Increased Sitosterol Absorption Is Offset by Rapid Elimination to Prevent Accumulation in Heterozygotes With Sitosterolemia

Gerald Salen, G.S. Tint, Sarah Shefer, Virgie Shore, and Lien Nguyen

Using plasma isotope-kinetic methods, we measured the absorption and turnover rates of cholesterol and sitosterol (24-ethylcholesterol) in two obligate heterozygotes (parents) and their homozygous daughter with sitosterolemia with xanthomatosis. Diets contained approximately 500 mg/day cholesterol and 100 mg/day sitosterol. In the homozygote, plasma cholesterol and apolipoprotein B concentrations were slightly higher, but sitosterol levels were 22 and 58 times higher than in her heterozygous parents. Cholesterol absorption was at the high end of the normal range in both heterozygotes (59% and 84%) and in the homozygote (62%) (value in the control subject 48%). In contrast, cholesterol synthesis was severely depressed in the homozygote (28% and 26% as great as in the heterozygotes and the control, respectively). Sitosterol absorption in the homozygote (34%) was 12 and 2.0 times greater than in the heterozygotes and 6.8 times greater than in the control. Cholesterol turnover rate, calculated independently by mathematical analysis of specific-activity decay curves, amounted to 15 and 24 mg/day in the heterozygotes compared with 7.9 ± 2.3 mg/day in five control subjects. However, the total body sitosterol pool was 15 and 103 times larger in the homozygote (4,080 mg) than in her heterozygous parents because of extremely slow removal. The average sitosterol elimination constant in the heterozygotes (K_e = 0.11 day\(^{-1}\)) was 10 times that in the homozygote (K_e = 0.01 day\(^{-1}\)) but 35% less than that in the controls (K_e = 0.17 day\(^{-1}\)). These results demonstrate that despite enhanced sitosterol absorption, small body pools and low plasma concentrations result from rapid elimination associated with adequate cholesterol synthesis in sitosterolemic heterozygotes. In distinction, sitosterol accumulates and body pools are disproportionately enlarged because increased absorption is combined with decreased removal, which may compensate for reduced cholesterol synthesis in sitosterolemic homozygotes.

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KEY WORDS • sitosterol absorption • elimination • pool sizes • sitosterolemia • heterozygotes • homozygotes

Sitosterolemia with xanthomatosis is a rare lipid storage disease that presents clinically with accelerated atherosclerosis, tendon and tuberous xanthomas, hemolysis, and symmetrical arthritis and arthralgias involving the ankle and knee joints. Increased amounts of plant sterols (sitosterol, campesterol, and stigmasterol) and 5α-stanols (cholestanol, 5α-sitostanol, and 5α-campestanol) are present in plasma and all tissues except brain. Enhanced absorption of sitosterol and structurally related sterols has been suggested to account for the enlarged pools, while cholestanol and 5α-saturated plant sterol derivatives are produced endogenously because diets are virtually devoid of these stanols. The disease is inherited as an autosomal recessive trait. Although increased plant sterol absorption with slow removal has been demonstrated in some homozygotes, the absorption and turnover rate of sitosterol have not been studied in heterozygotes whose plasma plant sterol and 5α-stanol concentrations are not elevated and who do not show clinical features of the disease.

We measured sitosterol and cholesterol absorption and turnover rates in two obligate sitosterolemic heterozygotes (parents) and their homozygous daughter. Our objective was to uncover differences in the metabolism of cholesterol and sitosterol between homozygotes and heterozygotes with this disease.

Methods

Clinical

Studies were conducted in one sitosterolemic female homozygote (aged 28 years) who showed tendon and tuberous xanthomas, an aortic systolic murmur, and periodic episodes of arthralgias involving both ankles and knees. Her parents (mother aged 50 years and father aged 56 years) are obligate heterozygotes and are

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clinically normal. All subjects ate regular meals, and food selections were supervised by the Metabolic Unit dietician. Weekly food diaries were reviewed, and daily calorie and sterol intakes were calculated. A representative 24-hour food sample was collected, and the sterol composition measured by capillary gas–liquid chromatography (GLC) contained 520 mg cholesterol and 105 mg sitosterol. Caloric intake and weight remained constant throughout the study. Control subjects for the absorption and turnover studies included four men and one woman (aged 36–69 years) who consumed similar diets.

Blood was collected into tubes that contained solid EDTA (Becton Dickinson, Rutherford, N.J.) after the subjects had fasted overnight for 12 hours. Plasma was separated from erythrocytes by centrifugation at 5,000g for 10 minutes at 4°C. The research protocols were approved by the human studies committees of the University of Medicine and Dentistry of New Jersey–New Jersey Medical School, Newark, N.J., and the Veterans Administration Medical Center, East Orange, N.J.

**Experimental Design**

Plasma sterol concentrations in fasting specimens from the homozygote, her two heterozygous parents, and the control subjects were measured weekly for 10 weeks by capillary GLC. Plasma apolipoprotein (apo) A-I and B concentrations were determined immunochemically on at least five specimens. Percent and mass absorption of exogenous sitosterol and cholesterol were measured simultaneously by the plasma dual-isotope ratio method in the homozygote, the two heterozygotes, and a healthy 55-year-old man who consumed the same diet. The plasma turnover rates of sitosterol and cholesterol were estimated by mathematical analysis of specific activity–time curves after simultaneous intravenous pulse-labeling with [3H]sitosterol and [14C]cholesterol. Body pool sizes and production rates were calculated simultaneously by the plasma dual-isotope ratio method in the homozygote, the two heterozygotes, and a healthy 55-year-old man who consumed the same diet.

**Sitosterol and cholesterol absorption.** The absorption of sitosterol and cholesterol was measured by the plasma dual-isotope ratio method as described for cholesterol by Zilversmit and Hughes and adapted for sitosterol and cholesterol.

**Sterol turnover rates.** Sitosterol and cholesterol turnover rates were calculated according to the two-compartment model. This model was selected for sitosterol because after isotope labeling, plasma sitosterol specific-activity decay gives an excellent fit for two exponentials. For cholesterol, the decay of plasma specific activities can be described by two or three exponentials. Using the three-compartment model, cholesterol pool sizes and turnover rates are larger. However, because the goal of this investigation was to compare sitosterol and cholesterol turnover rates, we analyzed both sterol specific-activity decay curves by using the same two-compartment model with the understanding that cholesterol pool sizes may be underestimated and turnover rates will be about 8% lower than if calculated by the three-compartment model.

**Results**

**Plasma Sterol and Apolipoprotein Concentrations**

Plasma sterol concentrations are presented in Table 1. Cholesterol levels in the controls and the two heterozygotes were similar and tended to be slightly lower than in the homozygote consuming the same diet. In contrast, plasma sitosterol levels in the homozygote were 22 and 58 times greater, respectively, than in her heterozygous parents and 95 times greater than in the controls. Plasma cholesterol levels in the homozygote were 5.8 and 11 times greater than in her heterozygous parents and 19 times greater than in the controls. Although sitosterol and cholesterol plasma concentra-
TABLE 1. Plasma Sterol Concentrations

<table>
<thead>
<tr>
<th>Subject</th>
<th>Weekly specimens analyzed</th>
<th>Sterol</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (n=5)</td>
<td></td>
<td>185±6</td>
<td>0.20±0.20</td>
<td>0.22±0.20</td>
</tr>
<tr>
<td>Heterozygotes</td>
<td></td>
<td>210±26</td>
<td>0.65±0.21</td>
<td>0.95±0.17*</td>
</tr>
<tr>
<td>Mother</td>
<td></td>
<td>194±14</td>
<td>0.34±0.10</td>
<td>0.36±0.09</td>
</tr>
<tr>
<td>Father</td>
<td></td>
<td>233±12</td>
<td>3.8±1.4</td>
<td>21.0±2.0</td>
</tr>
</tbody>
</table>

Values are mean±SD in milligrams per deciliter.
*p<0.002 different from control value.

Sterol Absorption

Values for cholesterol and sitosterol absorption are presented in Table 3. The 3H to 14C ratios of sitosterol and cholesterol isolated individually from the plasma were compared with the ideal ratios. The ideal sterol ratio, achieved if absorption is 100%, is calculated by dividing the total radioactive oral dose by the total radioactive injected dose. About 34% of the orally administered sitosterol was absorbed in the homozygote compared with 5% in the similarly fed control. However, in the two heterozygotes, sitosterol absorption amounted to 15% in the mother and 17% in the father, three times that in the control but only about one half the value in the homozygote. The constancy of the 3H to 14C ratio in the plasma (as shown by the coefficient of variation) is evidence against any significant in vivo loss of 3H from [3o^3H]sitosterol as absorbed.

TABLE 2. Plasma Apolipoprotein Concentrations

<table>
<thead>
<tr>
<th>Subject</th>
<th>Samples analyzed</th>
<th>Apolipoprotein A-I</th>
<th></th>
<th>Apolipoprotein B</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (n=5)</td>
<td>147</td>
<td>137-154</td>
<td>69</td>
<td>52-81</td>
<td></td>
</tr>
<tr>
<td>Heterozygotes</td>
<td>140</td>
<td>133-150</td>
<td>72</td>
<td>52-88</td>
<td></td>
</tr>
<tr>
<td>Mother</td>
<td>147</td>
<td>138-157</td>
<td>77</td>
<td>59-88</td>
<td></td>
</tr>
<tr>
<td>Father</td>
<td>147</td>
<td>124-135</td>
<td>131</td>
<td>102-150</td>
<td></td>
</tr>
</tbody>
</table>

Values are in milligrams per deciliter.

Table 3. Sitosterol and Cholesterol Absorption

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sitosterol Absorbed</th>
<th>Cholesterol Absorbed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% mg/day (±%)</td>
<td>% mg/day (±%)</td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Heterozygotes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mother</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>Father</td>
<td>17</td>
<td>8</td>
</tr>
<tr>
<td>Heterozygote</td>
<td>34</td>
<td>6</td>
</tr>
</tbody>
</table>

Sitosterol intake, 100 mg/day; cholesterol intake, 500 mg/day. Control, healthy 55-year-old man who consumed similar diet. CV, coefficient of variation.

TABLE 4. Sitosterol Turnover Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls (n=5)</th>
<th>Heterozygotes</th>
<th>Homozygote</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1/2 (days)</td>
<td>3.8±0.2</td>
<td>3.0</td>
<td>5.2</td>
</tr>
<tr>
<td>T2/2 (days)</td>
<td>13.8±2.4</td>
<td>39</td>
<td>23</td>
</tr>
<tr>
<td>Ks (days^-1)</td>
<td>0.17±0.04</td>
<td>0.12</td>
<td>0.10</td>
</tr>
<tr>
<td>MA (mg)</td>
<td>80±36</td>
<td>75</td>
<td>152</td>
</tr>
<tr>
<td>MB (mg)</td>
<td>46±22</td>
<td>190</td>
<td>246</td>
</tr>
<tr>
<td>MM (mg)</td>
<td>126±32</td>
<td>265</td>
<td>398</td>
</tr>
<tr>
<td>PRs (mg/day)</td>
<td>7.9±2.3</td>
<td>15</td>
<td>24</td>
</tr>
<tr>
<td>PRs (mg/kg/day)</td>
<td>0.095±0.028</td>
<td>0.3</td>
<td>0.32</td>
</tr>
</tbody>
</table>

Values are mean±SD for controls.
*Weight reported in Table 5.

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methyl cholesterol absorption, as reported in rhesus monkeys by Bhattacharyya and Eggen.28

Sitosterol Turnover

Sitosterol turnover parameters were calculated by mathematical analysis of specific-activity decay curves according to the kinetics of the two-compartment model.3 Table 4 lists key values. The homozygote showed much longer half-times for both exponentials than her heterozygous parents and the five controls. Both heterozygotes also exhibited longer half-times for the second exponential (T2/2) than the controls. These differences can also be seen by examining the plots of sitosterol specific activity versus time (Figure 1). Daily production (PRs), which is equivalent to absorption because there is no endogenous sitosterol synthesis, was substantially greater in the homozygote and the two heterozygotes than in the five controls. However, total body sitosterol pools were significantly greater in the sitosterolemic homozygote than in the controls (32 times) and the heterozygotes (15 and 10 times). The explanation for the disproportionately large deposits of sitosterol in the homozygote and only slight accumulations in the two heterozygotes can be seen by examining the excretion rate constant from pool A (Ka). Sitosterol elimination in the two heterozygotes was 10 times that in the homozygote but 35% less than in the controls. Thus, the failure to accumulate sitosterol in the heterozygotes despite increased absorption (PRs) relates to efficient excretion. Apparently in the heterozygotes the liver retains the ability to preferentially excrete...
sitosterol. However, the slight increases in the sitosterol pool and plasma concentrations (Table 1) in the heterozygotes compared with the controls probably relate to increased absorption.

The plot of sitosterol production (absorption) versus total pool size (Figure 2) shows a linear relation in all controls and the two heterozygotes. In contrast, the calculated total pool size for the homozygote is 10 times the predicted pool size because of the marked retention of sitosterol in this disease.

Cholesterol Turnover

Cholesterol turnover parameters computed by mathematical analysis of cholesterol specific-activity decay curves are listed in Table 5. As noted previously, in sitosterolemic heterozygotes radioactive cholesterol decayed more slowly and was associated with delayed elimination (smaller $K_A$) compared with controls. Unlike in the homozygote, in whom sitosterol specific activities declined more slowly than those of cholesterol, the more rapid decay of sitosterol relative to cholesterol was maintained in the heterozygotes (Figure 3). Cholesterol pool sizes varied and did not distinguish the homozygote from the controls or the heterozygotes. Of major significance, $PRA$, which represents the combination of absorption and synthesis, was much smaller in the homozygote than in either the heterozygotes or the controls. Further, after cholesterol absorption was subtracted from $PRA$, synthesis was markedly diminished in the sitosterolemic homozygote. Thus, cholesterol turn-
over and synthesis were comparable in the heterozygotes and the controls, but very low synthesis characterized the sitosterolemic homozygote.

Discussion

The important new observations made in sitosterolemic heterozygotes include increased sitosterol absorption with only slightly larger sitosterol pools. Sitosterol absorption in the heterozygotes was almost three times that in controls but still smaller than that in the homozygote. However, sitosterol elimination was 10 times more rapid in the heterozygotes than in the homozygote, which prevented excessive sitosterol accumulation. In contrast, cholesterol synthesis was markedly depressed in the homozygote but was not different from that in the controls in the heterozygotes.

Plasma Sterols and Apolipoproteins

Plasma sterol concentrations in the sitosterolemic heterozygotes resembled those in the controls, with sitosterol and cholestanol accounting for <1% of the total sterol concentrations compared with about 11% plant sterols and 5α-stanols in the sitosterolemic homozygote. However, in the mother the sitosterol and cholestanol concentrations were significantly greater than the control mean (p<0.01), suggesting that in a few heterozygotes both cholestanol and sitosterol levels are higher than in normal individuals. This finding was previously noted in Chinese heterozygotes by Wang et al12 and in Japanese heterozygotes by Hidaka et al13 and may relate to the greater consumption of vegetables in those diets.

Concentrations of apo A-I and B in both heterozygotes were also similar to those in the controls and were different from the values in the homozygote, in whom apo B levels were elevated and apo A-I concentrations were reduced.3

Sterol Absorption

An important new observation made in this study was that sitosterol absorption as measured by two independent methods was increased in both sitosterolemic heterozygotes compared with the control. Both the plasma dual-isotope ratio method (which measures the ratio of fed to injected labeled sitosterol in the plasma) and the isotope-kinetic method (which gives the production rate, which is equivalent to absorption because there is no endogenous synthesis) gave comparable values in the heterozygotes. These values were more than twice control values and in most cases about one half that in the homozygote (Tables 3 and 4). Unfortunately, neither the absorption pathway nor the intestinal mechanism that normally discriminates sterol structure is known, so the factors responsible for enhanced absorption in these subjects still require elucidation.

Cholesterol absorption was similar in the sitosterolemic heterozygotes and the homozygote and tended to be at the high end of values for normal subjects.29 Thus, the increased absorption of sitosterol in sitosterolemic heterozygotes and the homozygote did not compete or interfere with cholesterol absorption.

Sitosterol Turnover

The kinetic parameters for sitosterol turnover (Table 4) in the heterozygotes were much more rapid than in the homozygote, but slower than in the controls. However, unlike in the homozygote (in whom sitosterol decayed more slowly than cholesterol), sitosterol decayed more rapidly than cholesterol in both heterozygotes (Figure 2). The explanation for the speedier turnover was fast removal, as evidenced by the elimination constant $K_a$, which in both heterozygotes ($K_a=0.11$) was 10 times that in their homozygous daughter ($K_a=0.01$) (Table 4). Thus, despite increased absorption (about three times that in the controls, as measured by PR), sitosterolemic heterozygotes do not accumulate sitosterol because removal (hepatic secretion) is more rapid. Apparently, tissue structural recognition, which is lost in the intestine and accounts for enhanced intestinal absorption of plant sterols, remains intact in the liver of heterozygotes. As a result, body sitosterol pools and plasma concentrations are only minimally increased in heterozygotes despite greater absorption than in the controls but are substantially smaller than the very large pools found in the homozygote (Figure 2).

Cholesterol PR calculated by mathematical analysis of plasma specific-activity decay curves in the two heterozygotes was similar to the values in four control subjects fed the same diet (Table 5). However, PR was much smaller in the sitosterolemic homozygote, and, when corrected for absorption, cholesterol synthesis was about 27% that in the controls and 34% and 22% that in the heterozygotes. Thus, diminished cholesterol biosynthesis, which has been noted previously in other homozygotes, is a consistent feature in sitosterolemia. We have postulated that diminished cholesterol synthesis relates specifically to a deficiency of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase,3,29 the rate-determining enzyme for cholesterol biosynthesis, and may be the inherited defect in this disease. The following observations support this contention.

Depressed cholesterol synthesis, as measured by either sterol balance or isotope kinetics, has been reported in other patients from unrelated families.3,4,6 The reduced cholesterol formation has been related to a severe deficiency of HMG-CoA reductase in the liver30 or mononuclear leukocytes31 from these subjects. Both HMG-CoA reductase activity and enzyme protein were about 10% of normal, and mRNA for HMG-CoA reductase was almost lacking in the liver of a homozygote.30 Thus, the low cholesterol synthesis noted in Table 5 corresponds to a markedly diminished formation of hepatic HMG-CoA reductase. In contrast, low density lipoprotein (LDL) receptor function, which normally is coordinately regulated with HMG-CoA reductase so that enzyme activity and LDL receptor function respond in the same direction, are opposite in sitosterolemic homozygotes.30-32 We have observed enhanced LDL receptor function in combination with low HMG-CoA reductase activity and enzyme protein in freshly isolated monocytes and liver from sitosterolemic homozygotes, and we believe that the increased expression of LDL receptors provides circulating (LDL) sterols for cells that cannot synthesize sufficient cholesterol. Finally, bile acid malabsorption induced either by treatment with sequestering resins (cholestyramine and colesteol) or by ileal bypass surgery lowers plasma sterol (cholesterol and sitosterol) concent-
trations by 25-50%. The decrease in plasma sterol concentrations is greater than expected and is not associated with the anticipated upregulation of cholesterol synthesis and HMG-CoA reductase activity that is usually noted in nonmural cells from subjects treated with cholestyramine. Importantly, clinical symptoms (xanthomas, aortic systolic murmurs, and arthritis) improved in one patient as plasma sterol concentrations declined, without the expected stimulation of cholesterol synthesis.

Finally, our observations of increased sitosterol absorption in heterozygotes associated with only minimal increases in plasma concentrations and pools because of rapid elimination were made in a single sitosterolemic family and obviously require confirmation in other unrelated families.

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References
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