Unexpected Failure of Bile Acid Malabsorption to Stimulate Cholesterol Synthesis in Sitosterolemia with Xanthomatosis
Comparison with Lovastatin

Lien Nguyen, Gerald Salen, Sarah Shefer, Virgie Shore, G. Stephen Tint, and Gene Ness

We examined the relationship between cholesterol synthesis and high affinity low density lipoprotein (LDL) catabolism in freshly isolated mononuclear leukocytes and plasma sterols and apolipoprotein concentrations in three homozygous and one heterozygous subject with sitosterolemia with xanthomatosis and in 12 control subjects. Observations in untreated subjects were compared during therapy with lovastatin or interruption of the enterohepatic circulation of bile acids. Plasma cholesterol, plant sterol, and apolipoprotein B concentrations declined more than 50% in the two homozygous sitosterolic subjects after ileal bypass surgery. In contrast, plasma cholesterol, plant sterol, and apolipoprotein B concentrations remained constant in a homozygous sitosterolic subject and declined only 7% in a heterozygous sitosterolic subject during 20 weeks of lovastatin (40 mg/day) treatment compared to a 28% decrease in similarly treated control subjects. Lovastatin treatment decreased cholesterol synthesis more than 60% but did not increase high affinity catabolism of LDL further in the sitosterolic cells, compared to a more than 20% rise in control mononuclear leukocytes. Conversely, bile acid malabsorption increased cholesterol synthesis 59%, total hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase activity 13%, and receptor-mediated LDL degradation 41% in control cells, but did not stimulate cholesterol synthesis or microsomal HMG-CoA reductase activity in sitosterolic mononuclear leukocytes although receptor-mediated LDL catabolism rose an additional 26%. These results demonstrate a greater than expected decrease in plasma sterols and apolipoprotein B concentrations in sitosterolic subjects after stimulation of bile acid synthesis because of the inability to up-regulate cholesterol production. We suggest that bile acid-sequestering drugs or ileal exclusion surgery may be more effective treatments to mobilize accumulated sterol deposits and prevent atherosclerosis in this disease. (Arteriosclerosis 10:289–297, March/April 1990)

Sitosterolemia with xanthomatosis is a rare inherited lipid storage disease that is characterized clinically by tendon and tuberous xanthomas, aortic stenosis, arthritis, hemolytic episodes, and accelerated atherosclerosis. Biochemically, plant sterol (campesterol, stigmasterol, and sitosterol) and 5α-stanol (cholestanol, 5α-campestanol, and 5α-sitostanol) concentrations are increased more than 100-fold and 30-fold, respectively, in plasma of sitosterolic compared to control subjects. Unrestricted intestinal absorption coupled with decreased hepatic removal account for the accumulation of plant sterols, since synthesis in mammalian organisms is nil. In distinction, cholestanol, 5α-campestanol, and 5α-sitostanol are probably produced endogenously from their unsaturated sterol precursors because human diets are virtually devoid of ω-saturated stanols. Although large amounts of cholesterol deposit in xanthomas and atherosclerotic lesions located in coronary arteries and aorta, whole-body cholesterol turnover and synthesis are subnormal in sitosterolic subjects. Furthermore, freshly isolated mononuclear leukocytes from sitosterolic subjects show markedly diminished cholesterol synthesis and microsomal hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase activity and enzyme protein that are coupled with increased high affinity LDL receptor function. We have, therefore, postulated that reduced cholesterol synthesis is the primary biochemical abnormality in sitosterolemia and may be compensated for by enhanced intestinal plant sterol absorption, lipoprotein sterol transport (elevated circulating low density lipoprotein [LDL]), and the augmented expression of tissue LDL receptors.
Recent reports have suggested that plasma sterol and 5α-stanol levels in sitosterolemic subjects are extremely sensitive to interruption of the enterohepatic circulation of bile acids. Treatment with the bile acid sequestering resins, cholestyramine or colestipol, induces bile acid malabsorption and promotes the enhanced conversion of cholesterol (or other sterols) to bile acids in the liver. In response, hepatic cholesterol synthesis is simultaneously up-regulated, so that the net reduction in plasma cholesterol concentrations reflects the balance between the input of newly synthesized cholesterol and its elimination as bile acids.

Lately, there has been considerable interest in the plasma-lowering effect of lovastatin. This new drug competitively blocks HMG-CoA reductase, the rate-controlling enzyme in cholesterol biosynthesis, and increases the expression of hepatic LDL receptors, thereby accelerating the clearance of plasma LDL. In hypercholesterolemic subjects, plasma cholesterol and LDL levels decline about 30% when 40 to 80 mg of lovastatin are given each day.

The objectives of this study were to compare the effects of lovastatin with interruption of the enterohepatic circulation of bile acids on plasma sterol and apolipoprotein B concentrations in sitosterolemic subjects. Measurements of mononuclear leukocyte cholesterol synthesis, microsomal HMG-CoA reductase activity, and receptor-mediated LDL degradation were related to plasma sterol and apolipoprotein concentrations to explain changes from the untreated state. Because lovastatin and bile acid malabsorption produce opposite effects on cholesterol biosynthesis, these treatments may intensify defects in cholesterol synthesis and regulation and help to better define the biochemical mechanism in this disease.

**Methods**

**Clinical**

Studies were conducted in three homozygous sitosterolemic sisters (KCN, TC, and KeC), ages 30, 27, and 25 years, respectively, and their heterozygous mother (AC) who was 50 years old. In two homozygous sisters (KCN and TC), Achilles tendon and tuberous xanthomas were present, while sister KeC showed small tendon xanthomas of the extensor tendons of the hand. An aortic systolic murmur was present, and coronary atherosclerosis was demonstrated by angiography in KCN. AC, the heterozygote, was clinically normal. All subjects consumed similar regular food diets, served as control subjects for baseline measurements of mononuclear leukocyte cholesterol synthesis, microsomal HMG-CoA reductase activity, and high affinity LDL catabolism. Six subjects from the control pool were treated with 20 mg of lovastatin twice a day (three subjects) or 5 g of colestipol twice a day (three subjects) for 20 weeks; during the final 5 weeks of each treatment period, plasma sterol concentrations and mononuclear leukocyte cholesterol synthesis, microsomal HMG-CoA reductase activity, and high affinity LDL receptor degradation were reevaluated. To minimize the differences in plasma sterol concentrations, mononuclear cell cholesterol synthesis, and HMG-CoA reductase activities related to meals, medication, and diurnal variation, blood was obtained from all subjects at 9 A.M., 12 hours after the last meal and evening dose of drug. Blood specimens were collected from each sitosterolemic subject into tubes that contained ethylenediaminetetraacetic acid (EDTA) at least five times when untreated and five times during each treatment period. An interval of at least 7 days separated each blood collection. Informed written consent was obtained from each subject before study. 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, and The Veterans Administration Medical Center, East Orange, NJ.

**Plasma Sterols and Apolipoproteins**

Sterol concentrations in plasma were measured by capillary gas-liquid chromatography. Immunoprotein B concentrations in plasma were quantitated by single radial immunodiffusion, and specific, competitive enzyme-linked immunosorbent assay (ELISA). The sensitivity of the ELISA ranged from 0.01 to 1.5 μg (100 μl per well) in microtiter plates. Concentrations of apolipoprotein A-I were determined by single radial immunodiffusion. Mononuclear leukocytes isolated from the same blood specimens were assayed for cholesterol synthesis, HMG-CoA reductase activity, and receptor-mediated LDL catabolism.

**Cell Separation**

Mononuclear leukocytes were isolated from 60 ml of venous blood according to the method of Boyum. After lyses of erythrocytes, the mononuclear leukocytes were washed twice with phosphate-buffered saline and were resuspended in incubation medium (RPMI-1640, Gibco, Grand Island, NY, containing 200 units/ml penicillin and streptomycin) in a volume equal to about 10% of the original blood volume. The total number of cells was counted and showed more than 98% viability by the trypan blue dye exclusion test. After myeloperoxidase staining, there were 19% ± 8% monocytes, with the remaining cells composed of lymphocytes. The same proportions of monocytes and lymphocytes were present in both sitosterolemic and control blood specimens and were not affected by the treatments. The cells were used immediately for the assays of cholesterol synthesis, HMG-CoA reductase activity, and LDL receptor function.
Assay of Cellular Sterol Synthesis

A cell suspension that contained 3 to 12×10⁸ mono-nuclear leukocytes was incubated in 1 ml of medium that contained 50% autologous plasma and 2.5 μmol 2,4-C-Na acetate (New England Nuclear, Boston, MA) diluted with unlabeled Na acetate to a specific activity of 15 dpm/μmol. Preliminary experiments with both control and sitosterolemic cells established that cholesterol synthesis was linearly related to cell number over this range and that substrate concentrations, co-factors, and incubation time were optimally suited for the assay. The incubation was carried out for 4 hours in a shaking water bath (46 rpm) at 37°C. After saponification, the labeled sterols were extracted with hexane and were separated on an alumina column, and radioactivity was determined by liquid scintillation spectroscopy. Sterol synthesis was expressed as the pmol of labeled acetate incorporated into sterols per 10⁶ cells per hour.

Measurement of Fasting Total Microsomal HMG-CoA Reductase Activity

Microsomes were prepared from 1 to 4×10⁸ mononuclear cells by differential ultracentrifugation in buffer TEDK (50 mM Tris, 1 mM disodium EDTA, 5 mM dithiothreitol, 70 mM KCl). Cell nuclei and debris were removed after centrifugation at 8000g for 10 minutes at 4°C, and the supernates were recentrifuged at 172,000 g for 90 minutes at 4°C to collect microsomal pellets.

The total HMG-CoA reductase activity was assayed according to the method of Harwood et al. after the microsomal protein concentration was determined on an aliquot by the method of Lowry et al. Between 50 and 200 μg of microsomal protein was pre-incubated for 10 minutes at 37°C in 150 μl of final volume of TEDK that contained 68 mM EDTA, a NADPH generating system (3.4 mM of NADP+, 30 mM of glucose-6-phosphate, and 0.3 unit of glucose-6-phosphate dehydrogenase), and 20,000 cpm of ³H-mevalonolactone (New England Nuclear) for the internal standard. The 30-minute incubation was started by the addition of 10 nmol of ³H-MG-CoA (Amersham, Arlington, IL), 114 dpm/μmol, and was stopped by the addition of 20 μl of 6 N HCl. After 3 mg of unlabeled mevalonolactone was added, the labeled products were isolated by thin-layer chromatography and counted by liquid scintillation spectroscopy. In other experiments, pre-incubation of the mononuclear leukocyte microsomes from untreated, lovastatin-treated, and ileal bypass-treated subjects with purified intestinal or E. coli alkaline phosphatases did not further increase total HMG-CoA reductase activity.

Determination of Receptor-mediated Low Density Lipoprotein Catabolism by Mononuclear Leukocytes

LDL fractions were prepared from sitosterolemic and control plasma by differential ultracentrifugation and were labeled with ¹²⁵I by the Iodine monochloride method. Sitosterolemic LDL was used with sitosterolemic cells and pooled control LDL, with control cells. The plasma used to isolate the LDL was obtained during the untreated baseline period. Between 1 to 4×10⁸ cells were incubated with 10 μg/ml of autologous ¹²⁵I-LDL in 0.5 ml medium (RPMI-1640 that contained 20% lipoprotein-free serum, 50 μmol/l of CaCl₂, 2% bovine serum albumin, and penicillin-streptomycin to prevent bacterial growth). Cells were incubated for 4 hours at 37°C in a shaking water bath (46 rpm). The cells were pelleted, and the incubation medium was assayed for radioactive noniodide trichloroacetic acid soluble degradation products. The quantity of autologous LDL degraded by the receptor-mediated pathway (high affinity binding) was expressed as ng/10⁶ cells/hour and was calculated as the difference between total LDL degradation (assayed in the absence of unlabeled LDL) and nonspecific LDL degradation (assayed in the presence of 40-fold excess of unlabeled LDL).

Statistical Analysis

Data were analyzed by comparing the mean value for each sitosterolemic subject to the 95% confidence interval of the mean value for the control subjects.

Results

Table 1 presents the effects of ileal exclusion surgery, colestipol (10 g/day), and lovastatin (40 mg/day) on plasma sterol concentrations. After 20 weeks of lovastatin treatment, plasma cholesterol levels declined 28% in three control subjects, but only 7% in the sitosterolemic heterozygote, and remained constant in the sitosterolemic homozygote. Interestingly, plant sterol concentrations rose in plasma in both the control and heterozygous sitosterolemic subjects, but did not change in the sitosterolemic homozygote during lovastatin therapy.

Both ileal exclusion surgery and colestipol treatments dramatically decreased total plasma sterol levels in the homozygous sitosterolemic subjects. The effect was more pronounced after ileal bypass surgery, presumably because bile acid malabsorption was greater than with the bile acid sequestering resin, which may be dose-dependent. Plant sterol and 5α-stanol levels declined by approximately the same extent as cholesterol after ileal bypass surgery. In KCN, tendon xanthomas softened and became smaller, painful arthritic attacks and arthralgias of the ankle and knee joints were abolished, and the aortic systolic murmur disappeared during the 140 week period after ileal exclusion surgery. In three control subjects treated with colestipol, plasma cholesterol levels decreased 14%. Plasma cholestanol and sitosterol concentrations, which were barely detectable in the control subjects, did not increase during this treatment.

Table 2 gives the plasma apolipoprotein B and A-I concentrations in the homozygous sitosterolemic subjects before and during treatment with either lovastatin or ileal bypass surgery. In untreated subjects, plasma apolipoprotein B concentrations were increased and reflected elevated plasma sterol levels. In distinction, apolipoprotein A-I levels tended to be low and to correspond to the below normal levels of high density lipoprotein (HDL) often found in patients with this disease. After ileal exclusion surgery, plasma apolipoprotein B levels de-
The table shows the effect of ileal exclusion surgery, colestipol, and lovastatin on plasma sterol concentrations in different subjects. Controls, n=3

<table>
<thead>
<tr>
<th>Subject (diagnosis)</th>
<th>Treatment* (wks)</th>
<th>Cholesterol† (mg/dl)</th>
<th>Plant sterols and 5α-stanols‡ (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls, n=3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AC</td>
<td>Lovastatin (20)</td>
<td>216±9 156±15 -28</td>
<td>0.2±0.1 0.6±0.1 +200</td>
</tr>
<tr>
<td>KeC</td>
<td>Lovastatin (20)</td>
<td>219±18 204±16 -7</td>
<td>0.4±0.2 1.4±0.2 +250</td>
</tr>
<tr>
<td>TC</td>
<td>Colestipol (20)</td>
<td>216±9 185±6 -14</td>
<td>0.2±0.1 0.2±0.1 0</td>
</tr>
<tr>
<td>KCN</td>
<td>Ileal exclusion</td>
<td>274±17 207±1 -24</td>
<td>38.7±1.7 27.8±1.1 -28</td>
</tr>
<tr>
<td></td>
<td>(140)</td>
<td>274±17 126±10 -54</td>
<td>38.7±1.7 18.2±1.0 -53</td>
</tr>
</tbody>
</table>

*The doses for lovastatin and colestipol were 40 mg/day and 10 g/day, respectively.
†Sterol measurements were made on plasma specimens obtained five times before and five times during the final weeks of the treatment period.
‡The plant sterols consisted of sitosterol, campesterol, stigmasterol, and avenasterol, and the 5α-stanols were cholestanol, 5α-campestanol, and 5α-sitostanol. In the homozygous sitosterolemic subject, control and heterozygote plasma contained small amounts of cholestanol and sitosterol. The other sterols and stanols found in the homozygous sitosterolemic subjects were not detected.
§0=untreated, T=treatment.

Table 2 contains measurements of cholesterol synthesis as determined by the conversion of 14C-acetate to cholesterol in freshly isolated mononuclear leukocytes from fasting control and sitosterolemic subjects during lovastatin treatment produced virtually no change in plasma apolipoprotein B or A-I levels in the homozygous sitosterolemic subject, which is consistent with the observed absence of effect of this drug on plasma sterol concentrations (Table 1).

Table 1. Effect of Ileal Exclusion Surgery, Colestipol, and Lovastatin on Plasma Sterol Concentrations

Table 2. Effect of Ileal Exclusion Surgery and Lovastatin on Plasma Apolipoprotein A-I and B Levels

*The average of four to five samples over a 3-year period before treatment.
†The numbers in parentheses represent the range of determinations. The values after treatment are the average of two measurements.

The changes in apolipoprotein B mirrored the drop in total plasma sterol concentrations (Table 1). Coincidentally, plasma apolipoprotein A-I levels rose substantially after ileal bypass surgery: 27% in KCN and 34% in TC, respectively, which probably reflected increased HDL sterol concentrations. Thus, stimulating bile acid synthesis improved plasma sterol and apolipoprotein concentrations both qualitatively and quantitatively. In contrast,
Table 3. Effect of Meal Exclusion, Colestipol, and Lovastatin on Mononuclear Leukocyte Cholesterol Synthesis

<table>
<thead>
<tr>
<th>Subject (diagnosis)</th>
<th>Treatment (wks)</th>
<th>Dose</th>
<th>MNL* sterol synthesis (pmol/10^7/h)</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Lovastatin (20)</td>
<td>40 mg/day</td>
<td>DT: 5.2±0.5 n=12 T: 1.3±0.3 n=3</td>
<td>-75</td>
</tr>
<tr>
<td>AC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(sitosterolemic heterozygote)</td>
<td>Lovastatin (20)</td>
<td>40 mg/day</td>
<td>2.3±0.3 (4)† n=4 T: 0.4±0.2 (4) n=3</td>
<td>-83</td>
</tr>
<tr>
<td>KeC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(sitosterolemic homozygote)</td>
<td>Lovastatin (20)</td>
<td>40 mg/day</td>
<td>3.0±0.5 (4)§ n=4 T: 1.6±0.4 (4) n=3</td>
<td>-47</td>
</tr>
<tr>
<td>Control</td>
<td>Colestipol (20)</td>
<td>10 g/day</td>
<td>DT: 5.2±0.5 n=12 T: 8.3±1.1† n=3</td>
<td>+59</td>
</tr>
<tr>
<td>TC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(sitosterolemic homozygote)</td>
<td>Colestipol (20)</td>
<td>10 g/day</td>
<td>2.7±0.5 (6)‡ n=6 T: 1.9±0.3 (3) n=3</td>
<td>-30</td>
</tr>
<tr>
<td>ileal exclusion (20)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KCN</td>
<td></td>
<td>ileal exclusion (140)</td>
<td>ND T: 2.7±0.5 (6)‡ n=6</td>
<td>0</td>
</tr>
</tbody>
</table>

* MNL=Mononuclear leukocytes composed of 81% lymphocytes and 19% monocytes in control and sitosterolemic preparations.
†=baseline, T=treatment period, ND=not determined. The number of weekly measurements are in the parentheses.
‡Significantly different than baseline control, p<0.01.
§Significantly less than baseline control, p<0.05.

Lovastatin treatment and bile acid malabsorption. Mononuclear leukocyte cholesterol synthesis was subnormal in both untreated heterozygous and homozygous sitosterolemic subjects (40% to 60% less than the mean for 12 control subjects). Nevertheless, mononuclear cells from all subjects responded similarly to treatment with lovastatin: cholesterol synthesis declined 75% in control cells and 47% in homozygous sitosterolemic cells. In comparison, when bile acid malabsorption was induced by colestipol, mononuclear leukocyte cholesterol synthesis rose 59% in control cells. Unexpectedly, cholesterol synthesis, which was subnormal, remained flat 20 weeks after colestipol or ileal bypass surgery in a sitosterolemic subject and was even lower in a second subject 140 weeks after this operation.

Table 4 lists the measurements of total microsomal HMG-CoA reductase activity in freshly isolated mononuclear leukocytes from fasting sitosterolemic subjects treated with lovastatin and after ileal exclusion surgery. Total fasting HMG-CoA reductase activity was significantly reduced in mononuclear leukocyte microsomes from both the untreated heterozygous and homozygous sitosterolemic subjects, 60% and 50% less, respectively, than the mean for 12 control subjects, which is consistent with the low rates of cholesterol synthesis reported for these cells in Table 3. After lovastatin treatment, microsomal HMG-CoA reductase activity increased 80% in the control cells, 20% in the heterozygote cells, and 83% in the homozygote sitosterolemic cells despite an almost 50% reduction in cellular cholesterol synthesis. Nevertheless, despite the rise, total HMG-CoA reductase activity was still 46% less in the sitosterolemic cells on lovastatin than in the untreated control cells. The explanation for the paradoxical increase in HMG-CoA reductase activity may relate to the fact that cellular cholesterol synthesis is measured in the presence of autologous serum, which may contain the active drug. In contrast, microsomes are prepared by differential ultracentrifugation, which involves repeated washings that remove the inhibiting drug from the microsomes. Also, in response to the competitive inhibition of HMG-CoA reductase, more enzyme may be produced.23,24,25

The total microsomal HMG-CoA reductase activity failed to increase in the mononuclear cells from the sitosterolemic subjects and remained depressed 20 and 140 weeks after ileal bypass surgery. In contrast, total microsomal HMG-CoA reductase activity rose 13% in mononuclear cells from control subjects treated with colestipol. Thus, cholesterol synthesis and HMG-CoA reductase activity remained subnormal in sitosterolemic mononuclear leukocytes and were unable to increase after stimulation of bile acid synthesis. Conversely, both cholesterol synthesis and HMG-CoA reductase activity rose substantially in control cells after bile acid malabsorption was induced.

The effect of lovastatin and bile acid malabsorption on receptor-mediated LDL catabolism by control and sitosterolemic mononuclear leukocytes is given in Table 5. As
Table 4. Effect of Ileal Exclusion Surgery, Colestipol, and Lovastatin on Mononuclear Leukocyte Microsomal Hydroxymethylglutaryl Coenzyme A Reductase Activity

<table>
<thead>
<tr>
<th>Subject (diagnosis)</th>
<th>Treatment (wks)</th>
<th>Dose</th>
<th>HMG CoA reductase (pmol/mg/min)</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0*</td>
<td>T*</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AC (sitosterolemic heterozygote)</td>
<td>Lovastatin (20)</td>
<td>40 mg/day</td>
<td>3.1±0.5 (n=12)</td>
<td>5.4±0.8 (n=3)</td>
</tr>
<tr>
<td>KeC (sitosterolemic homozygote)</td>
<td>Lovastatin (20)</td>
<td>40 mg/day</td>
<td>1.5±0.1 (3)†</td>
<td>1.8±0.5 (3)</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC (sitosterolemic homozygote)</td>
<td>Colestipol (20)</td>
<td>10 g/day</td>
<td>3.0±0.5 (n=12)</td>
<td>3.4±1.1 (n=3)</td>
</tr>
<tr>
<td>KeC (sitosterolemic homozygote)</td>
<td>Ileal exclusion (20)</td>
<td>10 g/day</td>
<td>1.3±0.4 (4)†</td>
<td>1.4±0.5 (5)</td>
</tr>
<tr>
<td>KCN (sitosterolemic homozygote)</td>
<td>Ileal exclusion (140)</td>
<td>ND</td>
<td>1.4±0.3 (4)</td>
<td></td>
</tr>
</tbody>
</table>

*0=baseline, T=treatment period, ND=not determined. The number of weekly measurements are in parentheses. †Significantly less than baseline control, p<0.01.

Table 5. Effect of Lovastatin, Ileal Exclusion Surgery, and Colestipol on Degradation of 125I-Low Density Lipoprotein by Freshly Isolated Mononuclear Leukocytes

<table>
<thead>
<tr>
<th>Subject (diagnosis)</th>
<th>Treatment (wks)</th>
<th>Dose</th>
<th>Receptor-mediated LDL degradation (ng/10^7 cells/4h)</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0*</td>
<td>T</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AC (sitosterolemic heterozygote)</td>
<td>Lovastatin (20)</td>
<td>40 mg/day</td>
<td>3.9±0.4 (n=5)</td>
<td>4.9±0.1 (n=3)</td>
</tr>
<tr>
<td>KeC (sitosterolemic homozygote)</td>
<td>Lovastatin (20)</td>
<td>40 mg/day</td>
<td>3.4±0.9 (3)</td>
<td>4.0±0.4 (2)</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC (sitosterolemic homozygote)</td>
<td>Colestipol (20)</td>
<td>10 g/day</td>
<td>3.9±0.4 (n=5)</td>
<td>5.5±0.8 (n=3)</td>
</tr>
<tr>
<td>KeC (sitosterolemic homozygote)</td>
<td>Ileal bypass (20)</td>
<td>10 g/day</td>
<td>4.9±0.5 (3)</td>
<td>6.4±2.2 (3)</td>
</tr>
<tr>
<td>KCN (sitosterolemic homozygote)</td>
<td>Ileal bypass (140)</td>
<td>ND</td>
<td>6.7±3.7 (3)</td>
<td>8.1±0.7 (3)</td>
</tr>
</tbody>
</table>

*0=baseline, T=treatment period. The number of weekly measurements are in parentheses.

noted previously, receptor-mediated LDL degradation was enhanced in mononuclear leukocytes from untreated homozygous sitosterolemic subjects compared to control cells, while in cells from the heterozygous sitosterolemic subject, receptor-mediated LDL degradation was similar to control cells. When cellular cholesterol synthesis was inhibited by lovastatin treatment, receptor-mediated LDL degradation rose 26% in the controls cells...
and 18% in the heterozygote sitosterolemic mononuclear leukocytes. In contrast, receptor-mediated LDL catabolism remained constant in the mononuclear leukocytes from the homozygous sitosterolemic subject during lovastatin treatment. Twenty weeks and 140 weeks after ileal exclusion surgery, receptor-mediated LDL catabolism increased 31% and 21%, respectively, in mononuclear leukocytes from the two homozygous sitosterolemic subjects. Similarly, receptor-mediated LDL degradation by mononuclear leukocytes from control subjects increased 41% compared to the untreated baseline after bile acid synthesis was stimulated. Thus, bile acid malabsorption induced greater receptor-mediated LDL degradation by both control and sitosterolemic cells.

Discussion

These results demonstrate a dramatic lowering of plasma sterols (cholesterol and plant sterols) and apolipoprotein B concentrations in homozygous sitosterolemic subjects after bile acid malabsorption was induced. Ileal bypass surgery or the resin, colestipol, reduced the return of bile acids to the liver via the portal blood, and up-regulated bile acid synthesis through the activation of hepatic microsomal cholesterol 7a-hydroxylase, the rate-controlling enzyme. As a consequence, cholesterol and presumably other sterols (plant sterols and cholestanol) were transformed to bile acids. To supply precursors for bile acid synthesis, more hepatic LDL receptors are expressed and de novo cholesterol biosynthesis is derepressed through the activation of HMG-CoA reductase, the rate-determining enzyme. Because of additional cellular receptors, plasma LDL particles are cleared more rapidly so that plasma sterol and apolipoprotein B levels decline. Up-regulation of HMG-CoA reductase provides newly synthesized cholesterol to serve as a precursor for bile acid production and to be incorporated into LDL via the formation and secretion of hepatic VLDL. The net reduction in circulating plasma sterols reflects the balance between receptor-mediated LDL catabolism and the input of newly synthesized cholesterol. In hypercholesterolemic subjects, bile acid sequestering resins or ileal exclusion surgery reduced plasma cholesterol levels approximately 10% to 20% and in the three normolipidemic control subjects reported in Table 1, plasma cholesterol decreased 14%. In contrast, total plasma sterols declined more than 50% in the two sitosterolemic subjects after ileal bypass surgery, and the effect persisted for more than 140 weeks after the operation. Similar reductions in plasma sterol concentrations have been reported in other sitosterolemic subjects treated with bile acid sequestering resins. Of importance, the markedly reduced plasma sterol levels were often associated with clinical improvement. As seen in one subject (KCN) after ileal exclusion surgery, the Achilles tendon xanthomas diminished in size, the aortic systolic murmur disappeared, and episodes of painful arthritis and arthralgia have not recurred. The greater than expected decline in plasma sterol and apolipoprotein B concentrations in the sitosterolemic subjects apparently resulted from two mechanisms: increased receptor-mediated LDL catabolism coupled to the failure to increase cholesterol synthesis. In contrast to control mononuclear leukocytes where cholesterol synthesis rose about 59% and HMG-CoA reductase activity increased 13% after bile acid malabsorption was induced, cholesterol synthesis and HMG-CoA reductase activity in sitosterolemic mononuclear leukocytes failed to increase (Tables 3 and 4). In other words, despite the stimulus to make more cholesterol, the production of cholesterol in sitosterolemic mononuclear cells could not increase. Thus, absorbed sterols (cholesterol and plant sterols) transported in plasma lipoproteins served as virtually the only source of precursors for increased bile acid synthesis. Previously, we demonstrated that reduced cholesterol synthesis and HMG-CoA reductase activity in sitosterolemic mononuclear leukocytes were due to decreased microsomal HMG-CoA reductase enzyme protein. Therefore, sitosterolemic cells depend on enhanced high affinity LDL degradation to meet cellular sterols needs. When even more sterols are required to serve as precursors for augmented bile acid synthesis, only plasma lipoproteins are available as evidenced by the marked decline in plasma sterols and apolipoprotein B concentrations. In support of this theory, Bell et al. have recently reported that the fractional catabolic rate of homologous LDL was 49% greater in a sitosterolemic than in three control subjects. These investigators interpret this finding of greater in vivo LDL catabolism as consistent with the enhanced expression of LDL receptors in the sitosterolemic subject. It should also be noted that intestinal cholesterol and plant sterol absorption, which are increased in sitosterolemic subjects, remained elevated after ileal bypass surgery (G. Salen, unpublished observations). Thus, although increased intestinal cholesterol and plant sterol absorption persists after bile acid malabsorption, plasma sterol concentrations decline because of the increased utilization of LDL sterols for bile acid synthesis.

The effect of bile acid malabsorption on increasing mononuclear cholesterol synthesis has been amply authenticated by McNamara et al. These investigators showed for the first time that the conversion of acetate to cholesterol by mononuclear cells increased almost 60% in subjects treated with the bile acid binding resin, cholestyramine. Therefore, mononuclear leukocyte cholesterol synthesis responds similarly to the liver when bile acid synthesis is stimulated. Although the mechanism has not been elucidated, reduced plasma sterol concentrations may provide the stimulus since the bile acid pool is almost totally confined to the enterohpetic circulation.

The results with the HMG-CoA reductase inhibitor lovastatin are consistent with the above interpretation. Lovastatin is a new cholesterol-lowering agent that works by competitively inhibiting HMG-CoA reductase and suppressing cellular cholesterol synthesis. As a consequence, cellular sterol needs are met through the expression of additional LDL receptors. Plasma sterol (LDL) concentrations fall in response to the increased uptake and degradation of circulating LDL. Our results (Tables 3 and 5) show that cholesterol synthesis was suppressed and associated with a rise in receptor-mediated LDL catabolism in mononuclear leuko-
cytes from control subjects during lovastatin treatment, and the latter findings are similar to those reported by Hageme-

However, despite a similar inhibition of cellular cholesterol synthesis in sitosterolemia mononuclear

leukocytes during lovastatin therapy, plasma cholesterol decreased only 7% in the heterozygote and did not change in the homozygote subject. Plasma sterol levels did not re-

spond to lovastatin in sitosterolemic subjects presumably because cholesterol synthesis was subnormal and LDL re-

ceptors functioned adequately to supply cellular sterol needs.

It was noteworthy that plasma plant sterol levels rose in the control and hyperchoyzygous sitosterolemic subjects during

lovastatin treatment. Although the increment was quantitatively small in the control subjects, it was suffi-

ciently large in the sitosterolemic heterozygote to serve possibly as a marker for the detection of the carrier state.

Whether increased plasma plant sterol levels in control or heterozygous sitosterolemic subjects reflect enhanced intestinal absorption (less structural discrimination), decreased hepatic removal, or the combination, the finding suggests an important relationship between de novo cho-

lesterol cellular synthesis and the structural recognition of plant sterols by the intestine (absorption) and liver cells.

In summary, prolonged interruption of the enterohepatic circulation of bile acids markedly decreased plasma sterol (cholesterol and plant sterol) and apolipoprotein B concentra-

tions and improved clinical symptoms in homozygous sitosterolemic subjects. Stimulating bile acid synthesis altered the balance between increased catabolism of plasma LDL and the input of new cholesterol via microsomal HMG-CoA reductase, since de novo cholesterol synthesis is abnormally suppressed and cannot be up-regulated. In contrast, further inhibition of HMG-CoA reductase with lovastatin was an ineffective treatment in a sitosterolemic subject, since cholesterol synthesis was already below normal and cellular LDL catabolism elevated. Enhanced plant sterol absorption coupled to increased receptor-

mediated LDL degradation may compensate for reduced cholesterol synthesis in sitosterolemia.

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L Nguyen, G Salen, S Shefer, V Shore, G S Tint and G Ness

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