Early Statin Therapy Restores Endothelial Function in Children With Familial Hypercholesterolemia

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OBJECTIVES
This study was designed to determine whether simvastatin improves endothelial function in children with familial hypercholesterolemia (FH).

BACKGROUND
Endothelial function measured by flow-mediated dilation of the brachial artery (FMD) is used as a surrogate marker of cardiovascular disease (CVD). Adult studies have shown that statins reverse endothelial dysfunction and therefore reduce the risk for future CVD.

METHODS
The study included 50 children with FH (9 to 18 years) and 19 healthy, non-FH controls. Children with FH were randomized to receive simvastatin or placebo for 28 weeks. The FMD was performed at baseline and at 28 weeks of treatment.

RESULTS
At baseline, FMD was impaired in children with FH versus non-FH controls (p < 0.024). In the simvastatin FH group, FMD improved significantly, whereas the FMD remained unaltered in the placebo FH group throughout the study period (absolute increase 3.9% ± 4.3% vs. 1.2% ± 3.9%, p < 0.05). In the simvastatin FH group, FMD increased to a level similar to the non-FH controls (15.6% ± 6.8% vs. 15.5% ± 5.4%, p = 0.958). Upon treatment, the simvastatin FH group showed significant absolute reductions of total cholesterol (TC) (−2.16 ± 1.04 mmol/l, 30.1%) and low-density lipoprotein cholesterol (LDL-C) (−2.13 ± 0.99 mmol/l, 39.8%). The absolute change of FMD after 28 weeks of therapy was inversely correlated to changes of TC (r = −0.31, p < 0.05) and LDL-C (r = −0.31, p < 0.05).

CONCLUSIONS
Our data show significant improvement of endothelial dysfunction towards normal levels after short-term simvastatin therapy in children with FH. These results emphasize the relevance of statin therapy in patients with FH at an early stage, when the atherosclerotic process is still reversible.

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Endothelial dysfunction is an early reversible stage in the development of atherosclerosis (4) and has predictive value for future cardiovascular events (5,6). It is characterized by an imbalance in favor of proatherogenic factors such as vasoconstriction, platelet activation, monocyte adhesion, thrombogenesis, and inflammation (7). In the past decade, a number of studies have shown that endothelial function measured as flow-mediated dilation (FMD) is impaired in children with FH (8–10). Hydroxymethylglutaryl coenzyme A reductase inhibitors (statins) have been shown to reverse endothelial dysfunction; but only in adult dyslipidemic patients (11–13). However, at later stages statin therapy is unable to normalize endothelial function in coronary arteries, likely because of structural changes.

In spite of the aggressive nature of FH, the recommended therapy for children consists of a cholesterol-lowering diet and, if deemed necessary, bile-acid binding resins (14). The use of statins in children has been evaluated to a certain degree, but is still under debate (15–17). In view of the limited timespan for cardiovascular prevention in patients with FH, improvement of endothelial function at an early reversible stage of the atherosclerotic process would provide a strong argument for implementation of statin therapy in childhood (4).

This placebo-controlled study was designed to evaluate the effect of simvastatin therapy on endothelial function in children with FH. At baseline, endothelial function was assessed as FMD of the brachial artery, a parameter for endothelium-dependent vasodilatation, in children with FH (n = 50) and non-FH controls (n = 19). In the children with FH, the measurements were repeated after 28 weeks of simvastatin (n = 28) or placebo (n = 22) therapy, respectively.

Familial hypercholesterolemia (FH) is an inherited autosomal dominant disorder of lipoprotein metabolism caused by a plethora of mutations in the low density lipoprotein (LDL) receptor gene (1). With a frequency of one in 400 persons, FH is one of the most common inborn errors of metabolism in the Dutch population (2). This disorder is associated with elevated levels of LDL cholesterol (LDL-C) and premature atherosclerosis, although the disease is usually asymptomatic in children (3).
PATIENTS AND METHODS

Patients. A total of 50 heterozygous children with FH, ages nine to 18, were randomized to receive either simvastatin or placebo at a 3:2 ratio, respectively. Twenty-eight children were randomized to simvastatin and 22 to placebo. Nineteen healthy, non-FH siblings between nine and 18 years of age participated in the study as controls. The following criteria were designed to select children with heterozygous FH: plasma LDL-C levels above 95th percentile for age and gender, a documented family history of hyperlipidemia with LDL-C levels above the 95th percentile for age and gender before treatment, or a personal diagnosis of FH by detection of a mutation in the LDL receptor gene. Exclusion criteria were smoking, current use of any vasoactive medications, and concomitant conditions such as serious illness, hypertension, or diabetes mellitus.

Study design. The dosage of simvastatin was doubled every eight weeks from 10 to 20 to 40 mg per day. The assessment of FMD of the brachial artery as parameter for endothelium-dependent vasodilation was performed at baseline (at entry of the study) and after 28 weeks of treatment (40 mg of simvastatin or placebo). Total serum cholesterol (TC), triglycerides (TG), LDL-C, and high-density lipoprotein cholesterol (HDL-C) were measured at both visits. Total cholesterol and TG were analyzed by enzymatic methods on a Hitachi 747 analyzer as previously described (18). High density lipoprotein cholesterol was isolated with heparin-2M manganese chloride (19). Safety measurements including hepatic transaminases (alanine aminotransferase [ALT] and aspartate aminotransferase [AST]) and creatine kinase (CK) were measured during each visit. Physical examination (height, weight, blood pressure) was performed during the first and the last visit. Each child or child’s parents gave written informed consent for his or her participation in the study, which was approved by the Institutional Review Board of the Academic Medical Center (Amsterdam).

Flow-mediated dilation. The assessment of FMD was performed as published previously (20,21). In summary, all measurements were performed during the morning in a fasting state. All children refrained from alcohol and caffeine-containing beverages. Patients were studied in supine position. The blood pressure cuff was placed just below the elbow of the right arm. After a 10 to 15 min rest, the brachial artery in the right antecubital fossa was visualized using a 7.5 MHz transducer (5,22). After an optimal image of the brachial artery wall was obtained, a wall tracking system was used to measure the lumen diameter. After two baseline vessel diameter measurements were obtained, reactive hyperemia was induced by inflating the blood pressure cuff to 200 mm Hg distal to the location of transducer. Upon release of the cuff after 4 min, the ensuing dilation of the brachial artery is predominantly mediated by endothelial NO release (23). Ultrasonography then continued for 5 min to allow for lumen diameter measurements at 20-s intervals. Wall track measurements were stored digitally and analyzed offline using the wall track system software analysis package (24). All measurements were performed by the same observer, unaware of clinical details and the stage of the experiment. Baseline vessel diameter was calculated as the average of two measurements. Flow-mediated dilation was calculated at each examination as ([maximal lumen diameter after ischemia diameter at baseline]/diameter at baseline) and expressed as a percentage (25). Intra- and intersession variation coefficients were 1.1% and 3.8% respectively (21). The total duration of this investigation was approximately 20 min.

Statistical analysis. Analyses were performed using SPSS 10.0 for Windows software. The baseline characteristics of the controls versus the treatment and placebo group were compared by analysis of variance. In case of a significant result a post hoc analysis was performed. Multiple comparisons were taken into account by using the Bonferroni method. Differences between two groups were tested by using Student t test for continuous data. Skewed data were log-transformed before testing. Mean values before and after therapy were compared using the paired sample t test. Correlations were tested by using the bivariate Pearson correlation test for continuous variables. A p value <0.05 was considered statistically significant.

RESULTS

Baseline characteristics showed no significant difference with regard to gender, age, body mass index (BMI), blood pressure, and baseline vessel size between the non-FH control group, placebo FH group, and simvastatin FH group (Table 1). However, TC and LDL-C were significantly higher in the children with FH compared with the non-FH controls (p < 0.0001). Flow-mediated dilation was impaired in the children with FH versus the non-FH controls (p < 0.024). There were no significant differences with regard to age, gender, BMI, and blood pressure between the simvastatin FH and placebo FH group. Also, lipid profile, baseline vessel size, and FMD were not significantly different between these two groups.

After 28 weeks, the FMD increased significantly in the simvastatin FH group (p < 0.0001). In the placebo FH
group, FMD remained unaltered throughout the study period (Fig. 1). There was no difference with regard to the baseline vessel sizes between both groups at 28 weeks (simvastatin: 2.96 ± 0.53 mm; placebo: 3.05 ± 0.38 mm, \( p = 0.592 \)). The mean absolute change in FMD was significantly higher in the simvastatin FH group compared with the placebo FH group (3.9% ± 4.3% vs. 1.2% ± 3.9%, \( p = 0.05 \)). Eight children in the placebo FH group (8/22; 36%), compared with 19 children in the simvastatin FH group (19/28; 68%), had an improvement of FMD >2.5%. In the simvastatin FH group, FMD increased to a level similar to that in non-FH controls (15.6% ± 6.8% vs. 15.5% ± 5.4%, \( p = 0.958 \)).

After 28 weeks of treatment there was a significant mean absolute reduction of TC (\(-2.16 ± 1.04\) mmol/l, \( p < 0.0001 \)), LDL-C (\(-2.13 ± 0.99\) mmol/l, \( p < 0.0001 \)) and TG (\(-0.19 ± 0.37\) mmol/l, \( p < 0.002 \)) in the simvastatin FH group (Table 2). These data match with a TC reduction of 30.1%, a LDL-C reduction of 39.8% and a TG reduction of 16.7%. There was an absolute increase in HDL-C (4.5%) in the simvastatin FH group; however, the change did not reach statistical significance. There were no significant changes in lipoproteins in the placebo FH group compared to baseline. The mean absolute changes for TC, LDL-C, and TG were significantly higher in the simvastatin FH group than in the placebo FH group. Except for LDL-C, the lipoproteins in the simvastatin FH group after 28 weeks were reduced to levels comparable to those of the non-FH control group. There were no significant differences with regard to safety measurements (ALT, AST, and CK) between simvastatin and placebo FH groups and no adverse events were reported (data not shown).

In the children with FH the change of FMD after 28 weeks of therapy was inversely correlated to absolute changes of TC (\( r = -0.31 \), \( p < 0.05 \)) and LDL-C (\( r = -0.31 \), \( p < 0.05 \)).

**DISCUSSION**

Our data clearly show that children with FH are characterized by impaired endothelial function. More importantly, we demonstrate a significant improvement of endothelial dysfunction in children with FH after short-term statin therapy.

Patients with FH are characterized by severely increased LDL-C levels, a major risk factor for premature atherosclerosis, and show symptoms of cardiovascular disease (CVD) at relatively young age, sometimes even before the age of 30 (1). Several population studies have demonstrated that, in the general population, the process of atherosclerosis begins in adolescence (26,27). In view of the aggressive nature of CVD in adult patients with FH, it is safe to assume that in these individuals atherosclerotic changes already begin in early childhood (3,26). In line, several reports have demonstrated the presence of endothelial dysfunction in children with FH (8–10), which clearly precedes the onset of morphological changes (28). In the present study, we verified the presence of endothelial dysfunction in children

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**Table 1. Baseline Characteristics of the FH Children and Controls**

<table>
<thead>
<tr>
<th></th>
<th>Controls (Non-FH) n = 19</th>
<th>Simvastatin (FH) n = 28</th>
<th>Placebo (FH) n = 22</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No (%) male</td>
<td>11 (58)</td>
<td>15 (54)</td>
<td>11 (50)</td>
<td></td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>14.2 ± 3.1</td>
<td>14.6 ± 2.0</td>
<td>14.6 ± 2.5</td>
<td>0.826</td>
</tr>
<tr>
<td>BMI</td>
<td>21.5 ± 5.4</td>
<td>21.1 ± 3.7</td>
<td>21.5 ± 4.0</td>
<td>0.943</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>121 ± 17</td>
<td>125 ± 14</td>
<td>125 ± 17</td>
<td>0.592</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>66 ± 11</td>
<td>66 ± 9</td>
<td>67 ± 8</td>
<td>0.988</td>
</tr>
<tr>
<td>TC (mmol/l)</td>
<td>4.32 ± 0.85</td>
<td>6.97 ± 1.24*</td>
<td>7.34 ± 1.44*</td>
<td>0.0001</td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td>1.42 ± 0.31</td>
<td>1.27 ± 0.22</td>
<td>1.40 ± 0.30</td>
<td>0.148</td>
</tr>
<tr>
<td>LDL-C (mmol/l)</td>
<td>2.51 ± 0.66</td>
<td>5.31 ± 1.14*</td>
<td>5.47 ± 1.44*</td>
<td>0.0001</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)†</td>
<td>0.76 (0.50–1.38)</td>
<td>0.79 (0.50–1.82)</td>
<td>1.07 (0.34–1.79)</td>
<td>0.222</td>
</tr>
<tr>
<td>Baseline vessel size (mm)</td>
<td>3.10 ± 0.53</td>
<td>3.19 ± 0.59</td>
<td>3.14 ± 0.41</td>
<td>0.769</td>
</tr>
<tr>
<td>FMD (%)</td>
<td>15.6 ± 6.8</td>
<td>11.7 ± 5.0*</td>
<td>11.6 ± 3.5*</td>
<td>0.024</td>
</tr>
</tbody>
</table>

All values are given as means (SD) except for triglycerides given as median (range).

BMI = body mass index; FH = familial hypercholesterolemia; FMD = flow-mediated dilation; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; TC = total cholesterol; †post hoc analyses \( p < 0.05 \), vs controls; *tested after log-transformation.

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**Figure 1.** Flow-mediated dilation (FMD) (%) placebo versus simvastatin (familial hypercholesterolemia [FH]) at baseline and 28 weeks. **Striped bar** = baseline; **white bar** = 28 weeks. \( * p < 0.0001 \) vs. baseline; \( † p < 0.05 \), Δ FMD placebo vs. Δ FMD simvastatin.
with FH without signs of macrovascular disease as compared with age and non-FH controls.

After short-term (28 weeks) statin therapy we show an improvement of endothelial dysfunction in the hypercholesterolemic children. To the best of our knowledge, this is the first placebo-controlled trial that assessed the effects of statin therapy on endothelial function in children with FH. Many researchers investigated the effect of cholesterol-lowering therapy on peripheral (11,13) and coronary endothelial function (29–32), albeit in adults. Both positive (11,29–31) and negative (32) results have been reported. We have previously demonstrated complete reversibility of peripheral endothelial dysfunction in adult patients with FH after short-term lipid-lowering therapy (11). In contrast, after long-term statin therapy in patients with coronary artery disease, clear attenuation of the acetylcholine-induced vasoconstriction has been demonstrated, but endothelium-dependent vasodilation could not be restored (30,31). These findings emphasize that in order to achieve normalization of endothelial function in “atherogenic” vascular beds, such as the coronary arteries, therapy should be initiated at an early stage, before the onset of severe macrovascular structural abnormalities.

In our study, the obtained reductions for TC, LDL-C, and TG are comparable to those in adults (33). Previous statin trials in children showed similar reductions in lipids, but these studies contained small numbers or included only boys (15–17). In the present cohort, no toxicity or serious adverse or side effects were reported by the children during the course of this study. However, the duration of the present study is too short to draw conclusions with regard to the safety of long-term use of simvastatin in children. The long-term safety and efficacy of statins in children is currently being evaluated in ongoing trials at our department.

The mechanism behind the beneficial effects of simvastatin on endothelial function can be twofold: 1) by the inhibition of hepatic HMG CoA reductase and the subsequent lowering of serum cholesterol levels (34), or 2) by a direct effect on the vascular wall (35). The correlation between FMD improvement and the degree of LDL cholesterol lowering in the current study implies a role for cholesterol lowering per se in mediating the beneficial effects of statins. However, evidence has cumulated to show that statins also exert lipid-independent pleiotropic effects on the vasculature; for instance, by upregulating endothelial cell NO synthase through stabilization of messenger ribonucleic acid levels. In this respect, it remains to be established whether and to what extent non-statin-induced lipid lowering will have similar effects on vascular reactivity.

Finally, it should be taken into account that changes in vascular smooth muscle cell reactivity cannot be excluded as a potential cause of the altered FMD response, as nitroglycerin was not administered to these young children. However, in previous studies we and others have shown normal vascular responses towards exogenous nitrates in hypercholesterolemia (8,11), making it unlikely that altered smooth-muscle cell reactivity is of relevance in the present study.

**Clinical implications.** Endothelial dysfunction represents one of the earliest stages of atherogenesis and has been shown to have a clear predictive value for future CVD. In the present study, we show an improvement of endothelial dysfunction in the forearm vasculature after short-term statin therapy in children with FH. These findings underscore the importance of lipid-lowering therapy in children with FH. Hence, until long-term safety data on the use of statins in children become available, the current data vigorously support the institution of statin therapy at early stages, when endothelial dysfunction is still amenable to complete normalization.

**Table 2. Lipid Measurements at Baseline, 28 Weeks, and Mean Absolute Change From Baseline for the Simvastatin and Placebo FH Group**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>28 Weeks</th>
<th>p Value</th>
<th>Absolute Change</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (mmol/l) Simvastatin</td>
<td>6.97 ± 1.24</td>
<td>4.80 ± 1.05</td>
<td>0.0001</td>
<td>−2.16 ± 1.04</td>
<td>0.0001</td>
</tr>
<tr>
<td>Placebo</td>
<td>7.34 ± 1.44</td>
<td>7.31 ± 0.33</td>
<td>0.854</td>
<td>−0.05 ± 1.17</td>
<td>0.808</td>
</tr>
<tr>
<td>HDL-C (mmol/l) Simvastatin</td>
<td>1.27 ± 0.22</td>
<td>1.32 ± 0.24</td>
<td>0.141</td>
<td>−0.05 ± 0.17</td>
<td>0.050</td>
</tr>
<tr>
<td>Placebo</td>
<td>1.40 ± 0.30</td>
<td>1.34 ± 0.33</td>
<td>0.300</td>
<td>−0.05 ± 0.22</td>
<td>0.014</td>
</tr>
<tr>
<td>LDL-C (mmol/l) Simvastatin</td>
<td>5.31 ± 1.14</td>
<td>3.17 ± 0.96</td>
<td>0.0001</td>
<td>−2.13 ± 0.99</td>
<td>0.0001</td>
</tr>
<tr>
<td>Placebo</td>
<td>5.47 ± 1.44</td>
<td>5.45 ± 2.03</td>
<td>0.819</td>
<td>−0.05 ± 1.06</td>
<td>0.004</td>
</tr>
<tr>
<td>Triglycerides (mmol/l) Simvastatin</td>
<td>0.79 (0.50–1.82)</td>
<td>0.60 (0.30–1.65)</td>
<td>0.002</td>
<td>−0.19 ± 0.37</td>
<td>0.041</td>
</tr>
<tr>
<td>Placebo</td>
<td>1.07 (0.34–1.79)</td>
<td>0.80 (0.40–2.55)</td>
<td>0.791</td>
<td>−0.10 ± 0.54</td>
<td>0.009</td>
</tr>
</tbody>
</table>

*Lipids baseline vs. 28 weeks, †absolute change simvastatin vs. placebo; ‡tested after log-transformation. All values are given as means (SD) except for triglycerides given as median (range).*
REFERENCES


