# PLANT STANOLS DO NOT RESTORE ENDOTHELIAL FUNCTION IN PRE-PUBERTAL CHILDREN WITH FAMILIAL HYPERCHOLESTEROLEMIA DESPITE REDUCTION OF LOW-DENSITY LIPOPROTEIN CHOLESTEROL LEVELS

LILY JAKULJ, MSC, MAUD N. VISSERS, PHD, JESSICA RODENBURG, MD, PHD, ALBERT WIEGMAN, MD, PHD, MIEKE D. TRIP, MD, PHD, AND JOHN J.P. KASTELEIN, MD, PHD

**Objective** **To examine the effect of plant stanols on lipids and endothelial function in pre-pubertal children with familial hypercholesterolemia (FH).**

**Study design** **Children with FH (n = 42), aged 7-12 years, were enrolled in a double-blind crossover trial, in which they consumed 500 mL of a low-fat yogurt enriched with 2.0 g of plant stanols and 500 mL of a low-fat placebo yogurt for 4 weeks, separated by a 6-week washout period. Lipid profiles and endothelial function were assessed after both consumption periods. Endothelial function was measured as flow-mediated dilation (FMD) of the brachial artery.**

**Results** **This daily intake of 2.0 g of stanols significantly decreased the levels of total cholesterol (TC) by 7.5% and low-density lipoprotein cholesterol (LDL-C) by 9.2% as compared with placebo. High-density lipoprotein cholesterol and triglyceride levels remained unaltered. The reduction of LDL-C levels did not improve FMD, which was 10.5% ± 5.1% after plant stanol consumption and 10.6% ± 5.0% after placebo consumption, respectively (*P* = .852).**

**Conclusion** **This study demonstrates that plant stanols reduce LDL-C levels in children with FH without improving endothelial function.** ***(J Pediatr 2006;148:495-500)***

Familial hypercholesterolemia (FH) is an inherited autosomal dominant disorder of lipoprotein metabolism caused by a variety of mutations in the low-density lipoprotein (LDL)-receptor gene.[1] The disorder is associated with elevated cholesterol levels from birth onward and premature atherosclerosis. Although children with FH are almost always asymptomatic, development of atherosclerosis has already started, as reflected by increased intima-media thickness [2] and endothelial dysfunction.[3-6] This supports the view that prevention of atherosclerotic development should be initiated in early childhood. In the past decade, food products enriched with plant sterols have been introduced to reduce cholesterol levels. Plant sterols, which are synthesized in plants, are structurally similar to cholesterol and naturally present in the diet. Beta-sitosterol, campesterol, and stigmasterol are the most common ones. Plant sterols and their saturated counterparts, stanols, inhibit the absorption of cholesterol in the small intestine by decreasing the incorporation of dietary and biliary cholesterol into micelles.[7] Sterols and stanols have been shown to safely reduce total cholesterol (TC) and LDL cholesterol (LDL-C) levels by approximately 10% in adults and children with FH.[8-10] We previously evaluated the effect of 2.3 g of plant sterols on LDL-C levels and endothelial function for 4 weeks in children with FH.[3] Although LDL-C levels decreased by 14%, endothelial dysfunction did not improve. This suggests a possible untoward effect of plant sterols on the vascular endothelium. Although serum plant sterol levels were not measured, it is known that serum plant sterol concentrations increase by 39% to 96% after daily consumption of 2.0 g of plant sterols.[11-13] Raised serum plant sterol concentrations were suggested as a potential risk factor for coronary heart disease.[14] Although serum sterol concentrations remain within the normal range after consumption of plant sterols,[15] it is not known whether raised sterol concentrations affect endothelial function and thereby counteract the beneficial effects of plant sterols on LDL-C levels. However, this should not apply to plant stanols, which are hardly absorbed and actually lower serum plant sterol concentrations.[12,16] Therefore, we designed a study to evaluate the effect

| | | | |
|---|---|---|---|
| FH | Familial hypercholesterolemia | NCEP | National Cholesterol Education Program |
| FMD | Flow-mediated dilation | TC | Total cholesterol |
| HDL-C | High-density lipoprotein cholesterol | TG | Triglyceride |
| LDL-C | Low-density lipoprotein cholesterol | | |

From the Department of Vascular Medicine, Academic Medical Centre, Amsterdam, the Netherlands; and Emma Children's Hospital, Academic Medical Centre, Amsterdam, the Netherlands.

Submitted for publication May 29, 2005; last revision received Oct 6,2005; accepted Nov 4, 2005.

Reprint requests: Lily Jakulj, Academic Medical Centre, Department of Vascular Medicine, University of Amsterdam, Meibergdreef 9 – Room F4-159.2, 1105 AZ Amsterdam.E-mail: l.jakulj@amc.uva.nl

0022-3476/$ - see front matter



10.1016/j.jpeds.2005.11.023

**Table I. Baseline characteristics**

| Characteristics | FH subjects |
|---|---|
| n | 41 |
| Male/female | 22/19 |
| Age (years) | 9.8 (1.5) |
| Length (m) | 1.43 (0.10) |
| Weight (kg) | 36.5 (8.3) |
| BMI (kg/m$^2$) | 17.7 (2.8) |
| Systolic blood pressure (mm Hg) | 108 (12) |
| Diastolic blood pressure (mm Hg) | 61 (9) |
| TC (mmol/L) | 6.92 (1.55) |
| LDL-C (mmol/L) | 5.38 (1.69) |
| HDL-C (mmol/L) | 1.32 (0.31) |
| TG (mmol/L) | 0.64 [0.51–0.95] |

All values are given as means (SD), except for triglycerides as median [interquartile range].
To convert cholesterol levels expressed in mmol/L to mg/dL, multiply by 38.67.
To convert triglyceride levels expressed in mmol/L to mg/dL, multiply by 88.57.
*n*, Number.

of short-term consumption of plant stanols on LDL-C levels and to evaluate whether the presumed LDL-C level reduction will improve endothelial function in pre-pubertal children with FH.

## METHODS

### Subjects

Children were recruited from the outpatient lipid clinic at Emma Children's Hospital in Amsterdam. Each subject and each subject's parents or guardians gave written informed consent for the child's participation in this study, which was approved by the institutional review board of our center.

We enrolled 42 pre-pubertal children with FH who were between 7 and 12 years of age. All subjects met these inclusion criteria: a personal diagnosis of FH by means of detection of a mutation in the LDL-receptor gene or a LDL-C level higher than the 95th percentile for age and sex,[17] and 1 parent with a confirmed diagnosis of FH. None of the children had clinical signs of hypercholesterolemia, and none of the children smoked. Three subjects received drugs for asthma, and 1 boy received medication for his attention deficit hyperactivity disorder. At the start of the study, none of the girls had reached menarche. Clinical characteristics at baseline are shown in Table I.

### Study Design

The study was a double-blind placebo-controlled crossover trial. Subjects started with a 2 week diet run-in period, in which they were not allowed to consume any plant sterol- or stanol-enriched food products. Twenty-four children, however, started with an extended run-in period of 6 weeks because they were already consuming these commercially available products on a regular basis. The extended run-in period also applied to 5 children who consumed dietary vitamin supplements, because short-term treatment with antioxidants has been shown to influence endothelial function in children with FH.[18] The children were restricted from these products and supplements during the study. After the run-in period, subjects were randomly assigned to consume either 500 mL of yogurt containing 2.0 g of plant stanols or 500 mL of the placebo low-fat yogurt without plant stanols, for 4 weeks each. Twenty children started with the plant stanol-enriched yogurt, and 22 children first ingested the control-yogurt during this first study period. A 6-week washout period followed, after which the children changed to the alternate yogurt for another 4 weeks. During the entire study, all children were on a low-saturated fat, low-cholesterol diet, compatible with the National Cholesterol Education Program Step II (NCEP-II) diet.[19] Endothelial function was assessed at the end of both consumption periods, and lipid profiles were measured at baseline and at the end of both consumption periods. A physical examination, including measures of height, weight, blood-pressure, and heart-rate, was performed at baseline and at the end of the study.

### Lipid Profiles

After an overnight fasting period of at least 12 hours, a capillary lipid profile (TC), high-density lipoprotein cholesterol (HDL-C), LDL-C, and triglyceride levels (TG) were measured. After 15 minutes in the sitting position, finger-stick samples were drawn into heparin-coated capillary tubes. The blood (35 $\mu$L) was dispensed immediately onto commercially available test cassettes (Cholestech, Hayward, Calif) for analysis in a portable lipid analyzer (Cholestech LDX, Cholestech Corporation, Hayward Calif).[20]

### Yogurt Composition and Administration

The stanol-enriched and the low-fat control yogurts were both produced and blinded by Campina, Woerden, the Netherlands. The yogurts were distributed in identical-looking 500-mL jars. The children were instructed to consume 1 jar daily with their meals. At the start of each consumption period, the children received yogurt for 1 week in a cooling bag. For the remaining 3 weeks, parents collected yogurt at the hospital on a weekly basis. Compliance was measured at each study-visit by collection and calculation of the empty and full jars of yogurt.

### Endothelial Function

Endothelial function was measured as flow-mediated dilation (FMD) according to a standardized protocol.[21,22] In brief, children were in the morning fasting state and were studied in the supine position. A blood pressure cuff was placed distal to the elbow of the right arm. After a 10-minute rest, the brachial artery in the right antecubital fossa was visualized by using a 7.5-MHz linear transducer. After obtaining an optimal view of the artery, the lumen diameter was measured by using a wall tracking system. Three baseline vessel diameter measurements were obtained, after which reactive hyperemia was induced by inflation of the cuff to a