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Original Article

The association between hypercholesterolemia and sitosterolemia, and report of a sitosterolemia kindred

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BACKGROUND: Sitosterolemia is associated with increases in intestinal sterol absorption, low-density lipoprotein cholesterol (LDL-C), and cardiovascular disease risk.

OBJECTIVE: We examined the relationship between hypercholesterolemia and sitosterolemia in a large population and report a new sitosterolemia case.

METHODS: Plasma sterol concentrations were measured by gas chromatography/mass spectrometry, and LDL-C by direct assay.

RESULTS: Of 207,926 subjects tested, 4.3% had LDL-C ≥190 mg/dL. Plasma β-sitosterol concentrations ≥8.0 mg/L (99th percentile) were found in 4.3% of these subjects vs 0.72% with LDL-C <130 mg/dL. Among all subjects, 0.050% had β-sitosterol levels ≥15.0 mg/L, consistent with sitosterolemia, while among those with LDL-C ≥190 mg/dL, 0.334% had this rare disorder. A 13-year-old boy with the highest LDL-C (679 mg/dL) of all subjects had planar xanthomas and a β-sitosterol level of 53.5 mg/L (normal <3.3 mg/L). He was a compound heterozygote for 2 *ABCG8* mutations (p.N409D and an intron 11+2T>A splice site mutation). On a low-cholesterol and plant-sterol diet, his LDL-C decreased to 485 mg/dL (−29%) and β-sitosterol to 44.6 mg/L (−27%). On atorvastatin 20 mg/d, his LDL-C decreased to 299 mg/dL (−38%). With added ezetimibe 10 mg/d, his LDL-C normalized to 60 mg/dL (−80% further decrease); and his β-sitosterol decreased to 14.1 mg/L (−68% further decrease).

CONCLUSIONS: Our data indicate that about 4% of subjects with LDL-C concentrations ≥190 mg/dL have plasma β-sitosterol concentrations above the 99th percentile and about 0.3% have concentrations consistent with sitosterolemia. Therefore, this diagnosis should be considered in such patients.

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Introduction

Sitosterolemia is a rare lipid disorder, first described in 1974 by Bhattacharyya and Connor in 2 young sisters with tendinous xanthomas and markedly elevated plasma β-sitosterol and campesterol levels.[1] Their plasma total cholesterol (TC) levels were normal at 195 and 206 mg/dL, and they were found to have significant increases in the intestinal absorption of the plant sterols β-sitosterol and campesterol.[1] Subsequently, plasma β-sitosterol and cholesterol kinetics and biliary and fecal excretion of sterols were studied by radioisotopic tracers in these patients while on a low–plant sterol diet.[2] The mean plasma half-life of the first exponential of plasma β-sitosterol turnover was 9.2 days, more than twice that in the normal subjects; and the mean half-life of the second exponential of plasma β-sitosterol turnover was 156 days, about 10 times longer than in normal subjects. In contrast, the mean cholesterol production rate into the fast turning-over pool was about 60% lower than in controls. TC synthesis was also measured by the sterol balance method and found to be about 70% lower than in controls. In 2 of the patients, treatment with a sterol-free diet resulted in 42% and 36% reductions in plasma β-sitosterol and cholesterol levels, respectively, while in a third patient, dietary treatment had no effect. The investigators concluded that sitosterolemia patients have a slow turnover of β-sitosterol, very low excretion of β-sitosterol into the bile and feces and low cholesterol synthesis rates.[2]

Salen et al in 1992 measured the absorption and turnover rates of cholesterol and β-sitosterol in a patient with homozygous sitosterolemia and in the patient's obligate heterozygous parents, while on controlled diets containing 500 mg/d of cholesterol and 100 mg/d of β-sitosterol.[3] In the homozygous proband, plasma TC and apolipoprotein (apo) B concentrations were slightly higher than in the heterozygous parents, whereas the plasma β-sitosterol level was more than 30-fold higher. Cholesterol absorption was at the high end of the normal range in all 3 individuals, and cholesterol synthesis was severely decreased. In contrast, β-sitosterol absorption in the homozygote was 2-fold greater than in the heterozygous parents and 7-fold greater than in non-sitosterolemic controls. The β-sitosterol turnover rate averaged 8 and 19 mg/d in the controls and heterozygotes, respectively, and was 27 mg/d in the homozygote. Moreover, the total body β-sitosterol pool size in the homozygote was 15-fold higher than in controls and 10-fold higher than in the heterozygous parents because of extremely slow removal. Salen et al concluded that, in contrast to sitosterolemic homozygotes, heterozygotes can compensate for their sterol overabsorption by enhancing their plasma clearance of plant sterols.[3]

In 2000 and 2001, a number of investigators reported different mutations in 2 adjacent, oppositely oriented genes that encoded for 2 newly discovered members of the adenosine triphosphate–binding cassette (ABC) transporter family, *ABCG5* and *ABCG8*.[4–6] These 2 genes were shown to code for separate sterol half-transporters, which were most highly expressed in the liver and intestine of mice. Cholesterol feeding upregulated their gene expression.[7] Studies in liver-specific [*L-G5G8*(−/−)], intestine-specific [*I-G5G8*(−/−)], and total [*G5G8*(−/−)] double knockout mice showed tissue β-sitosterol concentrations >90-fold higher in *G5G8*(−/−) mice than in wild-type animals.[7] Knock-in expression of *G5* and *G8* only in the intestine [*I-G5G8*(+/+)] or only in the liver [*L-G5G8*(+/+)] in whole-body knock-out mice decreased tissue plant sterol levels by 90%, compared with untreated double knockout, *G5G8*(−/−) animals. Compared with wild-type mice, with normal *G5G8* expression, biliary plant sterol secretion was reduced in *L-G5G8*(−/−) and whole-body *G5G8*(−/−) mice, but not in *I-G5G8*(−/−) mice. Conversely, absorption of plant sterols was increased in *I-G5G8*(−/−) and whole-body *G5G8*(−/−) mice, but not in *L*-G5G8(−/−) mice. Reverse cholesterol transport, as assessed from the fraction of intravenously administered ^{3}H-cholesterol appearing in feces, was reduced in all 3 models, whole-body *G5G8*(−/−), *I-G5G8*(−/−), and *L-G5G8*(−/−) mice. These data indicate that *ABCG5/G8* gene expression in both the liver and intestine protects animals from sterol accumulation, but by different mechanisms, and that both intestinal and liver *ABCG5/ABCG8* gene expressions are needed for full reverse cholesterol transport in mice.[7] It should be noted that in humans with sitosterolemia due to *ABCG5/ABCG8* mutations, there is a great deal of variability in plasma levels of low-density lipoprotein cholesterol (LDL-C). The reasons for this variability remain uncertain.[4–6]

In the current report, we provide an estimate of the prevalence of both elevated β-sitosterol (≥99th percentile, ≥8.0 mg/L) and frank sitosterolemia (≥15.0 mg/L) in a large diagnostic laboratory–based population, with special reference to patients with LDL-C values ≥190 mg/dL. In addition, we present a kindred with mutations in the *ABCG8* gene, in which the proband had a β-sitosterol concentration >50 mg/L and an LDL-C level >500 mg/dL. The former was reduced by 74%, and the latter was normalized with combined therapy of a low plant sterol, low-cholesterol diet, atorvastatin, and ezetimibe.

Methods

The population studied in this analysis was selected from among 451,843 blood samples sent by healthcare providers throughout the United States (by overnight express on cold packs) to Boston Heart Diagnostics over a 30-month period for the measurement of serum lipoproteins and plasma sterols. Excluded from the study population were subjects sampled more than once (only data from the first sample were used), those not sampled after an overnight fast, and those taking statins and/or ezetimibe. These subjects were excluded because nonfasting status can affect plasma sterol concentrations and because statins