Familial hypercholesterolemia in childhood
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Chapter 9

Plant Sterols Lower LDL cholesterol
Without Improving Endothelial Function in
Pre-pubertal Children with Familial
Hypercholesterolemia

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Submitted for publication
Abstract

Objectives: In adults with familial hypercholesterolemia (FH), cholesterol lowering with statins has been shown to improve endothelial function, a hallmark of early atherogenesis. Currently, therapeutic options for treating high cholesterol levels in FH children are limited. Plant sterols safely and effectively reduce serum cholesterol concentrations by inhibiting cholesterol absorption. Therefore, we evaluated the effect of plant sterols on cholesterol and vascular function in prepubertal children with FH.

Study design: We included 41 children (5-12 y) with FH in a double blind crossover trial using spreads containing 2.3 grams of plant sterols (mainly sitosterol and campesterol) per 15 gram spread and placebo spread for a 4-week period, separated by a 6 weeks wash-out period. Lipid levels and endothelial function were assessed after both 4-week treatment periods. Endothelial function was assessed as flow-mediated dilatation (FMD) of the brachial artery using a wall tracking system. Data were compared to those of 20 healthy controls.

Results: Intake of 2.3 grams plant sterols per day decreased total cholesterol (TC; -11%) and low-density cholesterol (LDL-C; -14%) as compared to placebo spread in FH children. FH children were characterized by an impaired FMD compared to healthy control children (7.2 ± 3.4 % versus 10.1 ± 4.2 %, p<0.005). However, the reduction of LDL-C in FH children did not improve FMD (placebo: 7.2 ± 3.4 % versus plant sterol: 7.7 ± 4.1%).

Conclusion: The present study shows a clear reduction of LDL-C by plant sterols therapy. However, short-term plant sterol therapy does not improve the endothelial function in FH children.
Effect of plant sterols on endothelial function

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. With a frequency of one in 400 persons, FH is one of the most common inborn errors of metabolism in the Dutch population. This disorder is associated with elevated levels of LDL cholesterol (LDL-C) and premature atherosclerosis. Affected persons show symptoms of atherosclerotic cardio-vascular disease at relatively young age, sometimes before the age of 30. However, the disease is usually asymptomatic in children.

In spite of the aggressive nature of the disease, the recommended therapy for FH children only consists of a cholesterol-lowering diet and, if necessary, bile-acid binding resins. However, clinical experience shows that the long-term cholesterol-lowering efficacy of dietary intervention in children is very limited and resins appear safe, but their lipid-lowering efficacy is modest and long-term compliance remains poor. Treatment with HMG CoA reductase inhibitors (statins) has been evaluated to a certain degree and is expected to result in a better therapeutic response, but is still under debate because of insufficient safety data in children.

Plant sterols and plant stanols effectively and safely reduce serum cholesterol by inhibiting cholesterol absorption in the small intestine. Studies with hypercholesterolemic and normocholesterolemic children, have shown that plant sterols/stanols can reduce the plasma levels of total cholesterol (TC) and LDL-C by 10% and 15%, respectively, without any clinical adverse events. However, it is unknown whether and to what extent this reduction of LDL-C will contribute to an improved clinical outcome in FH children.

The last decade, endothelial dysfunction has emerged as the reflection of an early, but reversible stage in the development of atherosclerosis. Several studies have shown that the presence of endothelial dysfunction has predictive value for future cardiovascular events. In addition, other studies have shown that endothelial function, measured as flow-mediated dilatation (FMD) of the brachial artery, is impaired in FH children. In view of the limited time-span for cardiovascular prevention in FH patients, restoration of endothelial dysfunction at an early stage of the atherosclerotic process would provide a strong argument for lipid-lowering therapy, initiated in early childhood even before puberty. Plant sterols might be a possible solution in addition to diet therapy for lipid-lowering in these children.
We therefore designed this placebo controlled cross-over study to determine whether pre-pubertal FH children are characterized by an impaired endothelial function of the brachial artery and whether short-term dietary intervention with plant sterols can improve endothelial dysfunction in young FH children.

**Patients and methods**

**Patients**

In present study we included 41 pre-pubertal heterozygous FH children between 5 and 12 years of age and 20 non-FH, controls. All FH children were on a low-saturated-fat, low-cholesterol diet (Step I). 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 for age and gender before treatment or a personal diagnosis of FH by detection of a mutation in the LDL receptor gene. Exclusion criteria for both the FH children and controls were: post menarche in girls; boys with a Tanner stage more than genital- and pubic hair stage 1; smoking; current use of any vaso-active medications or dietary supplements; and concomitant conditions such as serious illness, hypertension or diabetes mellitus.

**Study design**

The present study was a double blind placebo controlled cross-over trial. After a 2 week run-in period or 6 weeks run-in period for children who used plant sterol enriched products, all children were randomly assigned to consume either 15 grams of plant sterol spread or 15 grams of placebo spread. Twenty children started to consume the plant sterol spread for 4 weeks, whereas 21 children started to consume the placebo spread. After a 6-week wash-out period, in which the children consumed placebo spread, they crossed-over to the alternate spread for another 4 weeks.

Endothelial function was assessed by FMD after both 4-week periods. At baseline and after both 4-week treatment periods, capillary lipid profile was measured [TC, high density lipoprotein cholesterol (HDL-C), LDL-C and triglycerides (TG)]. Physical examination (including height, weight and blood pressure) was performed at the baseline visit and after the second treatment period. Compliance was measured during each visit by counting the returned full
and empty tubs. In the healthy controls FMD and capillary lipid profile was assessed once. Each 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).

**Spread composition and administration**
The plant sterol-enriched and control spread were produced and prepared by UBF, Purfleet, UK and Unilever Research, Vlaardingen, The Netherlands. Fat and sterol composition of the spreads were analyzed according to methods described by Weststrate et al.\(^2\) The spread was distributed to the patients in identical-looking 15 gram tubs and they were instructed to eat one tub daily. Preferably, the subjects should consume it as spread on sandwiches, but they could also eat it as part of a hot meal if the spread was mixed with the food on the plate. The tubs were labeled A or B and delivered to the children ones during each intervention period in boxes of 32 tubs (including spare tubs). Packaging and labeling were performed by persons not involved with the patients or data handling. All other personal and statisticians were blinded to the treatments. The plant sterol-enriched spread contained 5.7 grams of fat (composed of 24.3 % saturated fatty acids [SAFA], 24.2 % monounsaturated fatty acids [MUFA] and 51.0 % polyunsaturated fatty acids [PUFA]), and 2.3 grams of sterols in free sterol equivalents (composed of 46.9 % sitosterol, 27.3 % campesterol, 16.3 % stigmasterol and 9.5 % other sterols) per 15 grams of spread. The control spread contained 5.4 grams of fat (composed of 23.2 % SAFA, 25.5 % MUFA and 50.8 % PUFA) per 15 grams of spread.

**Flow-mediated dilatation**
The assessment of flow-mediated dilatation (FMD) was performed as published previously.\(^23,\)\(^24\) 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.\(^16,\)\(^25\) 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 offline 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 \( \frac{\text{(maximal lumen diameter after ischemia - diameter at baseline)}}{\text{diameter at baseline}} \) 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. Assuming that the common standard deviation of FMD is 2.0%, a sample size of 17 children in each group will have 80% power to detect a difference in means of 2%, using a two group t-test with a 0.05 two-sided significance level. Skewed data were tested using the Mann Whitney test. Mean values before and after therapy within the FH group were compared using the paired Student t-test. A p-value < 0.05 was considered significant.

**Results**

A total of 41 FH children (age: 8.2 years) and 20 controls (age: 9.2 years) were included in this study (table 1). The baseline characteristics showed no significant differences between the FH children and the controls with regard to gender, length, weight and blood pressure. However, as expected in FH, TC and LDL-C were significantly higher (p<0.001) and HDL-C was significantly lower in the FH children. All 41 FH children completed the study. Based on the amount of returned empty and full tubs the compliance was 97 % in both the placebo and treatment group. The FH children did not report any adverse effects throughout the whole study.
Table 1. Baseline characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Controls</th>
<th>FH (home diet)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>20</td>
<td>41</td>
</tr>
<tr>
<td>Male/female</td>
<td>9-nov</td>
<td>20/21</td>
</tr>
<tr>
<td>Age (years)</td>
<td>8.2 (2.2)</td>
<td>9.2 (1.6)</td>
</tr>
<tr>
<td>Length (m)</td>
<td>1.34 (0.14)</td>
<td>1.37 (0.10)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>31.5 (8.0)</td>
<td>33.3 (8.6)</td>
</tr>
<tr>
<td>Syst. bloodpressure (mmHg)</td>
<td>95 (12)</td>
<td>97 (9)</td>
</tr>
<tr>
<td>Diast. bloodpressure (mmHg)</td>
<td>57 (11)</td>
<td>57 (6)</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>4.03 (0.63)</td>
<td>7.30 (1.51)*</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>2.20 (0.65)</td>
<td>5.68 (1.53)*</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.52 (0.41)</td>
<td>1.25 (1.25)*</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>0.73 [0.51-1.34]</td>
<td>0.74 [0.46-2.20]</td>
</tr>
</tbody>
</table>

All values are given as means (SD), except for TG given as median [range]. TC; total cholesterol, LDL-C; low-density lipoprotein cholesterol, HDL-C; high-density lipoprotein cholesterol, TG; triglycerides. *p<0.001, †p<0.05

There was no carry-over effect in the FH group (placebo and plant sterol) with regards to the lipid and FMD data, and therefore, all data were analyzed as planned. After 4 weeks of treatment with plant sterol, TC significantly reduced by -0.79 mmol/L (95%CI: -1.02 to -0.60) and LDL-C by -0.78 mmol/L (95%CI: -1.00 to -0.60) (table 2); as percentages these reductions were 11% for TC and 14% for LDL-C. HDL-C and TG did not change.

Table 2. Lipids and absolute changes in lipids in FH children

<table>
<thead>
<tr>
<th>Lipids</th>
<th>Placebo spread</th>
<th>Plant sterol spread</th>
<th>Mean Absolute change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=41</td>
<td>n=41</td>
<td>(95% CI)</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>7.06 (1.35)</td>
<td>6.27 (1.12)</td>
<td>-0.79 (-1.02 to -0.60)</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>5.40 (1.37)</td>
<td>4.58 (1.13)</td>
<td>-0.82 (-1.00 to -0.60)</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.29 (0.29)</td>
<td>1.31 (0.31)</td>
<td>0.02 (-0.06 to 0.10)</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>0.90 (0.40)</td>
<td>0.85 (0.36)</td>
<td>-0.05 (-0.20 to 0.09)</td>
</tr>
</tbody>
</table>

All values are given as means (SD), except for the mean absolute change given as mean (95 % CI)
TC; total cholesterol, LDL-C; low-density lipoprotein cholesterol, HDL-C; high-density lipoprotein cholesterol, TG; triglycerides
The FMD was significantly lower in the FH placebo group than in the healthy controls (7.2 ± 3.4% versus 10.1 ± 4.2%, p<0.005) (figure 1), while the baseline vessel size was not significantly different between these two groups (FH placebo: 2.7 ± 0.9 mm versus controls: 2.2 ± 1.0 mm). However, within the FH children the FMD between the placebo treatment and the plant sterol treatment phase remained essentially similar (7.2 ± 3.4 % versus 7.7 ± 4.1 %, p=0.592). There were no significant differences in baseline vessel size between the placebo and the plant sterol treatment (2.7 ± 0.9 mm versus 2.7 ± 1.0 mm).

**Figure 1.** Mean FMD (SD) of the FH treatment groups and the controls

* p<0.01 versus Controls

**Discussion**

In the present study we show that pre-pubertal FH children are already characterized by impaired endothelial function. Whereas the use of plant sterol spread leads to a 14% decrease of LDL-C, this reduction is not associated with restoration of endothelial dysfunction over a 4-week intervention period.

As expected in FH, our data show that pre-pubertal FH children already have severely elevated plasma levels of LDL-C. The use of plant sterol ester enriched spread (2.3 gram sterols/day) induced a 14% reduction in LDL-C. These data are in line with the few studies previously described in FH children. One study in 7 young children showed a reduction of 17% of LDL-C, using a three-fold higher dosage of free plant sterols, compared to the present study. Another study performed by the same authors showed an exceptional LDL-C reduction of 20 %. However, the
mean LDL-C level in this study group was much higher (7.87 mmol/L) than in the present study, which may explain the 20 % reduction. In a larger study by Gyling et al, the LDL-C reduction by a sitostanol margarine spread was similar to the LDL-C reduction in our study 11. Recently, another study with 41 FH children using a sterol spread (1.6 g/day) showed a LDL-C reduction of 10.2% 29. Clinically relevant adverse effects were absent in all of the studies. Thus, plant sterols/stanols spreads seem both efficacious and well tolerated in hypercholesterolemic children, and offer an additional tool for cholesterol-lowering therapy in these children.

In the general population, the process of atherosclerosis begins in adolescence 30. In view of the aggressive nature of cardiovascular disease (CVD) in FH patients, it reasonable to assume that atherosclerotic changes arise during early childhood. In line with these findings, we have recently demonstrated the presence of endothelial dysfunction in another cohort of 50 adolescent FH children 31. Other studies have also demonstrated an impaired endothelial function in FH children. However, these studies were either small or concerned children with a higher mean age than present study 32-34. To the best of our knowledge the current study is the first concerning only pre-pubertal FH children.

After 4 weeks of plant sterol treatment we showed a significant reduction of LDL-C and TC. However, this reduction did not restore the endothelial dysfunction. Reduction of LDL-C by short-term treatment with lipid-lowering drugs (statins) is associated with complete normalization of endothelial dysfunction in adults 35,36. In a placebo controlled study we recently showed that short-term statin therapy (28 weeks) normalizes endothelial dysfunction in 50 FH adolescents (de Jongh et al. JACC in press). Two factors may contribute to the observed difference between sterols and statins: First, statins reduce LDL-C by 40 % in hypercholesterolemic adults 37 and adolescents 9, whereas plant sterols only mildly reduce LDL-C (10-15%). The absence of vascular effects during sterol therapy might imply a threshold of LDL-C lowering before improvement of endothelial function can occur. Second, statins have been shown to exert direct 'pleiotrophic' effects on the vasculature 38. The absence of such an effect on FMD during sterol use might also be the consequence of a lack of pleiotrophic effects of sterols. However, the improvement of endothelial function after LDL-C aphaeresis 39 and the correlation between LDL-C levels and endothelial function clearly show a major role for changes in LDL-C per se. Hence, the difference in potency between statins and sterols is most likely to explain the difference in vascular effects.
Overall, these data lend further support to aggressive lipid-lowering therapy already in pre-pubertal FH children, when endothelial dysfunction is still amenable to complete normalization. It remains to be established if, in spite of the lack of vascular effects, the use of long-term sterols can improve future cardiovascular outcome in this high-risk pediatric group.

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References


