Noncholesterol Sterols and Cholesterol Lowering by Long-Term Simvastatin Treatment in Coronary Patients: Relation to Basal Serum Cholestanol

T.A. Miettinen, T.E. Strandberg, H. Gylling, for the Finnish Investigators of the Scandinavian Simvastatin Survival Study Group

Abstract—Coronary patients with low baseline ratios of serum cholestanol and plant sterols to cholesterol (indicating low cholesterol absorption) but not those with high ratios (high absorption) experienced reduced recurrences of coronary events during simvastatin treatment in the Scandinavian Simvastatin Survival Study. Thus, in the present study, serum cholesterol, its precursor sterols (reflecting cholesterol synthesis), plant sterols (campesterol and sitosterol), and cholestanol were measured before and during a 5-year period of placebo treatment (n=433) and simvastatin treatment (n=434) in patients from a subgroup of the Scandinavian Simvastatin Survival Study to determine whether changes in cholesterol synthesis and serum levels were related to cholesterol absorption. Serum cholesterol level was unchanged, the ratios of cholesterol precursor sterols to cholesterol were decreased, and the ratios of plant sterols to cholesterol were increased in relation to increasing baseline ratios of cholestanol quartiles. The latter predicted 5-year ratios and simvastatin-induced reductions of the precursor sterols, with the lowering of the ratios (cholesterol synthesis reduction) being almost twice higher in the lowest versus the highest quartile. The ratios of plant sterols, especially campesterol, to cholesterol were markedly increased during simvastatin treatment, mostly in subjects with the highest baseline cholestanol quartiles. Simvastatin reduced serum cholesterol more (P=0.003) in the lowest versus the highest cholestanol quartile during the 5-year treatment period. The results show for the first time that baseline cholesterol metabolism, measured by serum noncholesterol sterols, predicts the effectiveness of simvastatin in reducing cholesterol synthesis and serum levels of cholesterol. The drug suppresses the synthesis of cholesterol markedly more effectively in subjects with high than with low baseline synthesis but reduces respective serum cholesterol levels less markedly than synthesis. Subjects with high cholesterol absorption and low synthesis may need a combination therapy to lower more effectively their serum cholesterol levels and prevent an increase in the levels of plant sterols. (Arterioscler Thromb Vasc Biol. 2000;20:1340-1346.)

Key Words: simvastatin ■ cholesterol lowering ■ cholestanol ■ lathosterol ■ sitosterol

Human serum contains small amounts of the precursors of cholesterol synthesis, including squalene and methylated and demethylated precursor sterols.1,2 From among the latter sterols, cholestenol, desmosterol, and lathosterol can easily be quantified by gas liquid chromatography (GLC),3 and the whole precursor sterol group is positively related to cholesterol synthesis.1,2,4-9 In addition, there are other noncholesterol sterols in serum, the most prominent of which are cholestanol and plant sterols (campesterol, sitosterol, and avenasterol). The higher the ratio of these sterols to cholesterol in serum, the higher is cholesterol absorption efficiency.8,10,11 Accordingly, ratios of noncholesterol sterols to cholesterol reflect absorption and synthesis activity of cholesterol, especially their changes by dietary or drug treatments in humans. Preliminary studies of the Finnish subgroup of the Scandinavian Simvastatin Survival Study (4S)12 revealed that in relation to the placebo group, inhibition of cholesterol synthesis by simvastatin reduced the ratios of the cholesterol precursor sterols to cholesterol by up to one third for the 5-year period of the 4S, increasing correspondingly the ratios of cholestanol and plant sterols to cholesterol. Coronary patients with the highest basal ratios of cholestanol to cholesterol did not show reduced recurrence of coronary events during the simvastatin treatment, whereas those with the lowest respective ratios were benefited by simvastatin treatment compared with placebo.13 It was tempting to interpret the findings on the basis of the different effects of statin in patients with varying absorption and/or synthesis of cholesterol. Thus, patients with a low ratio of cholestanol to cholesterol were considered to have low absorption and high synthesis of cholesterol, a situation that could be ideal for inhibition of cholesterol synthesis by statins. On the other
hand, patients with a high ratio of cholestanol to cholesterol most likely had high absorption and low synthesis of choles-
terol, so that the condition could be less favorable for the use
of synthesis inhibitors. The purpose of the present study was
to investigate the associations of baseline cholestanol with
other noncholesterol sterols and cholesterol in the sera of a 4S
subgroup at baseline and during the placebo and simvastatin
treatment periods for up to 5 years. Major interest was
focused on the lowering of serum cholesterol precursor
sterols and on relating their changes to lowered serum
cholesterol during simvastatin treatment of patients with
variable basal ratios of cholestanol to cholesterol.

Methods

Patients

The 4S population and the intervention methods used have been
published previously for the entire 4S cohort. The present study
population included the Finnish 4S subgroup of 867 coronary
patients. The serum cholesterol window was 5.5 to 8.0 mmol/L, and
triglycerides were <2.5 mmol/L on a lipid-lowering diet before
randomization into the placebo and simvastatin groups. The simva-
statin dose was initially 10 mg/d and was increased to 20 mg/d, but after
6 weeks of treatment, the dose was increased to 40 mg/d if serum
cholesterol values were >5.2 mmol/L.

Analytical Methods

Serum squalene and sterols were measured by GLC. Thus, total
cholesterol, squalene, and noncholesterol sterols, available for the
entire present study, were determined from the same GLC run. For
GLC, serum was saponified after addition of an internal standard
(5α-cholestanol) followed by extraction of nonsaponified material
and conversion to trimethylsilyl derivatives. GLC analysis was
performed on a 50-m-long SE-30 column with use of an automated
Hewlett Packard instrument equipped with an automated peak
calculator. Each run quantified squalene, cholesterol, cholestanol,
cholesterol, desmosterol, lathosterol, campesterol, sitosterol, and
avenasterol, as noted in increasing order of GLC retention times. The
avenasterol peak contained trace amounts of sitostanol and a meth-
ylated precursor sterol.

The noncholesterol sterols are transported in serum by lipopro-
teins, mainly by LDL. Thus, the decrease in concentration of LDL
cholesterol by simvastatin also changes the concentrations of non-
cholesterol sterols. Therefore, we have expressed the noncholesterol
sterols in terms of millimoles per mole of cholesterol, ie, as ratios of
noncholesterol sterols to cholesterol, and expressed them in the text
as noncholesterol sterol ratios. Ratios of precursor sterols to plant
sterols (expressed only for lathosterol/campesterol) were also calcu-
lated because this variable reflects synthesis and absorption
simultaneously.

Experimental Design

Two serum samples were analyzed from each patient before ran-
domization, and the mean value was calculated for the baseline ratio.
Other analyses were made at 6 weeks, 1 year, and 5 years (indicates
the end of the 5.4-year study) after baseline assessment from a single
serum sample each. The cholesterol value of this method was 7.2% lower
than the value of the central laboratory that was used for the
entire 4S study.

The patients were ranked by baseline cholestanol ratios into
quartiles (Table 1). This division into quartiles was used throughout
the study for evaluation of the 6-week, 1-year, and 5-year cholesterol
and other noncholesterol sterol values and respective changes from
the baseline.

Statistical Analysis

Mean±SD or mean±SE values were calculated for each quartile.
The baseline cholestanol ratios were correlated with the ratios of
other variables at the baseline and at 1 and 5 years and with the
corresponding changes from the baseline values. The differences
between the quartiles, the changes from the baseline, and the
differences between the placebo and simvastatin groups in the 4
cholestanol quartiles were analyzed by ANOVA.

Results

Baseline Data

Table 1 shows the baseline values of different variables by the
basal quartiles of the ratio of cholestanol to cholesterol. The
cholesterol concentrations and squalene ratios were not dif-
ferent between the quartiles, whereas the ratios of choles-
terol, desmosterol, and lathosterol significantly decreased,
and those of plant sterols increased.

Baseline ratios of plant sterols to cholesterol were insig-
ificantly related to cholesterol concentrations, but those of
cholesterol precursors exhibited weak negative respective
correlations (eg, squalene r=—0.235, lathosterol r=—0.140;
P<0.001 for both). Also, the ratios of precursor sterols to
plant sterols were weakly negatively related to basal choles-

<table>
<thead>
<tr>
<th>Variables</th>
<th>Q1 (n=217)</th>
<th>Q2 (n=217)</th>
<th>Q3 (n=217)</th>
<th>Q4 (n=216)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol, 10² mmol/mol*</td>
<td>91.7±12.6</td>
<td>116.0±5.6</td>
<td>136.0±6.3</td>
<td>171.8±22.8</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>28.3±3.8</td>
<td>26.9±3.5</td>
<td>26.2±2.9</td>
<td>25.1±2.9</td>
</tr>
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<td>Cholesterol, mmol/L</td>
<td>5.87±0.57</td>
<td>5.99±0.60</td>
<td>5.93±0.58</td>
<td>5.95±0.60</td>
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<tr>
<td>Squalene, 10² mmol/mol*</td>
<td>32.8±8.9</td>
<td>32.7±8.2</td>
<td>32.1±10.0</td>
<td>31.8±8.1</td>
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<td>Cholesterol, 10² mmol/mol*</td>
<td>19.9±8.9</td>
<td>15.5±8.3</td>
<td>13.3±7.7</td>
<td>10.2±6.2</td>
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<tr>
<td>Desmosterol, 10² mmol/mol*</td>
<td>104.7±68.2</td>
<td>79.4±19.7</td>
<td>71.7±17.4</td>
<td>63.2±13.3</td>
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<tr>
<td>Lathosterol, 10² mmol/mol*</td>
<td>219.0±60.2</td>
<td>179.9±45.9</td>
<td>161.2±45.6</td>
<td>136.5±40.7</td>
</tr>
<tr>
<td>Campesterol, 10² mmol/mol*</td>
<td>133.8±50.9</td>
<td>170.7±57.9</td>
<td>199.2±61.0</td>
<td>259.6±99.6</td>
</tr>
<tr>
<td>Sitosterol, 10² mmol/mol*</td>
<td>96.4±31.9</td>
<td>118.8±35.6</td>
<td>137.1±36.8</td>
<td>174.4±56.5</td>
</tr>
<tr>
<td>Avenasterol, 10² mmol/mol*</td>
<td>36.2±9.6</td>
<td>40.2±10.4</td>
<td>44.1±11.0</td>
<td>52.8±14.2</td>
</tr>
<tr>
<td>Lathosterol/campesterol</td>
<td>2.01±1.42</td>
<td>1.22±0.65</td>
<td>0.91±0.44</td>
<td>0.62±0.36</td>
</tr>
</tbody>
</table>

Values are mean±SE. BMI indicates body mass index.
*Indicates 10² mmol/mol of cholesterol.
†P<0.05 by ANOVA.
TABLE 2. Correlations of Baseline, 6 wk, 1-y, and 5-y Variables With Baseline Cholesterol/Cholesterol Ratio in Simvastatin-Treated Subjects in Finnish Subgroup of 4S Study

<table>
<thead>
<tr>
<th>Variables</th>
<th>Baseline (n=434)</th>
<th>6 wk (n=422)</th>
<th>1 y (n=423)</th>
<th>5 y (n=397)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol, mmol/L</td>
<td>0.074</td>
<td>0.195*</td>
<td>0.039</td>
<td>0.099*</td>
</tr>
<tr>
<td>Cholesterol, 10² mmol/mol†</td>
<td>−0.434*</td>
<td>−0.336*</td>
<td>−0.304*</td>
<td>−0.327*</td>
</tr>
<tr>
<td>Desmosterol, 10² mmol/mol†</td>
<td>−0.399*</td>
<td>−0.407*</td>
<td>−0.383*</td>
<td>−0.362*</td>
</tr>
<tr>
<td>Lathosterol, 10² mmol/mol†</td>
<td>−0.565*</td>
<td>−0.402*</td>
<td>−0.424*</td>
<td>−0.364*</td>
</tr>
<tr>
<td>Cholesterol, 10² mmol/mol†</td>
<td>1.00</td>
<td>0.919*</td>
<td>0.880*</td>
<td>0.765*</td>
</tr>
<tr>
<td>Campesterol, 10² mmol/mol†</td>
<td>0.589*</td>
<td>0.543*</td>
<td>0.531*</td>
<td>0.492*</td>
</tr>
<tr>
<td>Sitosterol, 10² mmol/mol†</td>
<td>0.603*</td>
<td>0.534*</td>
<td>0.682*</td>
<td>0.511*</td>
</tr>
</tbody>
</table>

*P<0.05. In placebo group, statistical significances were similar to those in simvastatin group, except that correlations of cholesterol were nonsignificant. †Indicates 10² mmol/mol of cholesterol.

Simvastatin and Placebo Treatments

Body Weight

Body weight was increased by 0.34±0.03 kg (P<0.001) in the whole population at 1 year and was similar in all cholestanol quartiles. At 5 years, body weight increased in the survivors by 0.70±0.06 kg (P<0.001) from the baseline value, and it increased gradually from the first to fourth cholestanol quartile by 0.50±0.12, 0.65±0.11, 0.68±0.14, and 0.97±0.13 kg, respectively, without specific simvastatin effect.

Cholesterol Precursors

Correlations of the baseline ratios of cholestanol to cholesterol with different lipid variables and their changes during the study period are shown in Tables 2 and 3, respectively, for the simvastatin group. The correlations remained significantly negative in the placebo group also (not shown) throughout the study for each precursor sterol but not for squalene, which was not changed by simvastatin. The higher ratios of cholestanol to cholesterol, the smaller were correlations in Table 2. The ratios in the placebo group tended to increase gradually from the baseline with time, especially in the fourth quartile (significantly so at 5 years). The mean 6-week reductions of cholestanol, desmosterol, and lathosterol by simvastatin were 33%, 21%, and 34% from the baseline, but the respective reductions were markedly higher in the first (42%, 26%, and 40%) than in the fourth (24%, 18%, and 30%) quartile. The reductions tended to decrease with treatment time, especially after the first year (Figure 1). However, the mean absolute reductions in the ratios of the precursor sterols to cholesterol from the respective placebo values were ~2 times higher in the first than in the fourth quartile over the 5-year treatment period (Figure 2). The time-dependent increase of the precursor sterol curves in Figure 1 was explainable by the respective body mass index adjustment only in the placebo group for the first quartile (P<0.05), but it was still significant for other curves (P<0.01).

TABLE 3. Correlations of Changes of 6-wk, 1-y, and 5-y Variables With Baseline Cholesterol/Cholesterol Ratio in Simvastatin-Treated Subjects in Finnish Subgroup of 4S Study

<table>
<thead>
<tr>
<th>Variables</th>
<th>Change in Sterols</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 wk (n=422)</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>0.125*</td>
</tr>
<tr>
<td>Cholesterol, 10² mmol/mol†</td>
<td>0.376*</td>
</tr>
<tr>
<td>Desmosterol, 10² mmol/mol†</td>
<td>0.296*</td>
</tr>
<tr>
<td>Lathosterol, 10² mmol/mol†</td>
<td>0.434*</td>
</tr>
<tr>
<td>Cholesterol, 10² mmol/mol†</td>
<td>−0.097*</td>
</tr>
<tr>
<td>Campesterol, 10² mmol/mol†</td>
<td>0.160*</td>
</tr>
<tr>
<td>Sitosterol, 10² mmol/mol†</td>
<td>0.139*</td>
</tr>
</tbody>
</table>

*P<0.05. In placebo group, the following correlations were significant: lathosterol at 5 y (r=−0.100), cholesterol at each time (r=−0.108 at 6 wk, −0.284 at 1 y, and −0.183 at 5 y), campesterol at 1 y (r=0.099), and sitosterol at 5 y (r=−0.178).
Cholestanol and Plant Sterols
The basal ratios of cholestanol to cholesterol were positively related to those of plant sterols and cholestanol in the placebo group and to changes of plant sterols throughout the study but were negatively related to their own changes (Tables 2 and 3) in the placebo group throughout the study. In the placebo group, the ratios of sitosterol and especially of cholestanol to cholesterol were reduced from the basal values with time significantly more in the fourth than in the first quartile, whereas those of campesterol were similarly increased in all quartiles (Figure 3).

Simvastatin increased the cholestanol ratios from the baseline values significantly more in the first than in the fourth quartile, whereas the highest plant sterol ratios and levels occurred in the fourth quartile (Figures 3 and 4). The increments from the respective placebo values were higher for cholestanol in the first than in the fourth quartile, whereas for the plant sterols, the increments were higher in the fourth than in the first quartile. The differences were virtually similar between the 2 quartiles during the entire treatment period, and the difference even increased with time for campesterol in the fourth quartile.

Serum Cholesterol
In the placebo group, cholesterol did not change significantly during the 5-year period, and it was not related to the baseline cholestanol ratios at any time point (Figure 5).

Serum cholesterol concentration of the survivors, defined by the first and fourth quartiles of basal cholestanol ratios, was reduced by simvastatin more in the first than in the fourth quartiles (Figure 5) despite the significantly lower number of patients (21 versus 36) on high doses of simvastatin. The difference between the 2 quartiles was slight but statistically significant by ANOVA ($F=8.62$, $P=0.003$); at 6 weeks, the difference between the 2 quartiles was significant ($P<0.001$), and the decrease of cholesterol was higher in the first than in the fourth quartile (29.4±0.9% versus 25.6±0.9%, $P<0.001$). In addition, the baseline cholestanol ratios were positively related to the cholesterol concentrations (Table 2) in the simvastatin but not in the placebo group at 6 weeks ($r=0.195$, $P<0.001$) and 5 years ($r=0.099$, $P=0.048$) and to cholesterol reduction at 6 weeks (Table 3; $r=0.127$, $P<0.001$).

Discussion
The present study relates for the first time baseline cholestanol metabolism to the effectiveness of statin (1) to lower cholesterol synthesis, (2) to increase serum plant sterols, and (3) to reduce serum cholesterol concentration. In the main 4S,
The patients needing titration of simvastatin doses from 20 to 40 mg/d had higher basal cholesterol levels than did those not needing the titration for the insufficient cholesterol lowering.17 Our unpublished findings on noncholesterol sterols suggest that the titrated subjects of the present series had high basal absorption and low synthesis of cholesterol and that the increase of the dose enhanced the inhibition of synthesis and lowered cholesterol.

The baseline cholestanol ratio was highly significantly related to other noncholesterol sterols (positively to the plant sterols and negatively to the precursor sterols) in the coronary subjects during the entire study period of 5 years. Similar findings were observed earlier in a small cross-sectional group of randomly selected male subjects on a normal western diet.8 Thus, the subjects in the highest cholestanol quartile have the highest absorption efficiency and the lowest synthesis of cholesterol also among the coronary patients on a cholesterol-lowering diet.

The marked fall in the ratios of the cholesterol precursor sterols can be ascribed to simvastatin-induced inhibition of cholesterol synthesis, a finding reported earlier for different statins in small groups of short-term studies.7,18–22 The baseline cholestanol ratios and quartiles predicted throughout the study the precursor sterol reductions. The correlations in the simvastatin-treated patients are most likely reflecting gradually decreasing cholesterol synthesis in the patients from the lowest to the highest cholestanol quartile. Thus, the markedly higher reduction of the precursor sterol ratios in the first than in the fourth quartile (Figure 1) indicates lower inhibitory action of simvastatin on cholesterol synthesis in the highest than in the lowest cholestanol quartile.

Increased body weight increases cholesterol synthesis.23 Thus, the slight gradual increase of body weight, in proportion to the basal cholestanol quartiles, would be expected to stimulate cholesterol synthesis during the 5-year period more in the highest than in the lowest quartiles of the placebo and simvastatin groups. Accordingly, the differences between the simvastatin and placebo curves of the respective quartiles remained virtually unchanged throughout the treatment period (Figure 2).

Statin treatment reduces cholesterol absorption in patients with familial hypercholesterolemia,20 in experimental animals on a high but not on a low cholesterol diet,24,25 and in patients with no familial hypercholesterolemia.18 The increased ratios of cholestanol and plant sterols during the simvastatin treatment have been interpreted in our earlier statin studies18–20,26 to indicate reduced turnover of cholesterol by decreased synthesis, resulting in reduced biliary elimination of sterols, especially in the fourth quartile, with highest sterol absorption. Reasons are not known for the different responses of cholestanol ratios, especially in relation to campesterol (Figure 3), and for the reduction of cholestanol and sitosterol in the placebo group during the 5-year period. It should be borne in mind that the increase of body weight reduces the absorption efficiency of cholesterol and serum plant sterol ratios.27,28

The increased ratios of the plant sterols during the simvastatin treatment in the fourth quartile raise a question about their atherogenicity. Namely, phytosterolemia is strongly atherogenic,29,30 and high plant sterol levels have been suggested to provoke coronary heart disease even in nonsitosterolemic subjects.31 Not only the ratios but also the final 5-year campesterol and sitosterol concentrations were increased in the survivors despite reduced LDL cholesterol concentration, from the usual basal values of <1 mg/dL up to 2.8 and 1.5 mg/dL, respectively. Exchange of this type of sterol mixture with cholesterol of cell membranes or transfer, for instance in LDL, in the arterial walls of the patients may be functionally harmful, as evidenced by phytosterolemia. The atherogenic
limit of plant sterol concentrations or of the ratios of plant sterols to cholesterol is not known, but in phytosterolemia, sitosterol concentrations, usually 10 to 30 mg/dL, are associated with coronary artery disease at a young age.

It is tempting to suggest that the high baseline absorption and low synthesis of cholesterol results in a less favorable cholesterol lowering in the highest cholestanol quartile. In fact, the higher the basal cholestanol quartile, the lesser was the fall in the serum cholesterol concentration and the higher was the final treatment level of cholesterol even in long-term simvastatin treatment of coronary patients (Figure 5). Despite markedly higher reduction of cholesterol synthesis (lowered precursor ratios) by simvastatin in the patients in the lowest versus the highest cholestanol quartile, the respective difference in the serum cholesterol level was markedly lesser. This cholesterol difference alone could not be responsible for the different clinical response; something else, eg, serum plant sterols, would have to be contributing. The change in cholesterol synthesis is weakly related to serum cholesterol lowering, as illustrated by sterol balance studies showing virtually no change or slight reduction in the overall synthesis of body cholesterol and a marked decrease in serum cholesterol by resins or plant stanol esters markedly increase cholesterol synthesis, but they do not consistently lower serum cholesterol.

A highly significant negative correlation of the basal cholestanol ratio with ratios of the cholesterol precursor sterols and a positive correlation with ratios of the plant sterols for up to 5 years indicated that the high basal cholestanol quartile still predicted high absorption and low synthesis of cholesterol even for the 5-year survivors in the simvastatin-treated group. Thus, these patients with the high basal cholestanol ratios should be treated with combined stimulation and inhibition of cholesterol synthesis from the very beginning of hypolipidemic treatment. For instance, starting treatment with resins or plant stanol esters stimulates synthesis by respective malabsorption of bile acids or cholesterol, followed by statin-induced inhibition of synthesis in resistant cases. Replacement of normal dietary fat intake by the same amount of plant stanol ester margarine during a chronic simvastatin treatment actually revealed a more consistent (≈10%) fall in the serum cholesterol level of coronary patients with a high versus a low basal cholestanol ratio.

Acknowledgments

This study was supported by grants from the Finnish Academy of Medical Sciences, the University Central Hospital of Helsinki, and Merck & Co, Inc.

References


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doi: 10.1161/01.ATV.20.5.1340

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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