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, quantitative and qualitative changes of LDL cholesterol had only a small effect on total in vitro LDL oxidizability in this population with familial combined hyperlipidemia.

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Familial combined hyperlipidemia is a metabolically and genetically heterogeneous lipid disorder,1-3 with affected persons exhibiting elevations of total cholesterol, triglycerides, or both, at least partially caused by very-low-density lipoprotein (VLDL)-apolipoprotein-B (apo-B) overproduction.4 Its prevalence is estimated to be 0.5% to 1.0%,5 and the trait predisposes to premature cardiovascular complications.6 Because of the absence of a specific metabolic parameter characteristic of familial combined hyperlipidemia, family studies are pivotal for diagnosis. The current diagnosis is based on the following criteria1-8: prevalence of multiple lipoprotein phenotypes in first-degree relatives, premature atherosclerosis, decreased high-density lipoprotein (HDL) cholesterol levels, elevated plasma levels of apo-B,7 impaired clearance of VLDL remnants,8 and an increased prevalence of small dense low-density lipoprotein (LDL).7 A predominance of small LDL and its enhanced susceptibility to copper-mediated oxidative modification9 is associated with atherogenesis.10,11 Because of the reported increased risk for premature atherosclerosis, treatment with lipid-lowering drugs is frequently indicated.12,13 In this report we described the baseline lipoprotein concentrations, LDL subfraction profiles, and LDL oxidizability of well-defined patients with familial combined hyperlipidemia, and compared the effectiveness of treatment with gemfibrozil or simvastatin on these parameters in a double-blind, placebo-controlled fashion.

METHODS

Subjects: In all, 81 outpatients with familial combined hyperlipidemia were selected by 3 participating centers to evaluate the effect of treatment with either gemfibrozil or simvastatin on serum lipids. 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-
degree relative with significant hypercholesterolemia,
hypertriglyceridemia, or both; a positive family history
of premature coronary artery disease; total apo-A 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 study 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 gemf­
brozil 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 (n = 23) or gemfibrozil 1,200
mg/day together with placebo matching simvastatin (n
= 22). 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 lipo­
protein (IDL) (density [dl] ≤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 Beck­
mann L7-55 ultracentrifuge (Beckman, Palo Alto, Cali­
fornia).14 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.15 Cholesterol and triglycerides that remained after VLDL + IDL removal were
determined by ultracentrifugation. After removal of VLDL + IDL, cholesterol and triglycerides were calculated by sub­traction. Apo-B concentrations in total plasma and in
different subfractions were determined by an enzyme-linked immunoassay. 

**Oxidation of low-density lipoproteins:** The oxidation experiments were performed as described by Esterbauer et al., with modifications by Kleinvekla et al.20

**Statistics:** Results are expressed as mean ± SD. Sta­
tistical analysis of alterations within 1 group of treat­
ment was performed with Wilcoxon’s signed rank test.
Differences in percentages between the 2 groups of treat­
ment were analyzed with Wilcoxon’s rank sum test. A
2-tailed probability value <0.05 was considered signifi­
cant. Statistical analyses were performed with proce­
dures available in the SPSS PC+ (Statistical Package for
the Social Sciences) software package version 4.0.

**RESULTS**

**Patients:** At entry into the placebo period, all 45
patients described in this study met the inclusion crite­
ia. Therefore, all patients had hyperlipidemia phenotype
IIb. The groups consisted of 5 women and 17 men in
the gemfibrozil group and 18 men and 5 women in the
simvastatin group (mean age 53.9 ± 9.8 vs 53.1 ± 10.3
years, respectively; body mass index 27.4 ± 3.1 vs 26.6
± 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 subtraction 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 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

![Figure 1](image1.png)

**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.


**Total low-density lipoprotein oxidizability:** Because of technical reasons, the results of only 17 patients treated with gemfibrozil versus 18 patients with simvastatin could be analyzed. This reduction had no effect on lipid and apo-B levels, or the value of parameter K before and after therapy in this subset when compared with the initial 45 patients. Although the lag time at the preoxidative phase tended to increase, the differences were not significant in any of the treatment groups. Oxidation rate decreased after simvastatin (p = 0.01), in contrast to gemfibrozil. Total amounts of produced conjugated dienes (malondialdehyde reactive products) per milligram of LDL protein were similar before and after treatment in both groups.

**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. A depletion in LDL cholesterol with simvastatin and gemfibrozil reduced only VLDL + IDL apo-B-containing particles and simvastatin reduced both VLDL + IDL and LDL apo-B-containing particles.

Initially, all patients had a dense LDL subfraction profile, both determined by cholesterol content in isolated LDL subfractions and described by a continuous variable, parameter K. This method of approach provides the opportunity to obtain more detailed information about small alterations in the LDL subfraction profile than the often-used dichotomous classification in pattern A (light) and pattern B (heavy). The increase in cholesterol in the buoyant LDL1 and LDL2 subfractions after gemfibrozil could be explained by the observed decrease in the ratio of triglyceride/protein of the LDL particle only after gemfibrozil, reflecting an overall reduction in triglycerides in the LDL particles. This triglyceride reduction, not observed after simvastatin, is more likely to be converted into LDL particles, is suggested to be a cause of the observed increase in LDL cholesterol after gemfibrozil.

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. In hypertriglyceridemic states, this correlation is less pronounced because of possible underestimation of apo-B, although the correlation between total cholesterol minus HDL cholesterol and apo-B in these patients was still significant. Both therapies reduced total apo-B to a similar extent, but just like the reduction in VLDL + IDL cholesterol/triglyceride and LDL cholesterol, gemfibrozil reduced only VLDL + IDL apo-B-containing particles and simvastatin reduced both VLDL + IDL and LDL apo-B-containing particles.

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**Table III** Change in Fatty Acid Composition and Vitamin E Content of Total LDL After Treatment With Gemfibrozil (n = 14) or Simvastatin (n = 15) in Patients With Familial Combined Hyperlipidemia

<table>
<thead>
<tr>
<th>Fatty Acids</th>
<th>Before</th>
<th>After</th>
<th>Change (%)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitic acid (16:0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>G</td>
<td>22.3 ± 1.9</td>
<td>21.7 ± 1.6</td>
<td>-2.8 ± 6.2</td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>23.4 ± 2.2</td>
<td>23.5 ± 2.4</td>
<td>+0.2 ± 5.7</td>
<td></td>
</tr>
<tr>
<td>Stearic acid (18:0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>6.6 ± 0.8</td>
<td>6.3 ± 0.7</td>
<td>-3.1 ± 5.9</td>
<td>0.04</td>
</tr>
<tr>
<td>S</td>
<td>6.6 ± 0.6</td>
<td>6.9 ± 0.7</td>
<td>+2.8 ± 6.3</td>
<td></td>
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<tr>
<td>Oleic acid (18:1)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>G</td>
<td>18.7 ± 1.9</td>
<td>17.8 ± 1.6</td>
<td>-4.8 ± 5.7</td>
<td>0.01</td>
</tr>
<tr>
<td>S</td>
<td>18.8 ± 1.8</td>
<td>19.0 ± 2.2</td>
<td>+0.9 ± 8.6</td>
<td></td>
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<tr>
<td>Linoleic acid (18:2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>44.1 ± 3.7</td>
<td>45.8 ± 3.2</td>
<td>+1.4 ± 4.3</td>
<td>0.01</td>
</tr>
<tr>
<td>S</td>
<td>43.4 ± 3.6</td>
<td>41.6 ± 4.1</td>
<td>-4.0 ± 6.0</td>
<td></td>
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<tr>
<td>Arachidonic acid (20:4)</td>
<td></td>
<td></td>
<td></td>
<td>0.04</td>
</tr>
<tr>
<td>G</td>
<td>8.2 ± 1.7</td>
<td>8.4 ± 1.6</td>
<td>+3.0 ± 14.5</td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>7.8 ± 1.4</td>
<td>9.1 ± 1.4</td>
<td>+20.1 ± 21.0</td>
<td></td>
</tr>
<tr>
<td>Vitamin E</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>4.06 ± 1.65</td>
<td>3.42 ± 1.66</td>
<td>-16.5 ± 18.2</td>
<td>0.004</td>
</tr>
<tr>
<td>S</td>
<td>3.65 ± 2.17</td>
<td>4.32 ± 3.15</td>
<td>+14.0 ± 45.9</td>
<td></td>
</tr>
<tr>
<td>PUFA/Vit. E</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>846 ± 596</td>
<td>1,102 ± 673</td>
<td>+22 ± 83</td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>801 ± 538</td>
<td>727 ± 339</td>
<td>+6 ± 52</td>
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</table>

*p < 0.05, fp < 0.01, f p < 0.001; gemfibrozil versus simvastatin.
Values of fatty acids are presented in percentage of total fatty acids as mean ± SD.
Vitamin E in mg/g low-density lipoprotein.
Ratios of polyunsaturated fatty acids/vitamin E in μmol/mg.
PUFA = polyunsaturated fatty acids (μmol/mg); Vit. = vitamin; other abbreviations as in Table I.

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(−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 LDL (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 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.
supported by a recent study by Hokanson et al.\textsuperscript{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.\textsuperscript{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,\textsuperscript{16} 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 fraction profile.\textsuperscript{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\textsuperscript{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 LDL fraction profile\textsuperscript{14} could be offset by a reduced protection of polyunsaturated fatty acids from oxidation by vitamin E.

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**PREVENTIVE CARDIOLOGY/EFFECT OF GEMFIBROZIL OR SIMVASTATIN IN FCH**