Comparison of Gemfibrozil Versus Simvastatin in Familial Combined Hyperlipidemia and Effects on Apolipoprotein-B-Containing Lipoproteins, Low-Density Lipoprotein Subfraction Profile, and Low-Density Lipoprotein Oxidizability

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We evaluated in a double-blind, placebo-controlled, randomized trial of 45 well-defined patients with familial combined hyperlipidemia, the effect of gemfibrozil (1,200 mg/day) or simvastatin (20 mg/day) on apolipoprotein-B (apo-B)-containing lipoproteins, low-density lipoprotein (LDL) subfraction profile, and LDL oxidizability. Although both drugs reduced plasma cholesterol and triglyceride concentrations, gemfibrozil reduced plasma triglycerides more effectively and simvastatin reduced plasma cholesterol more effectively. LDL cholesterol was reduced with simvastatin. With both drugs, total serum apo-B concentration decreased. With gemfibrozil, this was due to an exclusive reduction (−46%) of very low/intermediate-density lipoprotein (VLDL + IDL) apo-B, whereas simvastatin decreased apo-B in both VLDL + IDL and LDL (34% and 15%, respectively). Initially, a dense LDL subfraction profile was present in all patients. The decrease in LDL cholesterol with simvastatin was due to a decrease in all isolated LDL subfractions except LDL2; gemfibrozil increased LDL1 and LDL2 cholesterol (p = 0.001) and reduced LDL4 cholesterol, resulting in a more buoyant LDL subfraction profile compared with simvastatin. In both groups, a predominance of small dense LDL remained despite therapy. LDL fatty acid composition showed a shift from oleic acid to linoleic acid after gemfibrozil; arachidonic acid increased after simvastatin. Vitamin E was lower after gemfibrozil. In the measurements of LDL oxidation, only the oxidation rate was significantly reduced with simvastatin. Thus, qualitative and quantitative changes of LDL cholesterol had only a small effect on total in vitro LDL oxidizability in this population with familial combined hyperlipidemia.

Methods

Subjects: In all, 81 outpatients with familial combined hyperlipidemia were selected by 3 centers for treatment with either gemfibrozil or simvastatin. Forty-five patients from the 3 centers were randomly assigned for more extensive biochemical studies. They participated after informed consent was obtained. At entry into the placebo baseline period, patients met the following inclusion criteria: total serum cholesterol >6.5 mmol/L and triglyceride level between 2.3 and 5.6 mmol/L; at least 1 first-

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degree relative with significant hypercholesterolemia, hypertriglyceridemia, or both; a positive family history of premature coronary artery disease; total apo-B 100 levels >1,200 mg/dl; and age >30 years. Patients with secondary causes for dyslipidemia or with apolipoprotein phenotype E 2/2 were excluded.

**Study design:** This was a double-blind, placebo-controlled trial with a double-dummy design, divided into 3 consecutive periods over 20 weeks. During the first period (weeks -8 to -5), selected patients who had taken no lipid-lowering drugs for ≥24 weeks received a standard lipid-lowering diet. The second period (weeks -4 to day 0) was a baseline placebo period. Each patient received 2 bottles, 1 containing placebo matching gemfibrozil and 1 containing placebo matching simvastatin. During the third period of active treatment (day 0 to week 12), patients were randomly assigned to receive either simvastatin 20 mg/day together with placebo matching gemfibrozil or gemfibrozil 1,200 mg/day together with placebo matching simvastatin. In the present study, data obtained at the end of the placebo period (day 0) were compared with results obtained at the end of the period of active treatment (day 84).

**Plasma:** Blood samples were obtained after an overnight fast and collected in vacutainers containing 1 mg/ml of ethylenediaminetetraacetic acid. Plasma was isolated immediately and a saccharose solution (600 mg/ml H₂O) was added to prevent denaturation of LDL during freezing; samples were stored at -80°C for 4 to 15 weeks. All measurements were obtained in the lipid research laboratory of the University Hospital Nijmegen.

**Analytic methods:** VLDL + intermediate-density lipoprotein (IDL) (density [d] ≤ 1.019 g/ml) were isolated by ultracentrifugation for 16 hours at 40,000 rpm in a fixed angle rotor (TFT 45.6 rotor, Kontron, Zürich) in a Beckman L7-55 ultracentrifuge (Beckman, Palo Alto, California). After removal of VLDL + IDL, cholesterol and triglycerides were measured in the remaining plasma and in total plasma. HDL was isolated from whole plasma by the polyethylene glycol method, and Sera Pak, Miles, California [catalog no. 237574], and Sera Pak, Miles, Houten, The Netherlands, were determined by nephelometry. For oxidation of low-density lipoproteins: The oxidation experiments were performed as described by Esterbauer et al., with modifications by Kleinveld et al. Statistical analysis of alterations within 1 group of treatment was performed with Wilcoxon’s signed rank test. Differences in percentages between the 2 groups of treatment were analyzed with Wilcoxon’s rank sum test. A 2-tailed probability value <0.05 was considered significant. Statistical analyses were performed with procedures available in the SPSS PC+ (Statistical Package for the Social Sciences) software package version 4.0.
± 2.9 kg/m², respectively). Both age and body mass index were similar in both groups. The lipid and lipoprotein levels, body mass index, and age of the 45 patients were equal compared with the initial population.

Effect of treatment on plasma lipid and lipoprotein levels: The lipid and lipoprotein levels of the patients with familial combined hyperlipidemia before and after treatment with gemfibrozil (n = 22) or simvastatin (n = 23) are summarized in Table I. There were no significant differences in lipid concentrations between the 2 groups at baseline. Gemfibrozil significantly affected total triglyceride levels in plasma as well as in the VLDL + IDL fraction, whereas simvastatin induced the largest reduction in total plasma cholesterol. On the other hand, VLDL + IDL cholesterol was reduced with both therapies to the same extent. The largest contribution to the reduction in total cholesterol and triglycerides was generated by a decrease in the VLDL + IDL fraction. LDL cholesterol only decreased with simvastatin, and even tended to increase after gemfibrozil. HDL cholesterol levels increased with both drugs.

Effect of treatment on apolipoprotein-B lipoproteins: A significant correlation was found between apo-B and total apo-B–related cholesterol (total cholesterol minus HDL cholesterol) (Pearson's correlation coefficient 0.91; p = 0.001) (Figure 1). The concentrations of VLDL + IDL cholesterol and LDL cholesterol also correlated with their related apo-B content (correlation coefficient 0.70; p = 0.001; 0.63; p = 0.001, respectively). Both therapies reduced total apo-B to a similar extent. Gemfibrozil reduced apo-B only in the VLDL + IDL fraction, whereas simvastatin reduced apo-B in the VLDL + IDL and in the LDL fraction (Figure 2).

Effect of therapy on low-density lipoprotein subtraction profile and K value: Initially in all patients, LDL consisting of a limited number of LDL subfractions, with a predominance of intermediate dense (LDL2) and small dense (LDL3 and LDL4) subfractions. In 3 of 45 LDL subfraction profiles, a clear, very dense LDL5 band could be distinguished, which in all cases completely disappeared after treatment (1 after gemfibrozil and 2 after simvastatin). This sporadic LDL5 appearance, in 3 patients with lipoprotein levels comparable to the other subjects, was excluded from further statistical analysis. Gemfibrozil treatment induced a less dense LDL subtraction profile, without a reduction in total LDL cholesterol, consisting of LDL1 to LDL4, with a predominance of LDL1 to LDL3 as the main LDL subfractions (Figure 3). The ratio of cholesterol/triglyceride within the LDL particle increased, whereas that of triglyceride/apo-B decreased, probably due to a reduction in triglycerides per LDL particle. The ratio of cholesterol/apo-B did not change (Table II). Simvastatin treatment reduced total LDL, but did not induce a major shift to a less dense LDL subtraction profile as seen with gemfibrozil. The amount of cholesterol in all LDL subfractions, except LDL2, was significantly reduced (Figure 3). Neither the ratio of cholesterol/triglyceride nor the ratio of cholesterol/apo-B or triglyceride/apo-B

**TABLE I** Changes in Lipids and Lipoproteins in Subjects With Familial Combined Hyperlipidemia After Treatment With Gemfibrozil or Simvastatin

<table>
<thead>
<tr>
<th>Lipid/Lipoprotein</th>
<th>G (n = 22)</th>
<th>S (n = 23)</th>
<th>Change (%)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total cholesterol</strong></td>
<td>Before</td>
<td>After</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>7.54 ± 1.13</td>
<td>6.51 ± 0.91</td>
<td>-12.9 ± 11.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>S</td>
<td>7.15 ± 0.87</td>
<td>5.58 ± 1.12</td>
<td>-22.2 ± 9.4*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Triglycerides</strong></td>
<td>G</td>
<td>2.90 ± 0.91</td>
<td>1.42 ± 0.41</td>
<td>-48.1 ± 18.0</td>
</tr>
<tr>
<td>S</td>
<td>3.27 ± 1.19</td>
<td>2.61 ± 0.86</td>
<td>-15.9 ± 25.8</td>
<td>0.003</td>
</tr>
<tr>
<td><strong>VLDL + IDL cholesterol</strong></td>
<td>G</td>
<td>2.24 ± 0.77</td>
<td>0.96 ± 0.38</td>
<td>-54.5 ± 20.0</td>
</tr>
<tr>
<td>S</td>
<td>2.58 ± 1.02</td>
<td>2.04 ± 0.74</td>
<td>-15.4 ± 24.2</td>
<td>0.005</td>
</tr>
<tr>
<td><strong>LDL cholesterol</strong></td>
<td>G</td>
<td>2.26 ± 0.76</td>
<td>0.99 ± 0.47</td>
<td>-55.3 ± 30.3</td>
</tr>
<tr>
<td>S</td>
<td>2.65 ± 0.90</td>
<td>1.50 ± 0.51</td>
<td>-40.9 ± 18.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>HDL cholesterol</strong></td>
<td>G</td>
<td>4.36 ± 0.86</td>
<td>4.59 ± 0.92</td>
<td>+9.3 ± 36.6</td>
</tr>
<tr>
<td>S</td>
<td>3.68 ± 1.01</td>
<td>3.12 ± 0.98</td>
<td>-13.3 ± 21.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>apo-B</strong></td>
<td>G</td>
<td>0.92 ± 0.18</td>
<td>1.06 ± 0.26</td>
<td>+15.3 ± 20.9</td>
</tr>
<tr>
<td>S</td>
<td>0.82 ± 0.21</td>
<td>0.94 ± 0.21</td>
<td>+16.4 ± 13.1</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*p <0.01, t* <0.001; gemfibrozil versus simvastatin.

Values are presented in mmol/L as mean ± SD.

Apo-B = apolipoprotein-B; G = gemfibrozil (n = 22); HDL = high-density lipoprotein; IDL = intermediate-density lipoprotein; S = simvastatin (n = 23); VLDL = very-low-density lipoprotein.

**FIGURE 1.** Correlation between total apolipoprotein-B (apo-B)–related cholesterol (total plasma cholesterol minus high-density lipoprotein cholesterol) (in mmol/L) and total plasma apo-B (closed squares), very-low-density lipoprotein + intermediate-density lipoprotein apo-B (plus signs), and low-density lipoprotein apo-B (open squares) (in mg/L) in 45 patients with familial combined hyperlipidemia.
changed significantly (Table II). The value of parameter K increased more after gemfibrozil (-0.55 ± 0.18 to -0.32 ± 0.21; p <0.001) than after simvastatin (-0.55 ± 0.16 to -0.47 ± 0.22; p = 0.04; gemfibrozil vs simvastatin; p <0.05). In 5 of 45 patients treated with gemfibrozil, the dense subfraction profile was altered into an intermediate subfraction profile, with an equal amount of buoyant and dense LDL particles. The other patients retained a dense subfraction profile, expressed as a negative value for parameter K, despite lipid-lowering therapy.

**Fatty acid composition and vitamin E content of low-density lipoprotein:** The fatty acid composition of each isolated LDL was determined (Table III). For technical reasons, only the results of 14 patients treated with gemfibrozil and 15 patients with simvastatin could be analyzed. This reduction had no effect on lipid levels, apo-B levels, or the value of parameter K before and after therapy in this subset when compared with the initial 45 patients. In the gemfibrozil group, the relative amount of stearic acid (18:0) and oleic acid (18:1) decreased, whereas that of linoleic acid (18:2) increased. In the simvastatin group, the relative contribution of linoleic acid (18:2) decreased, with an increase in arachidonic acid (20:4). Vitamin E in LDL decreased significantly with gemfibrozil and was unaffected with simvastatin. The ratio of polyunsaturated fatty acids/vitamin E tended to increase after gemfibrozil, whereas simvastatin did not affect this ratio.

| TABLE II Changes in the Ratios Cholesterol/Triglyceride, Cholesterol/Apo-B, and Triglyceride/Apo-B of the LDL Particles and the Total Cholesterol/HDL Cholesterol Ratio After Treatment With Gemfibrozil (n = 19) or Simvastatin (n = 21) in Patients With Familial Combined Hyperlipidemia |
|-------------------|-------|-------|-------------|-------|
|                   | Before| After| Change (%) | p Value |
| Cholesterol/Triglyceride |       |       |            |        |
| G                 | 7.73 ± 2.27 | 10.26 ± 2.62 | 53.7 ± 67.0 | 0.001 |
| S                 | 6.14 ± 1.75  | 6.14 ± 1.87  | 1.6 ± 20.9  |        |
| Cholesterol/Apo-B  |       |       |            |        |
| G                 | 1.48 ± 0.17  | 1.54 ± 0.21  | 5.8 ± 22.0  |        |
| S                 | 1.34 ± 0.16  | 1.36 ± 0.16  | 0.5 ± 12.9  |        |
| Triglyceride/Apo-B |       |       |            |        |
| G                 | 0.47 ± 0.11  | 0.36 ± 0.08  | -17.5 ± 19.3 | <0.001 |
| S                 | 0.53 ± 0.13  | 0.55 ± 0.17  | 5.0 ± 20.2  |        |
| Total cholesterol/HDL cholesterol |       |       |            |        |
| G                 | 8.4 ± 2.0    | 6.5 ± 2.2    | -22.1 ± 16.5 | <0.001 |
| S                 | 9.3 ± 2.6    | 6.1 ± 1.5    | -32.7 ± 10.4 | <0.001 |

*p <0.01, †p <0.001, ‡p <0.005; gemfibrozil versus simvastatin.

Values are presented as mean ± SD.

Abbreviations as in Table I.

**FIGURE 2.** Effect of treatment with either gemfibrozil (n = 21) or simvastatin (n = 23) on apolipoprotein-B (apo-B)-containing lipoproteins in total plasma (TOTAL), low-density lipoprotein (LDL) fraction, and the very-low-density lipoprotein + intermediate-density lipoprotein (VLDL/IDL) fraction in patients with familial combined hyperlipidemia. *p = 0.003; ‡p <0.001.

**FIGURE 3.** Effect of treatment with either gemfibrozil (n = 20) or simvastatin (n = 21) on the cholesterol content (in mmol/L) of 4 low-density lipoprotein subfractions (LDL1 to LDL4) in patients with familial combined hyperlipidemia. *p <0.05; ‡p = 0.001; ‡‡p <0.001.
All patients had moderate to severe elevations of apo-B levels in accordance with their elevation of lipid levels. In normolipidemic subjects, total apo-B-related cholesterol concentration correlates highly with serum apo-B. 

A depletion in triglycerides in the VLDL fraction with gemfibrozil, leading to small, more dense VLDL + IDL particles that are more likely to be converted into LDL particles, is suggested to be a cause of the observed increase in LDL cholesterol after gemfibrozil.

**DISCUSSION**

The underlying cause of the increased tendency toward cardiovascular diseases in patients with familial combined hyperlipidemia is probably related to increased levels of small dense LDL and other atherogenic lipoprotein remnant particles. 

A predominance of small dense LDL is observed either as a physiologic response to lipid abnormalities, or as a distinct characteristic of the disease with a possible genetic basis. These small dense subfractions are associated with atherosclerosis because of enhanced susceptibility to copper-induced oxidative modification. 

In the present study, we therefore investigated the apo-B-containing lipoprotein concentrations, LDL subfraction profiles, and LDL oxidizability of 48 affected patients, and evaluated the effects of pharmacologic intervention on these parameters.

The observed reduction of total plasma cholesterol and plasma triglyceride concentrations with simvastatin (−22.2% and −15.9%, respectively) and gemfibrozil (−12.9% and −48.1%, respectively) after 12 weeks of treatment are in accordance with previous reports. 

Isolation of VLDL and LDL particles together (d <1.019 g/ml), instead of isolating LDL together with VLDL (d >1.006 g/ml), explains the relatively large contribution of VLDL + IDL cholesterol and the relatively small contribution of LDL cholesterol to total plasma cholesterol, and also the large impact of the 2 therapies on this VLDL + IDL fraction. The increase in HDL cholesterol with simvastatin similar to gemfibrozil, despite a less pronounced reduction of triglyceride concentration after simvastatin, is larger than previously reported. 

The decrease and increase in LDL cholesterol with simvastatin and gemfibrozil, respectively, are also in line with other reports. 

A (light) and pattern B (heavy) LDL subfraction profile. Only 5 patients had a dense LDL subfraction profile that were converted to an intermediate profile. These results are consistent with previous reports. 

A predominance of small dense VLDL + IDL particles that are more likely to be converted into LDL particles, is suggested to be a cause of the observed increase in LDL cholesterol after gemfibrozil.
supported by a recent study by Hokanson et al.\(^\text{29}\) in which they proposed that in familial combined hyperlipidemia, small dense LDL and hypertriglyceridemia appear as interrelated but separate characteristics and regulated as separate processes.

In general, the observed LDL fatty acid composition in this group with familial combined hyperlipidemia was similar to that found in normal subjects.\(^\text{18}\) After both therapies, only small alterations in this composition were seen. The total amount of polyunsaturated fatty acids (linoleic and arachidonic acids), most susceptible for oxidative modification,\(^\text{18}\) did not change. On the contrary, vitamin E as the major antioxidant in LDL was reduced only with gemfibrozil. This may have implications for total LDL oxidizability.

Our data show only little effect on LDL oxidizability after treatment, less than suspected on the basis of a more buoyant LDL LDL subtraction profile.\(^\text{14}\) However, some explanations for this lack of change in LDL oxidizability are possible: We determined LDL oxidizability in total LDL, which is the addition of maximal 5 LDL subfractions, so small changes might be undetected. Despite treatment, these patients still had a predominance of small dense LDL particles. The ratio of cholesterol/protein of LDL particles correlating with LDL oxidizability\(^\text{30}\) was unaffected after both therapies. Finally, the ratio of polyunsaturated fatty acids/vitamin E increased only with gemfibrozil. This implies that the expected diminished susceptibility to copper-induced oxidation because of a more buoyant LDL subtraction profile\(^\text{14}\) could be offset by a reduced protection of polyunsaturated fatty acids from oxidation by vitamin E.

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