Chapter 7

Early Statin Therapy Restores Endothelial Function in Children with Familial Hypercholesterolemia.

Saskia de Jongh, Marc R. Lilien, Jos op 't Roodt, Erik S. G. Stroes, Henk D. Bakker, and John J.P. Kastelein

1 Department of Vascular Medicine, 2 Emma Children’s Hospital, Academic Medical Center Amsterdam, 3 Wilhelmina Children's Hospital, Utrecht, The Netherlands

Journal of the American College of Cardiology 2002, in press
Abstract

Objectives: To determine whether simvastatin improves endothelial function in children with familial hypercholesterolemia (FH).

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

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

Results: At baseline, FMD was impaired in FH children 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 % versus 15.5 ± 5.4 %, p=0.958). Upon treatment, the simvastatin FH group showed significant absolute reductions of TC (-2.16 ± 1.04 mmol/L, 30.1%) and 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).

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

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 and premature atherosclerosis, whereas the disease is usually a symptomatic in children\(^3\).

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 pro-atherogenic factors such as vasoconstriction, platelet activation, monocyte adhesion, thrombogenesis and inflammation\(^7\). In the last decade, a number of studies have shown that endothelial function measured as flow-mediated dilatation (FMD) is impaired in FH children\(^8\);\(^9\);\(^10\). Hydroxymethylglutaryl coenzyme A reductase inhibitors (statins) have been shown to reverse endothelial dysfunction, however only in adult dyslipidemic patients\(^11\);\(^12\);\(^13\). However, at later stages statin therapy is unable to normalize endothelial function in e.g. coronary arteries, likely due to structural changes.

In spite of the aggressive nature of the disease, the recommended therapy for FH 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\(^15\);\(^16\);\(^17\). In view of the limited time-span for cardiovascular prevention in FH patients, improvement of endothelial function at an early reversible stage of the atherosclerotic process would provide a strong argument for implementation of statin therapy already in childhood\(^4\).

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

Patients
A total of 50 heterozygous FH children, aged 9-18, 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 9-18 years of age participated in the study as controls. The following criteria were designed to select children with heterozygous FH: plasma LDL cholesterol levels above 95th percentile for age and gender; a documented family history of hyperlipidemia with LDL cholesterol levels above the 95th 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 vaso-active medications; and concomitant conditions such as serious illness, hypertension or diabetes mellitus.

Study design
The dosage of simvastatin was doubled every 8 weeks from 10 to 20 to 40 mg per day. The assessment of FMD of the brachial artery as parameter for endothelium-dependent vasodilatation 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), low-density lipoprotein cholesterol (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. HDL-C 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 dilatation
The assessment of flow-mediated dilatation (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 10-15 minutes rest, the brachial artery in the right ante-cubital fossa was visualized using a 7.5 MHz transducer. After an optimal image of the brachial artery wall was obtained, a wall tracking system was used to measure the lumen diameter. After obtaining 2 baseline vessel diameter measurements, reactive hyperemia was induced by inflating the blood pressure cuff to 200 mmHg, distal to the location of transducer. Upon release of the cuff after 4 minutes, the ensuing dilatation of the brachial artery is predominantly mediated by endothelial NO-release. Ultrasonography then continued for 5 minutes to allow for lumen diameter measurements at 20 seconds intervals. Wall track measurements were stored digitally and analyzed off-line by a blinded observer using the wall track system software analysis package. 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 2 measurements. Flow-mediated dilatation was calculated at each examination as \( \{(\text{maximal lumen diameter after ischemia} - \text{diameter at baseline}) / \text{diameter at baseline}\} \) and expressed as a percentage. Intra- and intersession variation coefficients are 1.1 and 3.8 % respectively. The total duration of this investigation is approximately 20 minutes.

**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 ANOVA. In case of a significant result a post hoc analysis were performed. Multi comparisons were taken into account by using the Bonferroni Method. Differences between 2 groups were tested by using Students’ t-test for continuous data. Skewed data were first 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 significant.

**Results**

Baseline characteristics showed no significant difference with regard to sex, 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 FH children compared to the non-FH controls (p<0.0001). FMD was impaired in the FH children versus the non-FH controls (p<0.024). There were no significant differences with regard to age, sex, 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.

Table 1. Baseline characteristics of the FH children and controls

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls (non-FH)</th>
<th>Simvastatin (FH)</th>
<th>Placebo (FH)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=19</td>
<td>n=28</td>
<td>n=22</td>
<td></td>
</tr>
<tr>
<td>No (%) male</td>
<td>11 (58)</td>
<td>15 (54)</td>
<td>11(50)</td>
<td></td>
</tr>
<tr>
<td>Age (year)</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 bloodpressure (mmHG)</td>
<td>121 ± 17</td>
<td>125 ± 14</td>
<td>125 ± 17</td>
<td>0.592</td>
</tr>
<tr>
<td>Diastolic bloodpressure (mmHG)</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>TG (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, TC=total cholesterol, HDL=high-density lipoprotein, LDL-C=low-density lipoprotein FMD=flow-mediated dilatation, †Tested after log-transformation, * post hoc analyses p<0.05, versus controls.

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 (figure 1). There was no difference with regard to the baseline vessel sizes between both groups at 28 weeks (simvastatin: 2.96 ± 0.53 mm versus placebo: 3.05 ± 0.38 mm, p=0.592). The mean absolute change in FMD was significantly higher in the simvastatin FH group compared to the placebo FH group (3.9 ± 4.3 % versus 1.2 ± 3.9 %, p=0.05). Eight children in the placebo FH group (8/22; 36 %) compared to 19 children in the simvastatin FH group (19/28; 68 %) had an improvement of FMD larger than 2.5%.
In the simvastatin FH group, FMD increased to a level similar to that in non-FH controls (15.6 ± 6.8 % versus 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 FH children 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).
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>Lipids</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)</td>
<td>simvastatin</td>
<td>6.97 ± 1.24</td>
<td>4.80 ± 1.05</td>
<td>0.0001</td>
<td>-2.16 ± 1.04</td>
</tr>
<tr>
<td></td>
<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>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>simvastatin</td>
<td>1.27 ± 0.22</td>
<td>1.32 ± 0.24</td>
<td>0.141</td>
<td>0.05 ± 0.17</td>
</tr>
<tr>
<td></td>
<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>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>simvastatin</td>
<td>5.31 ± 1.14</td>
<td>3.17 ± 0.96</td>
<td>0.0001</td>
<td>-2.13 ± 0.99</td>
</tr>
<tr>
<td></td>
<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>
</tr>
<tr>
<td>TG (mmol/L)*</td>
<td>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>
</tr>
<tr>
<td></td>
<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>
</tr>
</tbody>
</table>

All values are given as means (SD) except for triglycerides given as median (range), TC=total cholesterol, HDL=high-density lipoprotein, LDL-C =low-density lipoprotein, * Teste d after log-transformation, † lipids baseline vs. 28 weeks, ‡ absolute change simvastatin vs. placebo

Discussion

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

FH patients 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 FH patients, 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 FH children \(^8-10\), which clearly precedes the onset of morphological changes \(^28\). In the present study, we
verified the presence of endothelial dysfunction in FH children without signs of macrovascular disease as compared to 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 FH children. Many researchers investigated the effect of cholesterol-lowering therapy on peripheral \(^{11,13}\), and coronary endothelial function, albeit in adults \(^{29-32}\). Both positive \(^{11,29-31}\) and negative \(^{32}\) results have been reported. We have previously demonstrated complete reversibility of peripheral endothelial dysfunction in adult FH patients after short-term lipid-lowering therapy \(^{11}\). In contrast, after long-term statin therapy in patients with coronary artery disease (CAD), clear attenuation of the acetylcholine-induced ‘paradoxical’ vasoconstriction has been demonstrated but, endothelium-dependent vasodilatation 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 \(^{35}\). 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- and/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: (a) by the inhibition of hepatic HMG CoA reductase and the subsequent lowering of serum cholesterol levels \(^{34}\), and/or (b) by a direct effect on the vascular wall \(^{35}\). The correlation between FMD improvement and the degree of LDL-C 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 up regulating endothelial cell NO synthase (eNOS) through stabilization of mRNA 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 potential cause for the altered FMD response, since nitroglycerine (NTG) 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 FH children. These findings underscore the importance of lipid-lowering therapy in FH children. Hence, as soon as long-term safety data on the use of statins in children have become available, the current data vigorously support the institution of statin therapy at early stages, where endothelial dysfunction is still amenable to complete normalization.
References


