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. The sitosterol turnover rate, calculated independently by mathematical analysis of specific-activity decay curves, amounted to 15 and 24 mg/day in the heterozygotes compared with 27 mg/day in the homozygote and 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_a=0.11 day^-1) was 10 times that in the homozygote (K_a=0.01 day^-1) but 35% less than that in the controls (K_a=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.

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
clini-
cally 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. 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. 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. 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.

**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 for sitosterol and cholesterol by Salen et al.3 Mass absorption for each sterol was calculated from the dietary intake of sitosterol and cholesterol multiplied by the mean fraction of sitosterol and cholesterol absorbed as determined by the plasma dual-isotope ratio method.

**Sterol turnover rates.** Sitosterol and cholesterol turnover rates were calculated according to the two-compartment model.17–20 This model was selected for sitosterol because after isotope labeling, plasma sitosterol specific-activity decay gives an excellent fit for two exponentials.21 For cholesterol, the decay of plasma specific activities can be described by two or three exponentials.17,18 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.18–20 The method of calculation, equations, and assumptions were the same as employed in earlier studies.2,21

**Radiolabeled sterols.** [4,14C]Cholesterol (specific activity, 57.5 mCi/mmoll and [1,2-3H]cholesterol (specific activity, 45 Ci/mmol) were obtained from Du Pont, New England Nuclear Corp., Boston, Mass. [4,14C]Sitosterol (specific activity, 57.5 mCi/mmoll was purchased from Amersham Corp., Arlington Heights, Ill. [3a-3H]Sitosterol was synthesized from sitosterol by the method of Dayal et al22 with a final specific activity of 1 Ci/mmol. All labeled sterols were >98% pure as tested by high-performance liquid chromatography (HPLC). The sitosterolemic heterozygotes, the homozygote, and one control subject were fed 2.4–17.1 µCi [3H]cholesterol and 8.8–10.5 µCi [3H]sitosterol and administered intravenously 3.0–6.3 µCi [14C]cholesterol and 1.2–8.5 µCi [3H]sitosterol. Four control subjects received only 50 µCi [3H]sitosterol and 25 µCi [14C]cholesterol intravenously. The radiolabeled sterols given intravenously were dissolved in 1.0 ml absolute ethanol, suspended in 150 ml physiological saline, and infused immediately. The radiolabeled sterols fed were dissolved in 1.0 ml absolute ethanol and dispersed in 120 ml milk.

**Chemical studies.** Plasma sterol concentrations were measured by capillary GLC as described.23 The retention times relative to 5α-cholestane of the sterol trimethylsilyl ether derivatives were 1.86 for cholesterol, 1.71 for cholestanol, and 2.86 for sitosterol. Cholesterol and cholestanol were isolated individually by HPLC from plasma specimens to measure their respective 3H to 14C ratios and 3H and 14C specific activities.3

**Radioactive assay.** Measured portions of the purified sterols were dissolved in toluene phosphor (4.2%; Liquidifluor, Du Pont) and assayed for radioactivity in a model LS 250 liquid scintillation system (Beckman Instruments, Inc., Fullerton, Calif.). The efficiencies for counting 3H and 14C were 43% and 69%, respectively. The samples were counted for 10–100 minutes so that at least 100 cpm above background were recorded. Apolipoproteins. Apo A-I concentrations were quantified by a specific, competitive enzyme-linked immunosorbent assay.23,24 Concentrations of apo A-I were determined by single radial immunodiffusion.25

**Statistical Analysis**

Data were analyzed by using Student's t test and by comparing the homozygote's and heterozygotes' values with the 95% and 99% confidence intervals for the control group as described by Gardiner and Altman.26

**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 19 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 cholestanol plasma concentra-
The apo A-I and B measurements are given in Table 2. Values in the heterozygotes were similar to those in the controls consuming the same diet. In contrast, apo A-I concentrations were slightly lower and apo B levels substantially higher in the homozygote than in her heterozygous parents or the controls.

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.

Cholesterol absorption in the father was higher than in either the homozygote, the heterozygous mother, or the control. However, in contrast to sitosterol absorption, for which there were differences among the subjects, cholesterol absorption did not distinguish the sitosterolemic heterozygotes from the homozygote. In this regard, it is also important to note that the plasma dual-isotope ratio method may occasionally overestimate cholesterol absorption, as reported in rhesus monkeys by Bhattacharyya and Eggens.

Sitosterol Turnover

Sitosterol turnover parameters were calculated by mathematical analysis of specific-activity decay curves according to the kinetics of the two-compartment model. 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 (T1/2) than the controls. These differences can also be seen by examining the plots of sitosterol specific activity versus time. Daily production (PRa), 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 (PRa) relates to inefficient excretion. Apparently in the heterozygotes the liver retains the ability to preferentially excrete

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

Values are mean ± SD in milligrams per deciliter.

*Significantly different from control value.

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**Table 2. Plasma Apolipoprotein Concentrations**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Samples analyzed</th>
<th>Apolipoprotein A-I</th>
<th>Apolipoprotein B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>147</td>
<td>137–154</td>
<td>69 52–81</td>
</tr>
<tr>
<td>(n = 5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heterozygotes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mother</td>
<td>5</td>
<td>140 133–150</td>
<td>72 52–88</td>
</tr>
<tr>
<td>Father</td>
<td>5</td>
<td>147 138–157</td>
<td>77 59–88</td>
</tr>
<tr>
<td>Homozygote</td>
<td>5</td>
<td>131 124–135</td>
<td>131 102–150</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>CV (n = 5)</th>
<th>Cholesterol Absorbed</th>
<th>CV (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5 5</td>
<td>48 460</td>
<td>4 460</td>
<td></td>
</tr>
<tr>
<td>Heterozygotes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mother</td>
<td>15 15</td>
<td>59 295</td>
<td>6.7</td>
<td></td>
</tr>
<tr>
<td>Father</td>
<td>17 17</td>
<td>84 425</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Homozygote</td>
<td>34 34</td>
<td>6 310</td>
<td>7.6</td>
<td></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.

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**Table 4. Sitosterol Turnover Parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls (n = 5)</th>
<th>Heterozygotes</th>
<th>Heterozygotes</th>
<th>Heterozygotes</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1/2 (days)</td>
<td>3.8 ± 0.2</td>
<td>3.0 5.2</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>T1/2 (days)</td>
<td>13.8 ± 2.4</td>
<td>39 23</td>
<td>135</td>
<td></td>
</tr>
<tr>
<td>K (days−1)</td>
<td>0.17 ± 0.04</td>
<td>0.12 0.10</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>M (mg)</td>
<td>80 ± 36</td>
<td>75 152</td>
<td>1,980</td>
<td></td>
</tr>
<tr>
<td>M (mg)</td>
<td>46 ± 22</td>
<td>190 246</td>
<td>2,100</td>
<td></td>
</tr>
<tr>
<td>M + M (mg)</td>
<td>126 ± 32</td>
<td>265 398</td>
<td>4,080</td>
<td></td>
</tr>
<tr>
<td>PRa (mg/day)</td>
<td>7.9 ± 2.3</td>
<td>15 24</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>PRa (mg/kg/day)</td>
<td>0.095 ± 0.028</td>
<td>0.3 0.32</td>
<td>0.39</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SD for controls.

*Weight reported in Table 5.
TABLE 5. Cholesterol Turnover Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls (n=4)</th>
<th>Heterozygotes</th>
<th>Homozygote</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>83±20</td>
<td>50</td>
<td>70</td>
</tr>
<tr>
<td>T_{1/2} (days)</td>
<td>6.7±0.6</td>
<td>2.9</td>
<td>7.7</td>
</tr>
<tr>
<td>T_{2/3} (days)</td>
<td>56±6</td>
<td>49</td>
<td>74</td>
</tr>
<tr>
<td>K_{A} (days')</td>
<td>0.045±0.006</td>
<td>0.043</td>
<td>0.027</td>
</tr>
<tr>
<td>M_A (g)</td>
<td>29±8</td>
<td>20.1</td>
<td>65.9</td>
</tr>
<tr>
<td>M_B (g)</td>
<td>48±16</td>
<td>34.4</td>
<td>88.3</td>
</tr>
<tr>
<td>PR_A (mg/day)</td>
<td>1,450±560</td>
<td>870</td>
<td>1,780</td>
</tr>
<tr>
<td>Synthesis (mg/kg/day)</td>
<td>17.5±6.8</td>
<td>17</td>
<td>24</td>
</tr>
</tbody>
</table>

Values are mean±SD for controls. Synthesis = (PR_A - absorption)/weight; absorption estimated at 50% of intake of controls.
*Below lower limit of 95% confidence interval for controls (6.6-22.6 mg/kg/day).

controls. Further, after cholesterol absorption was subtracted from PR_A, synthesis was markedly diminished in the sitosterolemic homozygote. Thus, cholesterol turn-

FIGURE 2. Scatter plot of sitosterol absorption versus pool size. In controls and heterozygotes, sitosterol pool sizes are linearly related to absorption. However, in homozygote, sitosterol pool size is disproportionately enlarged and reflects sitosterol accumulation. Values include controls from Table 4 plus controls reported in References 3 and 6.

FIGURE 3. Sitosterol (---) and cholesterol (—) turnover curves. Sitosterol decays more rapidly than cholesterol in controls and heterozygotes but more slowly than cholesterol in homozygote.

FIGURE 1. Normalized sitosterol specific activity versus time curves. Sitosterol decays more slowly in homozygote than in her heterozygous parents. However, turnover is much more rapid in control subject.

Arteriosclerosis and Thrombosis Vol 12, No 5 May 1992

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 homozygotes 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, PR_{A}, 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 PR_{A}, synthesis was markedly diminished in the sitosterolemic homozygote. Thus, cholesterol turn-

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.
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 3). The explanation for the speedier turnover was fast removal, as evidenced by the elimination constant Kα, which in both heterozygotes (Kα=0.11) was 10 times that in their homozygous daughter (Kα=0.01) (Table 4). Thus, despite increased absorption (about three times that in the controls, as measured by PRₐ), 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 PRₐ 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, PRₐ 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 liver50 or mononuclear leukocytes51 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.50 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.50-32 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 colestipol) 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 mononuclear 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 minimally increased plasma concentrations and pools because of rapid elimination were made in a single sitosterolemia family and obviously require confirmation in other unrelated families.

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References


Increased sitosterol absorption is offset by rapid elimination to prevent accumulation in heterozygotes with sitosterolemia.

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