Effects of Diet and Simvastatin on Fatty Acid Composition in Hypercholesterolemic Men
A Randomized Controlled Trial

Antti Jula, Jukka Marniemi, Tapani Rönnemaa, Arja Virtanen, Risto Huupponen

Objective—To explore the separate and combined effects of simvastatin and a low-saturated diet rich in $\alpha$-linolenic acid on serum fatty acids.

Methods and Results—120 hypercholesterolemic men were randomly allocated to a habitual diet or dietary treatment group and to receive, in random order, simvastatin 20 mg/d or placebo, each for 12 weeks, in a double-blind manner. Dietary treatment decreased proportions from total fatty acids of palmitic acid (C16:0) by 3.3% ($P<0.05$), stearic acid (C18:0) by 3.7% ($P<0.05$) and increased proportions of oleic acid (C18:1n-9) by 4.2% ($P<0.01$), and $\alpha$-linolenic acid (C18:3n-3) by 29.8% ($P<0.001$). Simvastatin decreased proportions from total fatty acids of palmitic acid by 2.0% ($P<0.01$), linoleic acid (C18:2n-6) by 5.3% ($P<0.001$), and $\alpha$-linolenic acid by 6.8% ($P<0.05$), and increased proportions of $\gamma$-linolenic acid (C18:3n-6) by 11.1% ($P<0.001$), dihomo-$\gamma$-linolenic acid (C20:3n-6) by 4.2% ($P<0.01$), arachidonic acid (C20:4n-6) by 14.2% ($P<0.001$), and the sum of long-chain polyunsaturated fatty acids (C20-22) by 9.0% ($P<0.001$). Simvastatin increased ratios of stearic to palmitic, $\gamma$-linolenic to linoleic, and arachidonic to dihomo-$\gamma$-linolenic acid by 7.6%, 17.0%, and 10.0% ($P<0.001$ for all), respectively, suggesting increased fatty acid elongase and $\Delta$6- and $\Delta$5-desaturase enzyme activities.


Key Words: fatty acid metabolism ■ diet ■ hypercholesterolemia ■ statins

The effects of simvastatin and a diet low in saturated fatty acids but enriched in monounsaturated and polyunsaturated fatty acids including $\alpha$-linolenic acid, cereals, and berries on circulating low-density lipoprotein (LDL) cholesterol are independent and additive, with the effect of simvastatin being 3-fold on LDL cholesterol and 2-fold on circulating oxidized LDL cholesterol compared with that of dietary treatment.1 In secondary prevention of coronary heart disease, dietary trials characterized by a low intake of saturated fats,2,3 an increased intake of omega-3 fatty acids of marine4 or plant origin,2,3 and a high intake of fresh fruits and vegetables, legumes, and cereals2,3 have reported a similar reduction in cardiovascular morbidity or mortality as observed by cholesterol-lowering treatment with statins.2,8 In addition to lowering of serum lipid concentrations, part of the effects of statins and most of the effects of a modified Mediterranean-type diet may be mediated by factors associated with platelet aggregation, hemostasis, fibrinolysis, endothelial function, and the propensity for arrhythmia.9,10

The mechanisms behind the pleiotropic actions of statins are largely obscured. However, by inhibiting 3-hydroxy-3-methylglutaryl (HMG)-coenzyme A (CoA) reductase, the key enzyme of the cholesterol synthesis pathway originating from acetyl-CoA, the statins may increase the availability of acetyl-CoA for other metabolic pathways such as fatty acid synthesis through malonyl-CoA, or oxidation of acetyl-CoA in the Krebs cycle. Fatty acids may affect vascular functions per se, or they may serve as precursors for active mediators. Long-chain fatty acids, especially those of the omega-3 class, appear to have beneficial effects on vascular endothelial function.11 A cascade of reactions induced by elongase and desaturase enzymes converts short-chain fatty acids to long-chain ones, of which some such as dihomo-$\gamma$-linolenic acid, arachidonic acid, and eicosapentaenoic acid, are precursors of eicosanoids with potent vasoactive, anti-/prothrombotic, anti-/proinflammatory, and possibly anti-/proatherogenic effects.

Studies using experimental animals and cultured cell lines suggest that simvastatin increases formation of long-chain polyunsaturated fatty acids.12-14 Only limited information exists on the effects of statins on serum fatty acid metabolism in humans.15 The aim of this study was to characterize the
Subjects and Study Design
Previously untreated hypercholesterolemic men (n=120), 35 to 64 years of age, with fasting serum cholesterol concentration between 232 and 309 mg/dL (6.0 and 8.0 mmol/L), and fasting serum triglyceride concentration between 232 and 309 mg/dL (6.0 and 8.0 mmol/L), and fasting serum cholesterol concentration between 112 subjects and a 10% increase in the formation of long-chain fatty acids (C20-22) with a sample size of 117 subjects.

At the end of a 4- to 6-week open placebo run-in period, participants were randomly allocated to a habitual diet or a dietary treatment group (Figure 1). In both groups, a second randomization was performed and the subjects received simvastatin 20 mg/d and a matching placebo for 12 weeks in a double-blind, crossover fashion.

The study was conducted after the latest revision of the Declaration of Helsinki and was approved by the appropriate Ethics Committee.

Measurements and Analyses
Diet was recorded and 12-hour fasting blood samples were taken before randomization at the end of the placebo run-in period (baseline), and at the end of the 2 12-week drug treatment periods.

All measurements and analyses were performed blinded to the treatment allocation of the subject. The serum samples were frozen and stored at −70°C until assayed. The baseline and follow-up samples were analyzed always in 1 analytical run. Diet was monitored through 7-day food records using household measures. The records were analyzed by means of the Nutrica food and nutrient calculation software and the databases on the nutrient composition of Finnish food. Serum cholesterol concentrations were determined by enzymatic methods (Merck Diagnostica, Darmstadt, Germany).

For determination of serum total fatty acid composition, lipids were extracted from the serum with chloroform-methanol (2:1). The fatty acid methyl esters were synthesized using 14% boron trifluoride in methanol. The methyl esters were analyzed using a gas chromatograph (Varian CP-3800; Varian Inc, Walnut Creek, Calif) equipped with a 30-m×0.25-mm glass capillary column (stationary phase 50% cyanopropylphenyl-methyloxypolysiloxane; J&W Scientific, Folsom, Calif). The oven temperature increased 5°C/min from 140°C to 220°C during the analysis run. The peaks were identified on the basis of retention times recorded for different standards. Heptadecanoic acid (C17:0) was used as an internal standard. The fatty acids were quantified by peak areas relative to heptadecanoic acid using Star Chromatography Workstation software (Star Toolbar, version 5.50; Varian Inc).

Product-to-precursor ratios of fatty acids and the proportion of polyunsaturated fatty acids from the total fatty acids were calculated to characterize the activities of the elongase and desaturase enzymes and the overall activity of fatty acid metabolism, respectively.

Dietary and Drug Treatments
The targeted composition of the weight-stable diet was: ≤10% energy from saturated plus transunsaturated fatty acids; cholesterol intake ≤250 mg/d; omega-3 fatty acid intake of plant origin (α-linolenic acid) plus marine origin ≥4 g/d and the ratio of omega-6/omega-3 polyunsaturated fatty acids <4; and increased intakes of fruit, berries, vegetables, and soluble fiber. The subjects randomized to the dietary treatment group were instructed in and advised to use the modified Mediterranean-type diet, as described elsewhere in detail.1 The subjects randomized to the habitual diet group were advised to continue their usual diet during the study period. Capsules containing simvastatin or placebo were prepared in a local pharmacy according to the European Pharmacopoeia, as described elsewhere.1

Statistical Analyses
Baseline (at the end of the placebo run-in period) comparisons between the dietary treatment and habitual diet groups were made by Student t test to verify the success of the randomization. Carry-over effects were tested separately for dietary and habitual diet groups for all fatty acids. Because no carry-over effects were observed ANOVA for repeated measures of variance, with contrasts between baseline and simvastatin or placebo treatment periods, was used to test the significance of dietary changes within the dietary treatment and habitual diet groups. Repeated analyses of covariance with baseline values as covariates, dietary treatment, and habitual diet, as between subject factors, and placebo and simvastatin treatment, as within subject factors, were included in the final models to test the significance of effects and interactions of simvastatin and dietary treatment. Models with insulin and cholesterol as time-dependent covariates were used to test associations of insulin and cholesterol on fatty acid ratios reflecting Δ6- and Δ5-desaturase and elongase enzyme activities. Validity of the models was evaluated with residual analysis. Normality of residuals was checked by the Shapiro–Wilk statistics and constancy of residuals by a graphic analysis. Log or square root transformations were applied, if necessary. Because statistical inferences after transformation were unchanged, raw results are reported. The data are given as mean±SE values with 95% CIs for the mean changes. All statistical analyses were conducted with SAS version 8.2 (SAS Institute, Cary, NC).

Results
The baseline characteristics of subjects randomized to the dietary treatment or habitual diet groups are summarized in Tables 1 and 2. The groups did not differ from each other in age and dietary intake and fasting serum levels of fatty acids (Tables 1 and 2). As compared with the habitual diet group, subjects in the dietary treatment group had slightly lower levels of total cholesterol (Table 1).

The effects of the treatments on serum lipids and antioxidants have been summarized elsewhere.1 Compliance to the drug treatment was good and, on the average, dietary treatment group achieved the predetermined target values.1 The proportion of fats in total energy intake remained unchanged. The percentage of energy derived from saturated fatty acids separate and combined effects on serum fatty acid composition of simvastatin, a HMG-CoA reductase inhibitor, and of a diet low in saturated fatty acids and enriched in monounsaturated and polyunsaturated fatty acids (PUFAs), especially α-linolenic acid, cereals, fruits, berries, and vegetables.
decreased to <10 because of decreased intake of myristic, palmitic, and stearic acids (Table 2). The intake of monoenoic acid (largely oleic acid), linoleic acid, and \(\alpha\)-linolenic acid increased, reflecting increased intake of rapeseed oil (Table 2). The mean dietary ratio of linoleic to \(\alpha\)-linolenic acid decreased from 6.6 to 3.2.

**Serum Fatty Acid Composition**

Dietary treatment did not change serum total fatty acid concentration (Table 2), but it decreased the proportion of palmitic acid (C16:0) from total fatty acids by 3.3% and that of stearic acid (C18:0) by 3.7% (Table 3). The proportions of oleic acid (C18:1), \(\alpha\)-linolenic acid (C18:3 n-3), and the sum of C18-22 polyunsaturated n-3 fatty acids were increased by 4.2%, 29.8%, and 9.3%, respectively (Table 3 and Figure 2). Simvastatin decreased serum total fatty acid concentration by 13.0% \((P<0.001)\) and the concentrations of several fatty acids, including \(\alpha\)-linolenic, eicosatetraenoic (C20:4 n-3), and docosahexaenoic (C22:6 n-3) acid (Table 2). It decreased the proportion of myristic acid (C14:0) from total fatty acids by 11.5%, and that of palmitic, linoleic (C18:2 n-6) and \(\alpha\)-linolenic acid by 2.0%, 5.3%, and 6.8%, respectively (Table 3 and Figure 2). The proportions of \(\gamma\)-linolenic acid (C18:3 n-6), dihomo-\(\gamma\)-linolenic (C20:3 n-6), and arachidonic acid (C20:4 n-6) from total fatty acids in serum were increased by 11.1%, 4.2%, and 14.2%, respectively (Table 3 and Figure 2).

The effects of dietary treatment and simvastatin on the proportions of serum fatty acids were independent and additive (Table 3 and Figure 2).

**Indices of Fatty Acid Metabolism**

Simvastatin increased the proportion of long-chain fatty acids (C20–22) from total fatty acids by 9.0%, and the ratios of stearic to palmitic acid (reflecting elongase activity) by 7.6%, \(\gamma\)-linolenic to linoleic acid (reflecting \(\Delta6\) desaturase activity) by 17.0%, and the ratios of arachidonic to dihomo-\(\gamma\)-linolenic and eicosapentaenoic to eicosatetraenoic acid, both reflecting \(\Delta5\) desaturase activity, by 10.0% and 26.9%, respectively (Table 3).

**TABLE 1. Age, Baseline Serum Cholesterol, Insulin, and Fatty Acids of Men Randomized Into the Dietary Treatment and Habitual Diet Groups**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Habitual Diet</th>
<th>Dietary Treatment</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects, N</td>
<td>60</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>48.4 (6.2)</td>
<td>48.0 (6.2)</td>
<td>0.72</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL*</td>
<td>259 (24)</td>
<td>250 (21)</td>
<td>0.04</td>
</tr>
<tr>
<td>Total fatty acids, g/L</td>
<td>4.2 (0.8)</td>
<td>4.0 (0.8)</td>
<td>0.23</td>
</tr>
<tr>
<td>Myristic acid (C14:0), % from TFA</td>
<td>0.6 (0.3)</td>
<td>0.5 (0.3)</td>
<td>0.12</td>
</tr>
<tr>
<td>Palmitic acid (C16:0), % from TFA</td>
<td>19.3 (2.6)</td>
<td>19.2 (2.4)</td>
<td>0.82</td>
</tr>
<tr>
<td>Palmitoleic acid (C16:1n-7), % from TFA</td>
<td>2.0 (0.8)</td>
<td>1.9 (0.7)</td>
<td>0.30</td>
</tr>
<tr>
<td>Stearic acid (C18:0), % from TFA</td>
<td>7.3 (1.0)</td>
<td>7.3 (1.0)</td>
<td>0.89</td>
</tr>
<tr>
<td>Oleic acid (C18:1n-9), % from TFA</td>
<td>24.5 (2.6)</td>
<td>23.9 (2.3)</td>
<td>0.18</td>
</tr>
<tr>
<td>Linoleic acid (C18:2n-6), % from TFA</td>
<td>27.5 (3.7)</td>
<td>28.2 (3.8)</td>
<td>0.31</td>
</tr>
<tr>
<td>(\gamma)-linolenic acid (C18:3n-6), % from TFA</td>
<td>0.4 (0.1)</td>
<td>0.4 (0.1)</td>
<td>0.37</td>
</tr>
<tr>
<td>Dihomo-(\gamma)-linolenic acid (C20:3n-6), % from TFA</td>
<td>1.6 (0.4)</td>
<td>1.7 (0.4)</td>
<td>0.59</td>
</tr>
<tr>
<td>Arachidonic acid (C20:4n-6), % from TFA</td>
<td>6.7 (1.6)</td>
<td>6.7 (1.3)</td>
<td>0.87</td>
</tr>
<tr>
<td>(\alpha)-linolenic acid (C18:3n-3), % from TFA</td>
<td>1.2 (0.3)</td>
<td>1.1 (0.4)</td>
<td>0.31</td>
</tr>
<tr>
<td>Eicosatetraenoic acid (C20:4n-3), % from TFA</td>
<td>0.2 (0.1)</td>
<td>0.2 (0.1)</td>
<td>0.72</td>
</tr>
<tr>
<td>Eicosapentaenoic acid (C20:5n-3), % from TFA</td>
<td>1.8 (0.8)</td>
<td>2.0 (1.1)</td>
<td>0.40</td>
</tr>
<tr>
<td>Docosahexaenoic acid (C22:6n-3), % from TFA</td>
<td>3.3 (1.1)</td>
<td>3.5 (1.1)</td>
<td>0.28</td>
</tr>
<tr>
<td>C20–22 polyunsaturated fatty acids†, % from TFA</td>
<td>12.8 (2.9)</td>
<td>13.1 (2.6)</td>
<td>0.53</td>
</tr>
<tr>
<td>C18:0 to C16:0 ratio‡</td>
<td>0.4 (0.1)</td>
<td>0.4 (0.1)</td>
<td>0.93</td>
</tr>
<tr>
<td>C16:1n-7 to C16:0 ratio§</td>
<td>0.1 (0.03)</td>
<td>0.1 (0.03)</td>
<td>0.23</td>
</tr>
<tr>
<td>C18:3n-6 to C18:2n-6 ratio¶</td>
<td>0.014 (0.005)</td>
<td>0.013 (0.005)</td>
<td>0.33</td>
</tr>
<tr>
<td>C20:4n-6 to C20:3n-6 ratio‖</td>
<td>4.2 (1.0)</td>
<td>4.2 (1.2)</td>
<td>0.79</td>
</tr>
<tr>
<td>C20:5n-3 to C20:4n-3 ratio‖</td>
<td>10.6 (6.8)</td>
<td>11.5 (8.0)</td>
<td>0.52</td>
</tr>
</tbody>
</table>

TFA indicates total fatty acids.

*To convert cholesterol to mmol/L, multiply values by 0.0259.
†Indicates the sum of fatty acids C20:4n-6, C22:4n-6, C20:5n-3, C22:5n-3, and C22:6n-3.
‡Indicates the activity of elongase enzyme.
§Indicates the activity of \(\Delta9\) desaturase enzyme.
¶Indicates the activity of \(\Delta6\) desaturase enzyme.
‖Indicates the activity of \(\Delta5\) desaturase enzyme.
Dietary treatment neither increased the proportion of long-chain fatty acids nor affected the indicators of fatty acid elongase and Δ6- and Δ5-desaturase enzyme activities (Table 3).

According to ANOVA for repeated measures including insulin and cholesterol as time-dependent variables, changes in serum insulin were positively associated with changes in the ratio of γ-linolenic to linoleic acid (indicator of Δ6 desaturase activity) \((P<0.02)\) and inversely with changes in the ratio of arachidonic to dihomo-γ-linolenic acid (indicator of Δ5-desaturase enzyme activity) \((P<0.02)\). No association was found between changes in insulin in one part and in the ratio of eicosapentaenoic to eicosatetraenoic acid (indicator of Δ5-desaturase enzyme activity), and the ratio of stearic to palmitic acid (indicator of elongase activity) in another part. Changes in cholesterol were not associated with changes in the indicators of Δ6-desaturase and Δ5-desaturase activities. A decrease in cholesterol during simvastatin was significantly \((P=0.0027)\) associated with an increase in the ratio of stearic to palmitic acid (indicator of elongase activity).

**Discussion**

We evaluated the separate and combined effects of diet and simvastatin on serum fatty acids in a representative patient group. The effects of dietary treatment on serum fatty acids were consistent with published data and reflected changes in the fatty acid composition of the diet. A new finding was that simvastatin increased the formation of long-chain polyunsaturated fatty acids with important functions in the membranes of several tissues, such as endothelium and thrombocytes. These functions may partly be mediated through eicosanoid metabolites of the membrane-bound 20-carbon fatty acids arachidonic (the precursor of 2-series prostaglandins and thromboxanes and 4-series leukotrienes), dihomo-γ-linolenic (the precursor of 1-series prostaglandins), and eicosapentaenoic (the precursor of 3-series prostaglandins and thromboxanes and 5-series leukotrienes) acid.

In our study, simvastatin decreased serum total fatty acid concentration by 13% while simultaneously increasing the proportion of long-chain polyunsaturated fatty acids from total fatty acids. From circulating total fatty acids, 45% are in
TABLE 3. Effects of Dietary Treatment and Simvastatin on the Proportion and Ratios of Serum Fatty Acids*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean (SE) [95% CI]</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Changes in the proportion from total fatty acids (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C14:0 (myristic acid)</td>
<td>4.7 (5.6) [-6.4 to 15.7]</td>
<td>-11.5 (3.9) [-19.2 to -3.7]</td>
</tr>
<tr>
<td>C16:0 (palmitic acid)</td>
<td>-3.3 (1.4) [-6.0 to -0.5]</td>
<td>-2.0 (0.8) [-3.5 to -0.5]</td>
</tr>
<tr>
<td>C16:1 (palmitoleic acid)</td>
<td>2.3 (4.3) [-6.1 to 10.8]</td>
<td>-6.2 (2.1) [-10.3 to -2.1]</td>
</tr>
<tr>
<td>C18:0 (stearic acid)</td>
<td>-3.7 (1.5) [-6.6 to -0.8]</td>
<td>4.1 (1.1) [2.0 to 6.3]</td>
</tr>
<tr>
<td>C18:1n-9 (oleic acid)</td>
<td>4.2 (1.9) [1.3 to 7.1]</td>
<td>1.9 (0.8) [0.3 to 3.5]</td>
</tr>
<tr>
<td>C18:2n-6 (linoleic acid)</td>
<td>-2.1 (1.8) [-5.6 to 1.4]</td>
<td>-5.3 (1.0) [-7.2 to -3.4]</td>
</tr>
<tr>
<td>C18:3n-6 (γ-linolenic acid)</td>
<td>4.4 (4.5) [-4.5 to 13.3]</td>
<td>11.1 (2.6) [5.8 to 16.3]</td>
</tr>
<tr>
<td>C20:3n-6 (dihomo-γ-linolenic acid)</td>
<td>-4.6 (2.0) [-8.7 to -0.5]</td>
<td>4.2 (1.4) [1.4 to 7.0]</td>
</tr>
<tr>
<td>C20:4n-6 (arachidonic acid)</td>
<td>-4.3 (2.3) [-8.9 to 0.4]</td>
<td>14.2 (1.4) [11.4 to 17.0]</td>
</tr>
<tr>
<td>C18:3n-3 (α-linolenic acid)</td>
<td>29.8 (5.4) [19.1 to 40.6]</td>
<td>-6.8 (3.2) [-13.3 to -0.4]</td>
</tr>
<tr>
<td>C20:4n-3 (eicosatetraenoic acid)</td>
<td>6.8 (5.2) [-3.5 to 17.1]</td>
<td>-17.7 (3.9) [-25.5 to -9.9]</td>
</tr>
<tr>
<td>C20:5n-3 (eicosapentaenoic acid)</td>
<td>8.2 (8.5) [-8.7 to 25.1]</td>
<td>6.6 (5.2) [-3.6 to 16.9]</td>
</tr>
<tr>
<td>C22:6n-3 (docosahexaenoic acid)</td>
<td>3.4 (4.2) [-4.9 to 11.7]</td>
<td>1.2 (2.4) [-3.5 to 5.9]</td>
</tr>
<tr>
<td>C20–22 polyunsaturated fatty acids‡</td>
<td>0.2 (2.7) [-5.1 to 5.5]</td>
<td>9.0 (1.7) [5.5 to 12.4]</td>
</tr>
<tr>
<td>C18–22n-3 fatty acids§</td>
<td>9.3 (4.3) [0.8 to 17.8]</td>
<td>1.1 (2.3) [-3.5 to 5.8]</td>
</tr>
</tbody>
</table>

Changes in the ratios of fatty acids (%) | | |
| C18:2n-3 to C18:2n-6 ratio§ | 9.0 (4.7) [-0.2 to 18.3] | 1.7 (2.6) [-3.5 to 7.0] | 0.06 | 0.51 | 0.76 |
| C18:0 to C16:0 ratio¶ | 2.7 (2.8) [-2.9 to 8.3] | 7.6 (1.4) [4.7 to 10.5] | 0.34 | <0.001 | 0.13 |
| C16:1n-7 to C16:0 ratio** | -2.3 (3.5) [-9.2 to 4.5] | -4.1 (1.8) [-7.6 to -0.6] | 0.50 | 0.02 | 0.09 |
| C18:3n-6 to C18:2n-6 ratio† | 6.3 (5.4) [-4.4 to 17.1] | 17.0 (3.2) [10.6 to 23.4] | 0.24 | <0.001 | 0.92 |
| C20:4n-6 to C20:3n-3 ratio | -1.0 (2.8) [-6.6 to 4.6] | 10.0 (2.3) [5.5 to 14.5] | 0.73 | <0.001 | 0.25 |
| C20:5n-3 to C20:4n-3 ratio | -9.4 (7.8) [-24.9 to 6.0] | 26.9 (5.8) [15.5 to 38.3] | 0.23 | <0.001 | 0.34 |

CI indicates confidence interval.
*In analysis of covariance with baseline values as covariates.
†Indicates the sum of fatty acids C20:4n-6, C22:4n-6, C20:5n-3, C22:5n-3, and C22:6n-3.
‡Indicates the sum of fatty acids C18:3n-3, C20:4n-3, C20:5n-3, C22:5n-3, and C22:6n-3.
§C18–22 n-6 indicates the sum of fatty acids C18:2n-6, C18:3n-6, C20:3n-6, C20:4n-6, and C22:4n-6.
†Indicates the activity of elongase enzyme.
¶Indicates the activity of Δ5 desaturase enzyme.
**Indicates the activity of Δ9 desaturase enzyme.
††Indicates the activity of Δ6 desaturase enzyme.

Triacylglycerols, 15% in cholesteryl esters, 35% in phospholipids, and <5% in the form of nonesterified free fatty acids. Approximately 75% to 80% of serum cholesterol is esterified with fatty acids and only 20 to 25% appears as nonesterified cholesterol. Thus, the observed 21% decrease in serum cholesterol and 14% decrease in serum triglycerides during simvastatin are expected to result in a 10% decrease in serum total fatty acid concentration, which is comparable to that observed. Because serum lipid levels were decreased by simvastatin, product-to-precursor ratios and proportions of individual fatty acids from total fatty acids rather than their absolute serum concentrations reflect the effects of the treatment on the metabolism and serum composition of fatty acids. For example, a decrease in the proportion from total fatty acids of eicosatetraenoic acid, a precursor of eicosapentaenoic acid, and an increase in the ratio of eicosapentaenoic acid to eicosatetraenoic acid during simvastatin should be interpreted as a result of increased Δ5 desaturase activity.

Our findings of increased formation of long-chain polyunsaturated fatty acids during simvastatin are in line with studies using experimental animals and cultured cell lines. HMG-CoA reductase inhibitors enhance fatty acid synthesis and peroxisomal activity and increase arachidonic acid and thromboxane production in cultured cells. In monocytic THP-1 cells, simvastatin enhanced the conversion of exogenous linoleic and eicosapentaenoic acids to their long-chain polyunsaturated fatty acid derivatives. In contrast to this in vitro study, we found that simvastatin 20 mg/d increased not only Δ5-desaturation step but also Δ6-desaturation and elongation steps of the fatty acid metabolism (Figure 3). The increased formation of long-chain polyunsaturated fatty acids and precursor fatty acids for eicosanoid pro-
proinflammatory 4-series leukotrienes. Thus, an increased intake of α-linolenic acid, linoleic acid, and oleic acid, may shift the desaturation and elongation of polyunsaturated fatty acids from omega-6 to omega-3 series fatty acids, and lead to increased formation of more antiaggregatory and vasodilatory omega-3–derived prostanoids compared with omega-6–derived prostanoids.

Simvastatin may increase nitric oxide (NO) production and/or release by endothelial cells by cholesterol-dependent and cholesterol-independent mechanisms. In cultured human endothelial cells, endothelial nitric oxide synthase is downregulated by oxidized LDL cholesterol. Thus decreased oxidized LDL cholesterol per se may increase NO production. It has been suggested that statins increase production and release of NO from the endothelium by inhibiting the production of mevalonate, thereby preventing the isoprenylation of the small GTPase Rho, which negatively regulates the expression of endothelial nitric oxide synthase. However, arachidonic acid–mediated microvascular dilatation in canine coronary arteries includes stimulation of NO production. Our findings suggest that simvastatin may increase NO production or release by increasing the formation of certain long-chain polyunsaturated fatty acids and their eicosanoid metabolites.

The mode of action of simvastatin on fatty acid cascade remains speculative. In theory, simvastatin may have direct or indirect stimulatory effects on fatty acid Δ5-desaturase, Δ6-desaturase, and elongase enzyme activities. According to animal studies, a high-cholesterol diet decreases the Δ5- and Δ6-desaturase steps, whereas a low-cholesterol diet has opposite effects. The Δ5- and Δ6-desaturase enzyme activities are decreased in insulin-deficient rats and are reactivated by insulin treatment, whereas in animal models for type 2 diabetes the effect of insulin is less clear. Our results in humans suggest that in the absence of insulin deficiency, Δ5- and Δ6-desaturase activities may even be influenced differently by insulin. Changes in insulin were not associated with changes in the elongase activity. According to the regression analyses with cholesterol and insulin as time-dependent variables, a decrease in serum cholesterol during simvastatin was associated with an increase in elongase activity but changes in cholesterol were not associated with changes in Δ5- and Δ6-desaturase activities. In an earlier report based on this material, we showed that simvastatin decreased serum cholesterol levels 3-fold compared with dietary treatment. At the same time, simvastatin increased fasting serum insulin levels by 13%, whereas dietary treatment alone decreased both fasting serum cholesterol and insulin. We now report that unlike simvastatin, the α-linolenic acid–rich diet alone has no effects on elongase, Δ5-desaturation, and Δ6-desaturation steps of the fatty acid metabolism. Therefore, changes in serum cholesterol and in serum insulin per se may not explain the changes observed in Δ5- and Δ6-desaturase steps of fatty acid metabolism during simvastatin treatment. Findings from the regression analyses suggest that a decrease in serum cholesterol may partly

Figure 2. Separate and combined effects of diet and simvastatin on serum fatty acid composition (adjusted for baseline values).
explain the increasing effect on fatty acid elongase activity of simvastatin. Simvastatin decreases serum testosterone levels, and testosterone decreases Δ5- and Δ6-desaturase enzyme activities. Decreased serum testosterone may partially explain increased fatty acid metabolism during simvastatin. One may assume that blocking of the mevalonoid acid pathway by simvastatin shifts the metabolism of acetyl-CoA to increased oxidation in the Krebs cycle and to increased formation of ketone bodies and fatty acids. Whereas increased fatty acid synthesis from acetyl-CoA may explain our finding of increased flux from palmitic to stearic acid, it cannot explain increased metabolism of the essential fatty acids linoleic and α-linolenic acid.

There are some limitations in our study. First, we measured only serum total fatty acids and we cannot assure that changes in fatty acid composition of triglycerides, cholesterol, and phospholipids are similar. Second, we did not measure fatty acid composition in important target tissues such as vascular endothelium and platelet membranes. In erythrocytes, a 2-month treatment with simvastatin is capable to induce measurable changes (an increase in arachidonic acid content of the membrane), but erythrocytes differ in many respects from other cells in the body. Third, the formation of eicosanoids and other relevant usually short-living mediators synthesized from arachidonic acid and other PUFAs were not measured here.

To conclude, simvastatin increased the formation of long-chain polyunsaturated fatty acids and precursor fatty acids of prostaglandins. Long-term studies combined with analyses of membrane fatty acid composition and characterization of the functional changes at the tissue level are needed to evaluate the mode of actions of simvastatin on platelet aggregation, hemostasis, fibrinolysis, and endothelial function.

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References


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