</td>
</tr>
<tr>
<td>Mean Age (years)</td>
<td>42</td>
</tr>
<tr>
<td>Range</td>
<td>20–66</td>
</tr>
<tr>
<td>Mean Men (%)</td>
<td>58</td>
</tr>
<tr>
<td>Familial hypercholesterolemia (%)</td>
<td>52</td>
</tr>
<tr>
<td>Body mass index (kg/m²)*</td>
<td>25±3.5</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)*</td>
<td>10.6±1.6</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)*</td>
<td>1.2±0.4</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)*</td>
<td>8.5±1.8</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)*</td>
<td>1.9±0.8</td>
</tr>
</tbody>
</table>

HDL, high density lipoprotein; LDL, low density lipoprotein.

*Mean±SD.
Venous blood samples were drawn after a 12-hour overnight fast. Enzymatic methods were used for the determination of serum levels of cholesterol and triglycerides (Menotest and Test-Combination, respectively; Boehringer Mannheim, Mannheim, FRG). The HDL cholesterol concentration was determined after precipitation of LDL with dextran sulfate and MgCl2. The LDL cholesterol concentration was calculated according to Friedewald's formula (LDL cholesterol = total cholesterol−HDL cholesterol−0.45 x triglycerides).

Serum total levels of the cholesterol precursors squalene, Δ5-cholestenol, lathosterol, and desmosterol; serum total levels of the plant sterols campesterol and β-sitosterol; and serum levels of cholestanol were quantified by gas-liquid chromatography on a 50-m-long SE-30 capillary column as described in detail elsewhere.56,57 In "Results" the serum levels of noncholesterol sterols are also given in relation to the serum level of cholesterol. This expression seems preferable for the precursors at least because the marked reduction of concentrations of LDL, which transports most noncholesterol sterols, could also reduce levels of precursors even in the absence of reduced cholesterol synthesis. Thus, a reduced precursor to cholesterol ratio can be considered to reflect diminished synthesis while an increased ratio suggests enhanced synthesis.

Routine laboratory methods operational at the Department of Clinical Chemistry, University of Kuopio, were used for the determination of serum thyroxine, creatinine, creatine kinase, and liver enzymes.

**Statistical Analysis**

The data were analyzed using SAS (SAS Institute Inc., Cary, N.C.). The Pearson product–moment correlation was used for examining the linear association of changes in the concentrations of cholesterol precursor sterols and plant sterols with the change in the serum cholesterol concentration. The proportional within-group changes are given as mean percent changes, together with 95% confidence intervals (CIs), based on the t distribution. The t test for paired samples was used for comparisons within groups. The treatment groups were compared with analysis of covariance, adjusting for the difference at baseline in the distribution of the respective outcome variable. All probability values given are two sided. There was no adjustment for multiple comparisons.

Approval for the study had been given by the Ethics Committee of the University of Kuopio. Informed consent was given by all patients examined.

### Table 2. Serum Total Cholesterol and LDL Cholesterol at 0, 18, and 36 Weeks by Treatment Group

<table>
<thead>
<tr>
<th>Variable</th>
<th>Lovastatin+guar gum</th>
<th>Lovastatin+cholestyramine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 Week (n = 31)</td>
<td>18 Weeks (n = 31)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>10.6±1.6</td>
<td>9.9±1.3*</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>8.5±1.8</td>
<td>5.0±1.6*</td>
</tr>
</tbody>
</table>

**Results**

Table 1 gives the baseline characteristics of the study population by treatment group. There were no significant differences in age, sex distribution, prevalence of FH, body mass index, or serum lipid levels between the patients treated with L+GG and those treated with L+C, but serum levels of triglycerides tended to be higher in the L+C group.

Serum total cholesterol and LDL cholesterol concentrations declined to similar extents in both groups during lovastatin treatment (Table 2). Combining guar gum or cholestyramine with lovastatin resulted in significant additional reductions in serum total cholesterol and LDL cholesterol levels. The additional mean reduction in the serum total cholesterol concentration was 13% (95% CI, 9–18%) for L+GG and 21% (95% CI, 16–26%) for L+C (p = 0.048 for difference between groups). Detailed data on serum concentrations of lipids and lipoproteins during different treatment periods have been reported elsewhere.

Table 3 shows the serum levels of noncholesterol sterols during lovastatin treatment and the two combination treatments. Baseline data of the two groups tended to differ slightly so that the precursor sterol levels tended to be lower (significant for desmosterol) and other sterol levels higher (significant for campesterol) in the L+GG group than in the L+C group. These differences do not, however, influence the interpretation of the main results. Levels of cholesterol synthesis precursors also showed reductions during lovastatin treatment in both groups when the values were expressed in relation to the serum cholesterol level. The lathosterol to cholesterol ratio declined by 42% (95% CI, 32–52%) and 38% (95% CI, 30–45%) in the L+GG and L+C groups (p < 0.001 for both groups), respectively, during lovastatin therapy and rose significantly (p < 0.001 for both groups) during both combination therapies, by 51% (95% CI, 35–67%) in the L+GG group and by 212% (95% CI, 137–286%) in the L+C group, the change being significantly greater in the L+C group (p < 0.001, Figure 1). Similarly, increases in the cholestenol to cholesterol and desmosterol to cholesterol ratios were more marked after the combination of cholestyramine with lovastatin than of guar gum with lovastatin.

The serum contents of cholestanol, campesterol, and sitosterol were reduced to similar extents by lovastatin treatment, but when the values were given in relation to the cholesterol concentration small increases were seen even in the absence of reduced cholesterol synthesis. These differences do not, however, influence the interpretation of the main results. Levels of cholesterol synthesis precursors also showed reductions during lovastatin treatment in both groups when the values were expressed in relation to the serum cholesterol level. The lathosterol to cholesterol ratio declined by 42% (95% CI, 32–52%) and 38% (95% CI, 30–45%) in the L+GG and L+C groups (p < 0.001 for both groups), respectively, during lovastatin therapy and rose significantly (p < 0.001 for both groups) during both combination therapies, by 51% (95% CI, 35–67%) in the L+GG group and by 212% (95% CI, 137–286%) in the L+C group, the change being significantly greater in the L+C group (p < 0.001, Figure 1). Similarly, increases in the cholestenol to cholesterol and desmosterol to cholesterol ratios were more marked after the combination of cholestyramine with lovastatin than of guar gum with lovastatin.

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Table 3. Serum Noncholesterol Sterols During Lovastatin and Both Combination Therapies

<table>
<thead>
<tr>
<th>Variable</th>
<th>No drugs 0 Week</th>
<th>Lovastatin 18 Weeks</th>
<th>Lovastatin+ guar gum 36 Weeks</th>
<th>No drugs 0 Week</th>
<th>Lovastatin 18 Weeks</th>
<th>Lovastatin+ cholestyraraine 36 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squalene (μmol/l)</td>
<td>1.17±0.64</td>
<td>0.67±0.22*</td>
<td>1.24±0.43</td>
<td>1.20±0.38</td>
<td>0.76±0.32</td>
<td></td>
</tr>
<tr>
<td>Cholesterol/C</td>
<td>1.50±0.67</td>
<td>0.67±0.36f</td>
<td>1.71±1.0</td>
<td>1.80±0.58f</td>
<td>1.73±0.98f</td>
<td></td>
</tr>
<tr>
<td>Lathosterol/C</td>
<td>11.2±4.8</td>
<td>3.95±1.97</td>
<td>11.1±4.3</td>
<td>11.1±4.3</td>
<td>11.1±4.3</td>
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</tr>
<tr>
<td>Desmosterol/C</td>
<td>4.13±1.22</td>
<td>1.71±0.66f</td>
<td>4.49±1.45</td>
<td>4.36±1.49f</td>
<td>8.91±4.49f</td>
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<tr>
<td>Cholesterolan/C</td>
<td>8.04±1.65</td>
<td>5.91±1.61†</td>
<td>7.86±2.64</td>
<td>5.79±1.60†</td>
<td>2.27±1.69†</td>
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<tr>
<td>Campesterol/C</td>
<td>21.0±7.9</td>
<td>17.4±6.2†</td>
<td>15.7±7.2</td>
<td>15.6±6.2†</td>
<td>4.20±1.19†</td>
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</tr>
<tr>
<td>Sitosterol/C</td>
<td>13.3±4.6</td>
<td>11.6±3.8†</td>
<td>12.1±5.5</td>
<td>12.1±5.5</td>
<td>14.6±6.9</td>
<td></td>
</tr>
<tr>
<td>Concentration/C</td>
<td>12.0±7.3</td>
<td>10.2±3.5</td>
<td>12.9±6.3</td>
<td>12.1±5.5</td>
<td>14.6±6.9</td>
<td></td>
</tr>
<tr>
<td>Cholesterolan/C</td>
<td>16.1±7.6</td>
<td>11.7±7.3*</td>
<td>18.3±10.3</td>
<td>13.5±10.8†</td>
<td>36.6±19.0†</td>
<td></td>
</tr>
<tr>
<td>Lathosterol/C</td>
<td>117.45</td>
<td>65.0±31.3†</td>
<td>121.48±8.8</td>
<td>71.0±22.3†</td>
<td>205±85.4†</td>
<td></td>
</tr>
<tr>
<td>Desmosterol/C</td>
<td>43.8±11.6</td>
<td>28.4±9.8†</td>
<td>48.8±18.3</td>
<td>37.6±30.9</td>
<td>66.2±75.7†</td>
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<tr>
<td>Cholesterolan/C</td>
<td>85.7±17.3</td>
<td>98.2±23.1†</td>
<td>82.3±24.7</td>
<td>95.0±27.2†</td>
<td>87.6±23.1†</td>
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<tr>
<td>Campesterol/C</td>
<td>230±84</td>
<td>290±90.3†</td>
<td>259±112†</td>
<td>259±112†</td>
<td>384±151†</td>
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</tr>
<tr>
<td>Sitosterol/C</td>
<td>140±43</td>
<td>196±63.9†</td>
<td>167±54.8</td>
<td>167±59.2†</td>
<td>241±95.7†</td>
<td></td>
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</tbody>
</table>

Values are mean±SD. *p<0.01, †p<0.001, ‡p<0.05 compared with previous values.

Discussion

Various cholesterol synthesis precursors have been shown to reflect the rate of cholesterol synthesis in humans, and the measurement of these precursors' concentrations has been applied to study endogenous cholesterol synthesis in different clinical situations. In previous studies lovastatin has resulted in a 30–40% reduction of urinary mevalonate and the serum lathosterol to cholesterol ratio, most correlations were small and nonsignificant. The change in the serum total cholesterol concentration was even inversely related to the change in the squalene to cholesterol ratio.

The results were also analyzed separately for non-FH and FH patients, but no marked differences were found in serum noncholesterol sterol levels between non-FH and FH patients during the trial.
A high serum level of cholesterol, the 5α-saturated derivative of cholesterol, reflects high efficiency of intestinal sterol absorption but is inversely related to endogenous cholesterol synthesis and fecal excretion of neutral sterols. In the present study, the increase in the cholestanol to cholesterol ratio could be due to reduced biliary secretion caused by lovastatin. The small reduction in the ratio caused by the combination treatments, on the other hand, could be associated with reduced absorption and/or enhanced biliary output.

A positive correlation has been described between cholesterol absorption efficiency and the serum plant sterol to cholesterol ratios for campesterol and sitosterol. However, the serum ratios are also inversely related to biliary cholesterol secretion and fecal output of endogenous steroids. In patients with gut resections and cholesterol and fat malabsorption, serum campesterol and sitosterol levels in relation to the cholesterol level are low, whereas in patients with ileal bypass the values are high. The increase in plant sterol to cholesterol ratios of the present study during lovastatin treatment could be due to lowered biliary secretion caused by reduced cholesterol synthesis.

Interestingly, the two combination therapies had different effects on plant sterol levels. There were reductions in the absolute levels of campesterol and sitosterol as well as in their respective ratios with the cholesterol level after adding guar gum to lovastatin, but an increase was found in these values after adding cholestyramine. Because the serum levels of campesterol and sitosterol are positively associated with fractional cholesterol absorption and dietary plant sterols but inversely with endogenous cholesterol synthesis and biliary cholesterol secretion, several possible mechanisms could account for this unexpected finding. The most convenient explanation is that guar gum with lovastatin may result in reduction of cholesterol absorption efficiency, despite the finding that guar gum alone does not appear to interfere with cholesterol absorption. Cholestyramine, on the other hand, increases serum plant sterol levels, effectively interrupts the enterohepatic circulation of bile acids, and reduces cholesterol absorption during regular use. It can be hypothesized that the increases in serum levels of plant sterols during the combination of lovastatin and cholestyramine could be mainly due to reduced biliary sterol secretion.

In conclusion, both guar gum and cholestyramine stimulate cholesterol synthesis in patients on lovastatin therapy. Despite that, both drugs further reduce serum cholesterol levels.

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