No Association Between Plasma Levels of Plant Sterols and Atherosclerosis in Mice and Men

Kenneth R. Wilund, Liqing Yu, Fang Xu, Gloria L. Vega, Scott M. Grundy, Jonathan C. Cohen, Helen H. Hobbs

Objective—Sitosterolemia is characterized by elevated plasma levels of plant sterols, hypercholesterolemia and premature coronary heart disease (CHD). CHD develops in some subjects with sitosterolemia, despite having normal plasma cholesterol levels, suggesting that high circulating levels of plant sterols may be atherogenic. We tested whether elevated plasma levels of plant sterols (sitosterol and campesterol) were associated with atherosclerosis in genetically modified mice and in middle-aged men and women.

Methods and Results—Wild-type and hypercholesterolemic female mice with >20-fold higher plasma levels of plant sterols because of inactivation of the ATP-binding cassette (ABC) half transporters G5 and G8 (G5G8−/− mice) were fed chow or Western diets for 7 months. No significant differences in aortic lesion area were found when the sitosterolemic mice were compared with littermate controls. To determine whether plasma levels of plant sterols were associated with coronary atherosclerosis in humans, the relationship between plasma plant sterols and coronary calcium (detected by electron beam computer tomography) was examined in 2542 subjects aged 30 to 67 years. Plasma levels of cholesterol, but not sitosterol or campesterol, were significantly higher in subjects with coronary calcium.

Conclusions—The results of this study do not support an association between elevated plasma levels of plant sterols and atherosclerosis. (Arterioscler Thromb Vasc Biol. 2004;24:2326-2332.)

Key Words: sitosterolemia ■ ATP-binding cassette transporters ■ atherosclerosis ■ plant sterols

The 2 major classes of dietary sterols are those derived from animals (cholesterol) and those derived from plants (phytosterols). The most abundant dietary phytosterols are sitosterol and campesterol, which differ from cholesterol by an ethyl or methyl group attached to the side chain at C24. Although animal-derived and plant-derived sterols are structurally similar, they are handled differently by mammals. Less than 5% of dietary sitosterol is absorbed by the intestine, whereas between 30% and 80% of dietary cholesterol is incorporated into chylomicrons and delivered to the liver. Moreover, the small fraction of dietary plant sterols that reaches the liver is preferentially secreted into the bile. As a result of the low fractional absorption and enhanced biliary secretion of plant sterols, phytosterols comprise <1% of circulating sterols.

Individuals with mutations in either of 2 ATP-binding cassette (ABC) half transporters, ABCG5(G5) or ABCG8(G8), have sitosterolemia. This autosomal-recessive disorder is characterized by a >50-fold elevation in the plasma levels of plant sterols and is frequently associated with the development of tendon and cutaneous xanthomas, as well as premature coronary atherosclerosis (coronary heart disease [CHD]). Although many subjects with sitosterolemia have hypercholesterolemia, the plasma levels of cholesterol can vary dramatically in these individuals and can sometimes be within the normal range. The presence of coronary atherosclerosis in the absence of hypercholesterolemia in some sitosterolemic patients has led to speculation that elevated plasma levels of plant sterols promote development of atherosclerosis, even in persons without sitosterolemia.

In normolipidemic individuals, plasma levels of plant sterols vary over a 5- to 10-fold range. Several studies have reported that plasma levels of plant sterols are associated with CHD risk or with a family history of CHD, suggesting that plant sterols may be atherogenic. Some cholesterol-lowering agents, such as statins and stanol esters, increase plasma levels of plant sterols, whereas other agents, such as ezetimibe, reduce circulating phytosterol levels. The effect of such changes in plant sterol levels on coronary atherosclerotic lesion development is not known.

To determine whether elevated levels of plant sterols promote the development of atherosclerosis, we examined the
aortas of mice lacking G5 and G8 (G5G8/−/− mice). These mice have 30- to 100-fold elevations in plasma plant sterol levels. We also examined whether elevated levels of plant sterols accelerate the development of atherosclerosis in hypercholesterolemic animals by comparing aortic atherosclerosis in mice lacking both G5 and G8 and the low-density lipoprotein receptor (LDLR) (G5G8/−/−; Ldlr/−/− mice) and in mice lacking only the LDLR (Ldlr/−/−). In addition, we tested for association between plasma levels of plant sterols and either family history of CHD or coronary atherosclerosis, assessed using electron beam computer tomography (EBCT) in a population-based sample of middle-aged Americans.

Methods

Animals and Diets

Feeding studies were performed in 4 groups of mice: G5G8+/+; Ldlr+/+ (wild-type), G5G8+/−; Ldlr+/−, G5G8−/−; Ldlr+/−, and G5G8−/−; Ldlr−/− mice. Males G5G8−/− on a mixed genetic background (C57BL/6d and 129SvEv) were crossed with female Ldlr−/− mice (C57BL/6d; 129Sv; Jackson Laboratories, Bar Harbor, Me; #002077). The F1, G5G8+/−; Ldlr+/− males and females were crossed to produce G5G8+/−; Ldlr+/− mice. The F2, G5G8+/+; Ldlr+/− mice were mated, producing G5G8−/−; Ldlr−/− and G5G8+/−; Ldlr−/− littersmates. C57BL/6d; 129Sv/SF2 mice (Jackson Laboratories, #101045) were used to generate the wild-type mice. Wild-type female mice were crossed with male G5G8−/−; Ldlr+/− and the F1, G5G8−/−; Ldlr+/− mice were mated to produce G5G8−/−; Ldlr+/− and G5G8+/−; Ldlr+/− littersmates.

Mice were housed in plastic cages in temperature-controlled rooms (22°C) with a 12-hour light/12-hour dark cycle. Animals were fed ad libitum with standard chow (Diet 7001; Harlan Teklad, Madison, Wis) containing 0.02% cholesterol and 4% total fat. Mice were switched to 100 mmol/L for 2 hours. Lipids were extracted in petroleum ether, 80°C. For en face analysis, the aortas were transverse sectioned into 1 mm segments, pinned on a wax tray, and dissected from the abdominal aorta. Digital images were captured and lesion area was quantified using Metamorph Imaging System software, version 6.0 (Universal Imaging Corporation).

Atherosclerosis Analysis in Mice

The amount of atherosclerosis in the aorta (en face analysis) was quantified in 10 female mice in each group after 7 months of the indicated diet. The mice were fasted for 4 hours during the light/dark cycle, anesthetized by intraperitoneal injection of pentobarbital (2 mg) before perfusing the heart with 50 mL phosphate-buffered saline. The heart and aorta (from the ascending aorta to the iliac bifurcation) were placed in 4% paraformaldehyde overnight. After fixation, the heart was dissected from the aorta, embedded in OCT, and frozen at −80°C. For en face analysis, the aortas were transferred to phosphate-buffered saline after fixation and stored overnight at 4°C. Adventitial tissue was removed and the intimal surface was exposed. The aortic arch was cut longitudinally from the ascending arch to the left subclavian artery. The aorta was pinned open on a wax tray, rinsed for 5 minutes in 75% ethanol, stained with 0.5% Sudan IV in 35% ethanol and 50% acetone (15 minutes), destained in 75% ethanol (5 minutes), then rinsed with distilled H2O. No lesions were detected in the abdominal aorta in any animals (data not shown), so analysis was restricted to the aortic arch and thoracic aorta. Digital images were captured and lesion area was quantified from the aortic arch to 5 mm distal to the left subclavian artery using Metamorph Imaging System software, version 6.0 (Universal Imaging Corporation).

Plasma Sterol Analysis in Mice

An aliquot of ethanol containing the internal standards 5α-cholestane (50 μg) and epiprostanol (2.5 μg) was added to 50 μL of plasma, and sterols were hydrolyzed by heating (100°C) in ethanolic KOH (100 mmol/L) for 2 hours. Lipids were extracted in petroleum ether, dried under nitrogen, and derivatized with hexamethyldisilazane-trimethylchlorosilane. Gas chromatography (GC) and mass spectrometry was performed using an Agilent 6890N gas chromatograph coupled to an Agilent 5973 mass selective detector. The derivatized sterols were separated on an HP-5MS 5%phenyl methyl polysiloxane capillary column (30 m ×0.25 mm inner diameter ×0.25 μm film) with carrier gas helium (1 mL/min). The column temperature was set at 150°C for 2 minutes, then increased by 20°C/min up to 280°C and held for 13 minutes. The injector was operated in the splitless mode and was kept at 280°C. The MS was operated in selected ion monitoring (SIM) mode. The masses of the extracted ions were 458.4 (cholesterol), 343.3 (desmosterol), 458.4 (lathosterol), 456.4 (zymosterol), 382.4 (campesterol), 393.4 (lanosterol), and 396.4 (β-sitosterol).

Human Subjects

The human subjects were participants in the Dallas Heart Study, a probability-based sample of Dallas County. This study was approved by the Institutional Review Board at the University of Texas Southwestern Medical Center. Ethnicity was self-assigned as non-Hispanic blacks (blacks), whites, Hispanics, and “other.” Subjects in the “other” ethnic group were excluded from the analysis. During a structured interview, participants were asked if a first-degree relative had experienced a myocardial infarction (MI). Subjects were classified into 5 groups: (1) those with no family history of MI in a first-degree relative (n=1682); (2) at least 1 first-degree relative with a premature MI (younger than 55 years in women, younger than 50 years in men) (n=338); (3) at least 1 first-degree relative with an MI that was not premature (older than 50 years in men, older than 55 years in women) (n=647); (4) at least 1 first-degree relative who had an MI, age unknown (n=31); and (5) those who were uncertain about their family history (n=423). Individuals in group 1 were classified as having a negative family history of CHD and those in groups 2 to 4 were classified as having a positive family history of CHD. Individuals in group 5 were excluded from the analysis.

Fasting venous blood samples were collected into tubes containing citrate–EDTA and maintained at 4°C until the plasma was isolated and stored at −80°C. Plasma plant sterol levels (including campesterol and sitosterol) were measured by GC.

Plasma samples were saponified in 3% potassium hydroxide/ethanol at 65°C for 3 hours, 5α-cholestane was added as an internal recovery standard, and the lipids were extracted using petroleum ether. Samples were dried under nitrogen and the lipids were redissolved in Tri-Sil reagent (produced by Pierce) for analysis by GC. All plasma levels of plant sterols are expressed as a ratio to circulating cholesterol levels.

A total of 2542 subjects who met the inclusion criteria for the analysis obtained an EBCT scan of the heart to quantify coronary artery calcium levels. Subjects were defined as having a “positive” EBCT test if the EBCT measurement had a value >10 Agatston units.

Sequencing Exons and Flanking Introns of ABCG5 and ABCG8

The coding regions and intron/exon boundaries of ABCG5 and ABCG8 were sequenced as described in a single Dallas Heart Study participant with markedly elevated plasma levels of sitosterol and campesterol.

Statistics

Data are reported as means±SEM, or as medians and interquartile ranges. Differences in group means were tested by 2-tailed Student t tests or by ANOVA when means from 3 or more groups were being compared. Because the distribution of plant sterol levels in the population was highly skewed, the values were log-transformed before ANOVA analysis. Differences in median plant sterol levels between the various gender and ethnic groups were tested using Kruskal–Wallis 1-factor analysis of variance.

The relationships between sterol levels and other physiological variables were tested using Spearman ranked correlation test. Statistical tests were performed using SPSS (version 10.0) software.
Results

No Association Between Plant Sterol Levels and Aortic Atherosclerosis in Genetically Modified Mice

The mean plasma levels of cholesterol, sitosterol, and campesterol were compared in G5G8+/+; Ldlr+/+ (wild-type), G5G8+/+; Ldlr−/−, G5G8+/−; Ldlr+/+, and G5G8+/−; Ldlr−/− mice (Table 1). Total plasma sterol levels did not differ significantly between the chow-fed G5G8−/− and wild-type mice (79.6±6.3 versus 83.4±8.5 mg/dL), but the G5G8−/− mice had an 88-fold higher plasma level of sitosterol, a 13-fold higher plasma level of campesterol, and a 34% reduction in mean plasma level of cholesterol. As a consequence of these differences in sterol levels, plant sterols comprised 31% of the circulating sterols in the chow-fed G5G8−/− mice, compared with <1% in the wild-type mice.

As expected, inactivation of the Ldlr gene was associated with a significant increase in plasma levels of total sterols and ingestion of a Western diet caused further increases in sterol levels. Plant sterols comprised ∼12% of the total circulating sterol in the G5G8−/−; Ldlr−/− mice, compared with <0.2% in the G5G8+/−; Ldlr−/− mice, but the plasma levels of cholesterol were similar in the 2 strains of mice (771±84 mg/dL versus 765±39 mg/dL).

After 7 months of the indicated diets, the aortas of the female mice were excised and stained with Sudan IV, and the aortic lesion area (extending from the aortic arch to 5 mm distal to the left subclavian artery) was quantified as described in Methods. No significant atherosclerotic lesions was seen in the aortas of the chow-fed G5G8−/−; Ldlr−/−, G5G8+/−; Ldlr−/−, G5G8−/−; Ldlr+/+, or G5G8+/−; Ldlr−/− mice (Figure 1). Histological studies of thin sections from the proximal aorta were performed in these mice and either trace or no Oil Red O staining was detected (data not shown). Virtually no lesions were detected either en face (Figure 1) or in the aortic root (data not shown) in the G5G8−/−; Ldlr−/−, and G5G8+/−; Ldlr−/− mice on the Western diet.

In contrast to these results, 20% to 25% of the lumenal surface of the aortic arch and thoracic aorta stained positive for Sudan IV in both the G5G8−/−; Ldlr−/− and G5G8+/−; Ldlr−/− mice fed the Western diet (Figure 2). Despite the G5G8−/−; Ldlr−/− mice having a 116-fold higher plasma sitosterol and a 19-fold higher plasma level of campesterol than the G5G8+/−; Ldlr−/− mice, no significant differences in aortic lesion areas were found between these 2 strains of mice.

No Relationship Between Plant Sterol Levels and Family History of CHD or Coronary Calcium in Dallas Heart Study

Plasma cholesterol, sitosterol, and campesterol levels were measured by GC in 3252 black, white, and Hispanic participants (1828 women and 1424 men, ages 30 to 65 years) from the Dallas Heart Study. In contrast to plasma cholesterol

### Table 1. Plasma Sterol Levels in 8-Week-Old Female Mice (n=10 in each group) Fed the Indicated Diet for 7 Months

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Diet</th>
<th>Total</th>
<th>Cholesterol</th>
<th>Sitosterol</th>
<th>Campesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>G5G8+/+; Ldlr+/+</td>
<td>Chow</td>
<td>83.4±8.5</td>
<td>82.7±8.5</td>
<td>0.2±0.0</td>
<td>0.5±0.1</td>
</tr>
<tr>
<td>G5G8−/−; Ldlr+/+</td>
<td>Chow</td>
<td>79.6±6.3</td>
<td>55.2±4.8*</td>
<td>17.6±4.2*</td>
<td>6.8±0.8*</td>
</tr>
<tr>
<td>G5G8+/+; Ldlr−/−</td>
<td>Chow</td>
<td>189.0±19.6</td>
<td>186.6±19.5</td>
<td>0.8±0.1</td>
<td>1.6±0.3</td>
</tr>
<tr>
<td>G5G8−/−; Ldlr−/−</td>
<td>Chow</td>
<td>216.7±14.9</td>
<td>167.5±13.9</td>
<td>33.0±5.3*</td>
<td>16.2±2.0*</td>
</tr>
<tr>
<td>G5G8+/+; Ldlr+/+</td>
<td>Western</td>
<td>149.1±19.7</td>
<td>148.6±19.6</td>
<td>0.2±0.0</td>
<td>0.3±0.0</td>
</tr>
<tr>
<td>G5G8−/−; Ldlr+/+</td>
<td>Western</td>
<td>138.6±9.3</td>
<td>117.2±8.7</td>
<td>15.1±1.8*</td>
<td>6.3±0.6*</td>
</tr>
<tr>
<td>G5G8+/+; Ldlr−/−</td>
<td>Western</td>
<td>772.5±83.8</td>
<td>770.5±83.6</td>
<td>0.6±0.1</td>
<td>1.4±0.7</td>
</tr>
<tr>
<td>G5G8−/−; Ldlr−/−</td>
<td>Western</td>
<td>864.7±44.2</td>
<td>764.5±39.1</td>
<td>66.5±7.4*</td>
<td>33.7±3.4*</td>
</tr>
</tbody>
</table>

Values are means±SEM.

*P<0.05.

Plasma levels of sterols were compared between G5G8+/+ and G5G8−/− mice with each Ldlr genotype and diet.
levels, which were normally distributed, the distributions of sitosterol and campesterol were positively skewed, as previously described \(^* \) (Figure I, available online at http://atvb.ahajournals.org). The mean and median plasma levels of plant sterols (corrected for plasma cholesterol levels) were not significantly different between the sexes or between the ethnic groups (Table 2).

Spearman rank correlation coefficients between the plasma sterol levels and other physiological variables were calculated for all subjects (data not shown). Stratification of the data by gender or ethnicity did not significantly affect the strengths of the correlations observed, so the data were pooled before analysis (Table 3). Sitosterol and campesterol levels were positively correlated with each other \((r=0.892, P<0.001)\), with total cholesterol \((r=0.206 \text{ for sitosterol and 0.247 for campesterol})\), and with plasma LDL cholesterol levels. The plant sterol levels were inversely related to body mass index, fasting glucose, and fasting insulin levels, as has been previously observed.\(^{13,14,16,25}\) A modest, but significant negative correlation was also found between the plasma levels of sitosterol and plasma triglycerides \((r=-0.059; P=0.001)\).

One subject, a 31-year-old man of Pacific Islander and Asian descent, with previously undiagnosed sitosterolemia was identified. He had a plasma sitosterol level of 6.12 mg/dL, campesterol of 3.6 mg/dL, cholesterol of 229 mg/dL (80th percentile when compared with age- and sex-matched controls), triglycerides of 113 mg/dL (55th percentile), and LDL cholesterol of 170 mg/dL (85th percentile). The subject denied any family history of CHD and did not undergo an EBCT scan. Direct DNA sequencing of the exons and flanking introns of \(ABCG5\) and \(ABCG8\) revealed that he was homozygous for a novel substitution of thymidine for guanine in the consensus splice donor site immediately 3’ of exon 1 of \(ABCG5\) (IVS1.1 G>T) (Table I, available online at http://atvb.ahajournals.org).

The relationship between plasma levels of sitosterol and campesterol and a family history of CHD was examined (Table 4). Plasma levels of cholesterol were significantly higher in the participants who had a first-degree relative with premature coronary artery disease (MI in men younger than 50 years and women younger than 55 years) or who reported

**Table 2. Demographics and Plasma Levels of Sterol in Whites, Blacks, and Hispanics in the Dallas Heart Study**

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>White</td>
<td>Hispanic</td>
</tr>
<tr>
<td>Age, y</td>
<td>43.8±9.4</td>
<td>39.8±9.3*</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>28.4±5.2</td>
<td>28.9±4.5</td>
</tr>
<tr>
<td>Cholesterol, mg/dL</td>
<td>185±38.5</td>
<td>188±43.0</td>
</tr>
<tr>
<td>Mean sitosterol:C, μg/mg</td>
<td>0.9±0.6</td>
<td>0.8±0.5</td>
</tr>
<tr>
<td>Median sitosterol:C, μg/mg</td>
<td>0.72</td>
<td>0.66</td>
</tr>
<tr>
<td>Mean campesterol:C, μg/mg</td>
<td>1.6±1.1</td>
<td>1.4±0.8</td>
</tr>
<tr>
<td>Median campesterol:C, μg/mg</td>
<td>1.27</td>
<td>1.17</td>
</tr>
</tbody>
</table>

Table values are means±SEM, except when indicated.

*P<0.05 for comparison of means within genders.
†P<0.05 for comparison of means between genders.
C indicates cholesterol.
a history of MI in a first-degree relative at any age. In contrast, mean and median levels of plasma sitosterol and campesterol were not significantly different between those individuals with and those without a family history of MI or of premature CHD.

EBCT, a rapid noninvasive quantitative method that measures the amount of calcium in the coronary arteries, was performed on 2542 of the study participants (50% blacks, 32% whites, and 18% Hispanics) who obtained plasma sterol measurements (Table 5). Because the population was young (age 30 to 65), many subjects had little or no detectable coronary calcium. For this reason, subjects were classified as those with a history of MI in a first-degree relative at any age. In contrast, mean and median levels of plasma sitosterol and campesterol were not significantly different between those individuals with and those without a family history of MI or of premature CHD.

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Discussion

In this study, we examined the relationship between plasma levels of plant sterols and atherosclerosis in genetically modified mice that have markedly elevated levels of plant sterols, and in men and women from a population-based study. Despite very high plasma levels of plant sterols, little or no aortic atherosclerosis was apparent in the G5G8/−/− animals, irrespective of whether they were fed chow or Western diets. In mice with high plasma cholesterol levels because of inactivation of Ldlr, G5G8 deficiency did not exacerbate the atherosclerosis (Figures 1 and 2). Thus, even in severely hypercholesterolemic mice with significant atherosclerosis, increased levels of plant sterols were not associated with a greater aortic lesion area. It remains possible that the effect of the higher phytosterol levels on atherosclerotic lesion area in the G5G8/−/−; Ldlr−/− is not apparent because of the very high plasma levels of cholesterol in the Ldlr−/− mice fed a Western diet. However, these results strongly suggest that plant sterols are not more atherogenic than cholesterol, at least in mice.

The observation that some patients with sitosterolemia have normocholesterolemia at diagnosis and yet have premature CHD9–11,26–28 has stimulated the hypothesis that elevated plasma levels of plant sterols may promote atherosclerosis, even in individuals without sitosterolemia. Miettinen10 suggested that lipoproteins enriched in plant sterols might be taken-up more efficiently by tissues, thereby exacerbating atherosclerosis. This notion was supported by the finding that high plasma levels of plant sterols were associated with a positive family history of CHD and with coronary atherosclerosis in some studies.12,14 Glueck et al14 found that the prevalence of family history of CHD was elevated >2-fold in hyperphytosterolemic patients (defined as subjects having at least one plant sterol above the 95th percentile and a second above the 75th percentile). In the present study, we failed to find any evidence supporting an association between plasma levels of sitosterol and campesterol and a family history of CHD. The frequency of subjects with plasma levels of plant sterols greater than either the 90th or the 95th percentile was not shown). Alternatively, because plasma levels of plant sterols are positively corre-

lated with plasma cholesterol, individuals with higher plasma levels of plant sterols may be at greater risk for CHD because they are more likely to have hypercholesterolemia. We found no association between plasma levels of sitosterol or campesterol and family history of CHD in the Dallas Heart Study, irrespective of whether or not we corrected the levels of plant sterols for circulating levels of cholesterol.

We also did not find any evidence that elevated levels of plant sterols were a risk factor for the development of coronary atherosclerotic lesions in a middle-aged population. No relationship was found between the plasma levels of phytosterols and the presence of coronary calcium, as detected by EBCT, in either middle-aged men or in middle-aged women. However, these studies do not rule out the possibility that plant sterols may impact on CHD later in its course, by affecting plaque stability or by promoting thrombosis.

Ingestion of high levels of plant sterols reduces cholesterol absorption and lowers plasma cholesterol levels. One concern with the use of plant sterols as a cholesterol-lowering agent is that the increase in plasma plant sterols associated with dietary plant sterol supplementation may be atherogenic. Diets supplemented with high levels of plant sterols have been shown to prevent plaque formation in a variety of animal models, including apolipoprotein E knockout mice,29,30 apoE*3 Leiden transgenic mice,31 and in New Zealand White rabbits.32 In these animals, the reduction in atherosclerosis correlated with the reductions in plasma cholesterol levels associated with plant sterol supplementation. Increased circulating levels of plant sterols did not mitigate the beneficial effects of cholesterol reduction. These findings, together with the results of the present study, suggest that the modest increases in plasma plant sterol levels associated with dietary plant sterol supplementation are unlikely to confer increased risk of atherosclerosis.

In summary, our data provide no evidence that elevated plasma levels of plant sterols confer CHD risk, either in mice or in humans. Why, then, does premature coronary artery disease develop in some patients with sitosterolemia who have normal plasma cholesterol levels? One possibility is that sitosterolemic patients who have normal cholesterol levels at the time of diagnosis had high plasma levels of cholesterol at earlier stages in the course of the disease. The elevation in plasma cholesterol levels associated with sitosterolemia appears to be most severe in younger individuals; some children with sitosterolemia have plasma cholesterol levels as high as those seen in individuals with homozygous familial hypercholesterolemia.33,34 Perhaps elevated plasma cholesterol levels in early childhood are responsible for the development of premature coronary atherosclerosis later in life. Careful studies of the natural history of atherosclerosis in patients with sitosterolemia will be required to determine the relationship of the disease with plasma sterol levels.

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Supplementary Figure I

- **Median** = 0.71 µg/mg
- **Mean** = 0.89 µg/mg
- **Range**: 0.08 – 5.18 µg/mg

- **Median** = 1.23 µg/mg
- **Mean** = 1.48 µg/mg
- **Range**: 0.11 – 11.39 µg/mg

- **Median** = 177 mg/dL
- **Mean** = 180 mg/dL
- **Range**: 55 – 405 µg/dL

**Cholesterol (mg/dL)**

**Sitosterol:Cholesterol (µg/mg)**

**Campesterol:Cholesterol (µg/mg)**
Supplementary Figure Legend

Figure I. Distribution of plasma sterol levels in the Dallas Heart Study. Fasting blood samples were collected from all subjects and plasma was isolated by centrifugation and frozen at -80°C until analyzed. Plasma sterol levels were determined by GC analysis as described in Methods. The distribution of sterol levels in each gender and ethnic group was similar so pooled data from all subjects is represented here.
### TABLE I. Clinical characteristics and molecular defect of subject with sitosterolemia identified in Dallas Heart Study.

<p>| | |</p>
<table>
<thead>
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</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>31</td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>21.6</td>
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<tr>
<td>Ethnicity</td>
<td>Pacific Islander/East Indian</td>
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<tr>
<td>Sitosterol (mg/dL)</td>
<td>6.12</td>
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<tr>
<td>Campesterol (mg/dL)</td>
<td>3.6</td>
</tr>
<tr>
<td>Total Cholesterol (mg/dL)</td>
<td>229</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dL)</td>
<td>170</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dL)</td>
<td>37</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>113</td>
</tr>
<tr>
<td>Family history of CHD</td>
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</tr>
<tr>
<td>ABCG5 mutation</td>
<td>Intron 1: splice donor site</td>
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<tr>
<td></td>
<td>Normal: TACAGCGTCAGgt</td>
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<tr>
<td></td>
<td>Subject: TACAGCGTCAGtt</td>
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