Regulation of cholesterol biosynthesis in sitosterolemia: effects of lovastatin, cholestyramine, and dietary sterol restriction

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Abstract We investigated the effects of lovastatin, cholestyramine, and dietary sterol restriction on cholesterol synthesis and low density lipoprotein receptor function in freshly isolated mononuclear leukocytes from two unrelated sitosterolemic families. Total plasma sterol concentrations were elevated in the two homozygous sitosterolemic subjects (343 and 301 vs. 185 mg/dl in controls) and contained increased amounts of plant sterols and 5α-saturated stanols (20% and 8% vs. less than 1% in controls), but were not significantly different from controls in the two heterozygous subjects. The rates of conversion of acetate to cholesterol by mononuclear leukocytes were subnormal in all homozygous and heterozygous subjects and correlated with markedly reduced microsomal 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity. In the two homozygous subjects, cholestyramine treatment decreased plasma sterols 29% and 35%, and yet was associated with a paradoxical decline in mononuclear leukocyte HMG-CoA reductase activity. In contrast, plasma sterol concentrations decreased 14% and 5%, and mononuclear leukocyte HMG-CoA reductase activities increased 13% and 46% in three control and one heterozygous subjects treated with cholestyramine, respectively. Plasma sterol concentrations in the homozygous subjects unexpectedly failed to decline during treatment with lovastatin or a low sterol diet. In distinction, plasma sterol concentrations in three control and one heterozygous subjects dropped 28% and 31%, respectively, during treatment with lovastatin. Both cholestyramine and low dietary sterols stimulated low density lipoprotein receptor function. These results demonstrate a marked abnormality in cholesterol homeostasis in patients with homozygous sitosterolemia with xanthomatosis. *a*) Low cholesterol synthesis is caused by deficient HMG-CoA reductase activity; *b*) neither lovastatin nor a low sterol diet reduces plasma sterol concentrations in the homozygous sitosterolemic patients; and *c*) bile acid malabsorption produces a greater than expected decline in plasma sterol levels because of a failure to up-regulate the subnormal endogneous cholesterol synthesis.—**Nguyen, L. B., M. Cobb, S. Shefer, G. Salen, G. C. Ness, and G. S. Tint.** Regulation of cholesterol biosynthesis in sitosterolemia: effects of lovastatin, cholestyramine, and dietary sterol restriction. *J. Lipid Res.* 1991. **32:** 1941–1948.

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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 (1). The biochemical abnormalities include increased levels of plant sterols (campesterol, stigmasterol, and sitosterol) and their 5α-stanol derivatives in plasma and other tissues (2–4), and increased intestinal sterol absorption coupled to decreased hepatic removal of the plant sterols and 5α-stanols (5–7). Although large amounts of cholesterol deposit in xanthomas and atherosclerotic lesions in coronary arteries and aorta (7), whole body cholesterol turnover and synthesis are subnormal in sitosterolemic subjects (5, 7, 8). Freshly isolated mononuclear leukocytes from three sitosterolemic subjects from a single family showed markedly diminished cholesterol synthesis and a deficiency in both the activity and enzyme mass of the rate-controlling enzyme of cholesterol biosynthesis, HMG-CoA reductase (9). In these homozygous sitosterolemic patients, the deficiency in cholesterol synthesis is coupled with increased low density lipoprotein (LDL) receptor function (10) despite the accumulation of plant sterols and cholesterol in the cells.

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 (3, 5, 6, 10). Treatment with bile acid-sequestering resins (cholestyramine or colestipol) or ileal bypass surgery induces bile acid malabsorption and enhances the conversion of cholesterol to bile acids in the liver. Normally, hepatic cholesterol synthesis is up-regulated so that the net reduction in plasma cholesterol concentrations reflects the balance between the input of

Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; LDL, low density lipoprotein.

newly synthesized cholesterol and its elimination as bile acids (11). Lately, there has been considerable interest in lovastatin, a new hypocholesterolemic agent that competitively inhibits HMG-CoA reductase, increases the expression of hepatic LDL receptors, and accelerates the clearance of plasma cholesterol (12, 13). In a recent study on three sitosterolemic sisters (14), bile acid malabsorption (colestipol administration and ileal bypass surgery) induced a greater than expected decrease in plasma sterols and apolipoprotein B concentrations whereas lovastatin was ineffective.

The objectives of this study were to: *1*) examine endogenous cholesterol synthesis in two unrelated sitosterolemic patients to test the hypothesis that deficient cholesterol synthesis is a consistent basic biochemical defect in sitosterolemia; and *2*) evaluate the effects of a low sterol diet and lovastatin and cholestyramine treatments on cholesterol biosynthesis and LDL receptor function in sitosterolemia.

MATERIALS AND METHODS

Clinical

Studies were conducted in two homozygotes, two obligate heterozygotes for sitosterolemia, and 17 control subjects. The two homozygotes (GB, male, aged 28 years and DW, female, 9 years) were unrelated to each other and to the three sisters previously studied (9, 10, 14, 15). The heterozygotes were the sister (DB, 25 years) and father (RW, 47 years) of the homozygotes. The clinical presentations in the homozygotes included tendon and tuberous xanthomas and elevated plasma cholesterol, plant sterol and 5α-stanol concentrations. Homozygous patient GB suffered a myocardial infarction at age 25 and underwent coronary angiography and balloon angioplasty for atherosclerotic coronary stenosis. Homozygous patient DW was diagnosed as sitosterolemic at the age of 3½ years and her clinical picture was previously reported (16). The heterozygous patients were asymptomatic. The control group included 7 female and 10 male healthy subjects, aged 19 to 60 years.

One homozygote and her heterozygous father (DW and RW, respectively) were hospitalized at the metabolic ward at the Rockefeller University Hospital for a drug treatment study. Patient DW was given a metabolic diet (calorie composition: carbohydrate, 53%, protein 17%, fat, 30%) that contained 223 mg cholesterol/2000 kcal and 33 mg plant sterols/2000 kcal without or with one of the following drug treatments: lovastain (Mevacor, Merck), 15 mg b.i.d., and cholestyramine (Questran, Mead Johnson), 15 g/day. Each drug treatment was for 3 weeks followed by a 2-week washout period on the metabolic diet alone before the next treatment period. Her heterozygous father was treated similarly, except the dose for lovastatin was increased to 30 mg b.i.d. Constant body weights were maintained throughout the study.

The second homozygous subject (GB) and his heterozygous sister (DB) were on free-living diets (400–500 mg/day cholesterol and 100–150 mg/day plant sterols according to food diaries) and maintained the same caloric intakes and body weights. Sitosterolemic subject GB, who could not tolerate lovastatin therapy (rise in levels of liver enzymes after a few days), was studied without therapy, then was put on a low sterol diet (100 mg/day cholesterol and 50 mg/day plant sterols) for 3 weeks, followed by treatment with cholestyramine (15 g/day) after a 3-week washout period. The treatment of patient GB with cholestyramine was continued for more than a year. His heterozygous sister DB refused any drug treatment and was studied only in the untreated state.

Three control subjects (male healthy volunteers, aged 33 to 52), who were on free-living diets (400–500 mg/day cholesterol and 100–150 mg/day plant sterols) and maintained weights throughout the study, were treated with lovastatin (30 mg b.i.d.) and cholestyramine (15 g/day) for at least 3 weeks following untreated and washout periods, respectively.

Blood was collected at 9:00 AM after a 12-h fast during the second and third treatment weeks for plasma sterol analysis and separation of LDL and mononuclear leukocytes for LDL receptor and enzyme assays. All analyses were done in duplicate and repeated at least twice with samples collected at least 5 days apart. Measurements for control subjects were included each time determinations of individual patients were made. Witnessed consent was obtained from patients and normal control volunteers, and the experimental protocol was approved by the Human Subjects Review Committee of the Rockefeller University Institutional Review Board and 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.

Cell separation and determination of plasma sterols

Plasma sterol concentrations were measured by capillary gas–liquid chromatography as described previously (10). Mononuclear leukocytes were isolated from 30–60 ml of venous blood according to Boyum (17). After lysis of erythrocytes, the mononuclear leukocytes were washed twice with phosphate-buffered saline and resuspended in incubation medium (RPMI-1640, Grand Island Biological Co., Grand Island, NY, containing 200 units/ml penicillin and streptomycin) in a volume equal to 10% of the original blood volume. The total number of cells was counted with a hemacytometer and had greater than 98% viability by the trypan blue dye exclusion test. A portion of the cells was used immediately for the assay of cellular

sterol synthesis and receptor-mediated LDL degradation, and the remaining cells were stored as pellets at −70°C for the preparation of microsomes.

Assay of cellular sterol synthesis

A cell suspension containing 2–8 × 10^6 mononuclear leukocytes was incubated in 1 ml RPMI medium that contained 50% autologous plasma and 2.5 μmol Na[2-^{14}C] acetate (New England Nuclear Corp., Boston, MA), diluted with unlabeled Na acetate to a specific activity of 15 dpm/pmol. After incubation for 4 h at 37°C in a shaking water bath, the labeled sterols were extracted with hexane, separated on alumina columns, and radioactivity was determined by liquid scintillation spectrometry (18). The use of plasma from normal subjects in the assay of sitosterolemic cells gave results similar to those with autologous plasma, indicating that the short-term effect of plasma cholesterol on cholesterol synthesis is negligible. In order to validate the acetate to cholesterol conversion as a measure of cholesterol synthesis in the presence of autologous plasma, freshly isolated mononuclear leukocytes were incubated for 4 h at 37°C in RPMI medium containing 50% autologous plasma or lipoprotein-deficient serum (prepared by differential ultracentrifugation (19)). The cells were then washed with RPMI and quick-frozen prior to preparation of microsomes and assay for HMG-CoA reductase activity. The results showed no changes in HMG-CoA reductase activities in the homozygous, heterozygous, and four control subjects when autologous plasma was replaced by lipoprotein deficient serum for 4 h.

Determination of receptor-mediated low density lipoprotein degradation by mononuclear leukocytes

Low density lipoprotein (LDL) ($1.019 < d < 1.063$ g/ml) was isolated from venous blood by differential ultracentrifugation (20), analyzed for cholesterol content by capillary gas–liquid chromatography (10), and labeled with ^{125}I (New England Nuclear, Boston, MA) by the monohydrochloride method (21). Receptor-mediated LDL degradation was measured as the difference between total uptake and degradation (assayed in the absence of unlabeled LDL) and nonspecific uptake and degradation (assayed in the presence of 40-fold excess unlabeled LDL) as described previously (9, 14).

Assay for hydroxymethylglutaryl coenzyme-A reductase activity

Mononuclear leukocyte microsomes were prepared in the presence and absence of the phosphatase inhibitor, sodium fluoride (22). The assay for microsomal HMG-CoA reductase activity was based on the methods of Shefer et al. (23) and Harwood, Schneider, and Stacpoole (22). Briefly, 50–100 μg microsomal protein was preincubated at 37°C for 5 min in a total volume of 150 μl buffer (50 mM Tris, 68 mM EDTA, 5 mM DTT, 70 mM KCl or NaF, pH 7.5) containing an NADPH-generating system (34 mM $NADP^+$, 30 mM glucose-6-phosphate, and 0.3 units glucose-6-phosphate dehydrogenase) and 40,000 dpm [^{3}H]mevalonolactone as internal standard. The reaction was started with the addition of 10 nmol [^{14}C]HMG-CoA (Amersham, Arlington Heights, IL, 114–121 dpm/pmol). The reaction was stopped after 30 min at 37°C with the addition of 20 μl 6 N HCl. After lactonization of the products at 37°C for 30 min, the precipitates were pelleted by centrifugation (10,000 rpm in a Beckman microfuge) and the products in the supernates were separated by thin-layer chromatography on 0.25 mm-thick silica gel 60 plates (Merck, Darmstadt, Germany), developed with benzene–acetone 1:1 (v/v); the radioactivity was determined by liquid scintillation counting. Total and expressed HMG-CoA reductase activities were determined with microsomes prepared and assayed in the absence and presence of the phosphatase inhibitor, sodium fluoride, respectively. The addition of *E. coli* alkaline phosphatase did not further increase total HMG-CoA reductase activity (22). The protein concentration of the mononuclear leukocyte microsomes was determined by the method of Lowry et al. (24).

Data were analyzed statistically by the unpaired *t* test and comparison of patients' values under various treatments with the 95% and 99% confidence intervals for the means of the control group (25).

RESULTS

The plasma sterol concentrations and compositions from two homozygous and two obligate heterozygous subjects were compared to values from a group of control subjects (**Table 1**). Both homozygous patients showed elevated total plasma sterol concentrations (343 and 301 vs. 185 ± 6 mg/dl for controls) with abundant amounts of plant sterols and 5α-stanols (20% and 8% of total sterols vs. less than 1% in controls). In distinction, plasma sterol levels in the heterozygous subjects were normal to marginally high and, as in control subjects, only small levels of cholestanol and plant sterols were detected. Both homozygous sitosterolemic patients showed a significant and similar drop in plasma cholesterol (−28% and −35%), and plant sterol and 5α-stanol (−31% and −29%) concentrations during cholestyramine treatment whereas lovastatin treatment or dietary sterol restriction proved ineffective. In contrast, cholestyramine reduced plasma cholesterol concentrations only 14% and 5% in control and heterozygous subjects, respectively, while lovastatin significantly decreased plasma cholesterol levels in the control and heterozygous subjects (−28% and −31%, respectively).

Table 2 shows the rate of cholesterol synthesis in freshly

TABLE 1. Effects of lovastatin, cholestyramine, and dietary sterol restriction on plasma sterol concentrations

Subject/Treatment	Cholesterol[a]	Plant Sterols and 5α-Stanols[a,b]
	mg/dl	
Sitosterolemia		
Homozygote		
DW		
Untreated	275 (310, 250, 265)	68 (86, 59, 60)
Lovastatin	318 (306, 335, 312)	66 (63, 71, 63)
Cholestyramine	198 (194, 203, 198)[c]	47 (49, 45, 47)
GB		
Untreated	277 (272, 270, 288)	24 (28, 22, 23)
Low sterol diet	289 (291, 296, 281)	25 (25, 26, 24)
Cholestyramine	179 (165, 166, 191, 194)[d]	17 (17, 17, 17, 18)
Sitosterolemia		
Heterozygote		
RW		
Untreated	249 (261, 235, 250)	0.3 (0.4, 0.2, 0.4)
Lovastatin	172 (205, 156, 155)[c]	0.3 (0.4, 0.3, 0.1)
Cholestyramine	237 (230, 240, 240)	0.6 (0.9, 0.5, 0.3)
DB		
Untreated	204 (185, 223)	1.0 (1.0, 1.1)
Controls		
(n = 15) Untreated	185 ± 6	0.3 ± 0.1
(n = 3) Lovastatin	133 ± 15[d]	0.6 ± 0.1
(n = 3) Cholestyramine	159 ± 6	0.2 ± 0.1

[a]The measurements were repeated with blood samples collected at least 5 days apart. Means ± SEM are shown for control subjects and means with all sequential data in parentheses are shown for individual patients.
[b]Plant sterols and 5α-stanols in the homozygous sitosterolemic patients consisted of sitosterol (56.3%), campesterol (27.4%), 5α-sitostanol (7.2%), 5α-cholestanol (5.1%), 5α-campestanol (4.0%), and traces of stigmasterol and avenosterol. Control and heterozygote plasma contained <0.2% of 5α-cholestanol and trace amounts of sitosterol.
[c]Significantly different from values from untreated periods, $P < 0.05$.
[d]Significantly different from values from untreated periods, $P < 0.01$.

isolated mononuclear leukocytes. The rates of cholesterol synthesis in mononuclear cells from both untreated homozygotes and heterozygous subject DB were below the lower limit of the 99% confidence interval for the control mean.

TABLE 2. Conversion of [^{14}C]acetate to cholesterol by mononuclear leukocytes from sitosterolemic and control subjects

Subject	Cholesterol Synthesis[a]	
	pmol/10^6 cells/h	*% control*
Sitosterolemia		
Homozygote		
DW	3.5 (3.2, 3.8, 3.6)[b]	70
GB	1.5 (0.7, 2.2, 1.5)[b]	29
Sitosterolemia		
Heterozygote		
RW	3.5 (3.1, 4.7, 2.7)	69
DB	2.7 (1.8, 3.5)[b]	53
Controls		
(n = 16)	5.1 ± 0.4	100

[a]Measurements were repeated with cells collected at least 5 days apart. Means ± SEM are shown for controls and means with all sequential data in parentheses are presented for patients.
[b]All values from the two homozygotes and from heterozygote DB are below the lower limit of the 99% confidence interval for the control mean (3.9 to 6.2 pmol/10^6 cells/h).

Consistent with significantly reduced rates of cholesterol synthesis, HMG-CoA reductase activities in mononuclear leukocytes from untreated homozygous and heterozygous sitosterolemic subjects were only one-third the mean value observed with 17 control subjects (**Fig. 1**). In both controls and patients, more than half of the total HMG-CoA reductase activity was expressed. Neither lovastatin treatment nor a low sterol diet produced a significant effect on the reduced HMG-CoA reductase activities in the homozygous and heterozygous subjects whereas lovastatin increased HMG-CoA reductase activity 38% in the control subjects. In distinction, treatment with cholestyramine paradoxically decreased mononuclear leukocyte HMG-CoA reductase activity in the two homozygous sitosterolemic subjects (−8% and −43%) but resulted in 13% and 46% increases in the control and heterozygous subjects, respectively. However, despite this rise, total HMG-CoA reductase in the heterozygous subject treated with cholestyramine remained subnormal.

Receptor-mediated degradation of ^{125}I-labeled LDL by mononuclear leukocytes is presented in **Table 3.** As with three sitosterolemic sisters previously reported (9, 10, 14), homozygous patient GB had markedly elevated mononuclear leukocyte LDL receptor function (6.6 ng/10^7 cells

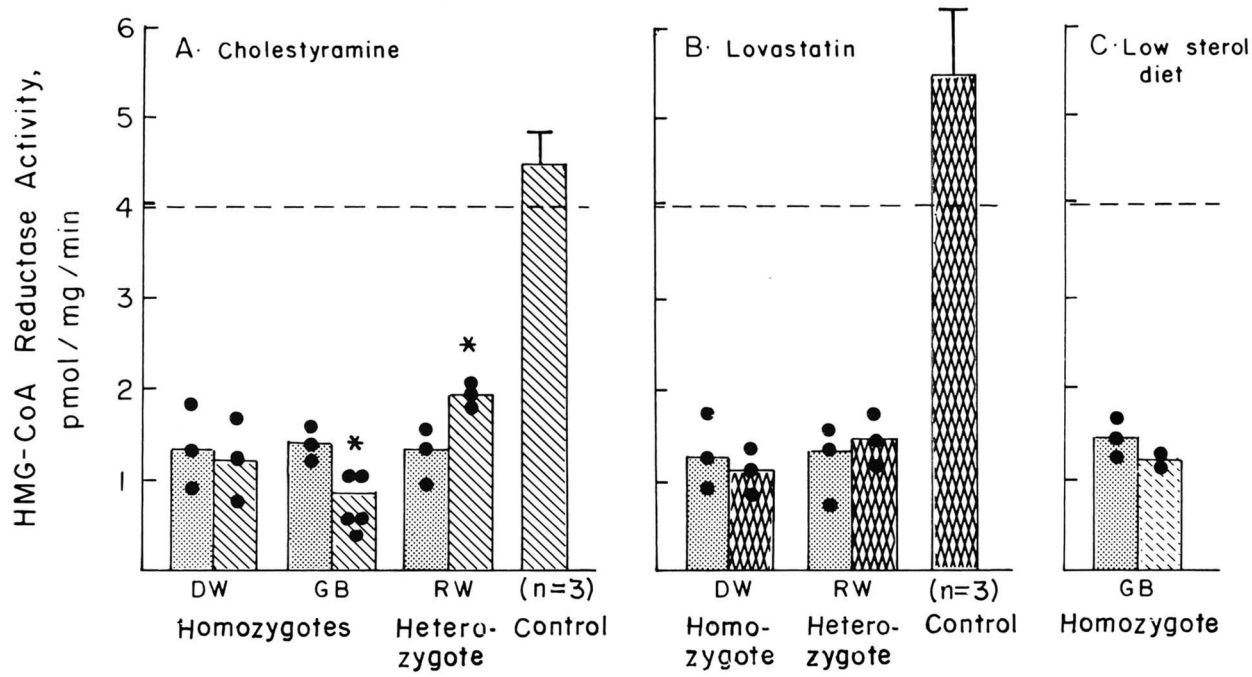

Fig. 1. Effects of cholestyramine (A), lovastatin (B), and low sterol diet (C) on HMG-CoA reductase activity in mononuclear leukocytes. The dotted line represents the mean for enzyme activity from 17 untreated control subjects. The shaded and hatched bars represent means from untreated and treated subjects, respectively. All measurements from homozygous and heterozygous subjects (dots) are below the lower limit of the 99% confidence interval for the mean of untreated controls (2.8 to 5.0 pmol/mg protein per min). *, Significantly different from values of the same subject during the untreated period ($P < 0.05$).

per 4 h, which is higher than the upper limit of the 99% confidence interval for the mean from the control group, 2.8 to 5.1 ng/10^7 cells per 4 h). His heterozygous sister (DB) also showed significantly elevated mononuclear leukocyte LDL function (10.4 ng/10^7 cells per 4 h vs. 4.0 ± 0.3 for controls). In homozygous subject GB, LDL receptor function was markedly stimulated by a low sterol diet and was also higher with cholestyramine treatment.

DISCUSSION

These results suggest that depressed cellular cholesterol synthesis due to a deficiency in HMG-CoA reductase activity that cannot be up-regulated by bile acid malabsorption or a low sterol diet is a basic biochemical abnormality in sitosterolemia (7, 9). In previous studies with mononuclear cells (9) and liver (15) from a single sitosterolemic family, reduced HMG-CoA reductase activity was related to the deficiency in HMG-CoA reductase protein as determined by immunoblotting. Furthermore, HMG-CoA reductase mRNA was barely detected by Northern blot analysis in sitosterolemic liver (15). This suggests that the molecular abnormality is insufficient mRNA to translate for HMG-CoA reductase synthesis.

It is very improbable that low cholesterol synthesis was related to feedback regulation by the accumulated plant sterols and 5α-stanols found in the sitosterolemic cells (9, 10). When fed (23, 26), infused intravenously to rats (27), or added in vitro to a fibroblast culture system (28), sitosterol and cholestanol were found to be ineffective in down-regulating HMG-CoA reductase. Recently, Ness, Keller, and Pendleton (29) have demonstrated that the feedback inhibition of hepatic HMG-CoA reductase activity by dietary cholesterol resulted from post-transcriptional modulation of the enzyme rather than a reduction in HMG-CoA reductase mRNA. In contrast, in sitosterolemic liver, HMG-CoA reductase mRNA is barely detected and enzyme protein is markedly deficient (15), which suggests a defect in enzyme synthesis.

In previous studies with a single family of three other homozygous sitosterolemic patients (9, 10, 14, 30), elevated LDL receptor function in the liver and mononuclear leukocytes was suggested to be a compensatory mechanism that provides needed cellular sterols because endogenous cholesterol synthesis was deficient. A similar up-regulation of cellular LDL receptor function was observed in patient GB. However, LDL receptor function may not be elevated in all sitosterolemic patients since fibroblasts from patient DW were deficient in LDL recep-

TABLE 3. Effects of lovastatin, cholestyramine, and dietary sterol restriction on the receptor-mediated LDL degradation by freshly isolated mononuclear leukocytes

Subject/Treatment	Receptor-Mediated LDL Degradation[a]	
	ng/10⁷ cells/4 h	*% control*
Sitosterolemia Homozygote		
GB		
Untreated	6.6 (6.4, 6.6)[b]	162
Low sterol diet	14.0 (12.9, 15.0)[b]	350
Cholestyramine	8.1 (6.5, 10.1, 5.5, 6.4, 12.0)[c]	202
Sitosterolemia Heterozygote		
DB		
Untreated	10.7 (11.0, 10.4)[b]	286
Controls		
(n = 6) Untreated	4.0 ± 0.3	100
(n = 3) Cholestyramine	7.4 ± 0.7[c]	185

[a]Receptor-mediated LDL degradation by control and sitosterolemic cells was 60 to 80% of total LDL degradation. Measurements for each treatment period were repeated with blood samples collected at least 5 days apart. Means ± SEM are shown for controls and means with all sequential data in parentheses are presented for individual patients.

[b]All values higher than the upper limit of the 99% confidence interval for the control mean (2.8 to 5.1 ng/10⁷ cells/4 h).

[c]Significantly different than values for untreated controls, $P < 0.01$.

tors (16). This may account for the higher amounts of plant sterols and 5α-stanols in her plasma (Table 1). Thus, the inherited abnormality can affect a different location in cholesterol biosynthesis and lead to a combination of reduced HMG-CoA reductase activity and LDL receptor function.

Although it has been suggested that sitosterolemic patients are quite sensitive to the intake of dietary cholesterol and plant sterols (1), not all patients responded predictably, as illustrated by patient GB. His plasma cholesterol failed to decline with a low sterol diet. Apparently, the biliary elimination of cholesterol from the body may be further reduced to conserve cholesterol (8).

A major finding was the demonstration that HMG-CoA reductase activity was not up-regulated in the sitosterolemic cells during cholestyramine treatment despite the significant reduction in plasma cholesterol levels and similar decline in plasma cholestanol, plant sterol, and 5α-stanol concentrations. In control subjects treated with cholestyramine, HMG-CoA reductase activity or cholesterol biosynthesis rate in mononuclear leukocytes increased up to 1.6-fold (14, 31) (Fig. 1). Furthermore, an important distinction between the homozygous and heterozygous sitosterolemic subjects is the response of HMG-CoA reductase to cholestyramine treatment. In heterozygous subjects (14) (Fig. 1), bile acid malabsorption increased mononuclear leukocyte HMG-CoA reductase activity, whereas in all similarly treated homozygous sitosterolemic subjects, mononuclear leukocyte HMG-CoA reductase activity either did not change or significantly declined (14) (Fig. 1). Since the patients in this study are unrelated to each other and the C family in the previous reports, the failure to up-regulate cholesterol synthesis during bile acid malabsorption is a common biochemical abnormality in sitosterolemia.

With bile acid malabsorption, a reduced hepatic bile acid flux stimulates bile acid synthesis via an up-regulation of cholesterol 7α-hydroxylase (32, 33). The decrease in plasma cholesterol and plant sterol concentrations may reflect the increased utilization of the sterols for bile acid synthesis and correlates with the stimulated expression of LDL receptor function (34, 35). However, because cholesterol synthesis in homozygous sitosterolemic individuals fails to increase (HMG-CoA reductase activity paradoxically declined), the effect of bile acid malabsorption on plasma sterol concentrations is magnified. Thus, the inability to increase normally endogenous cholesterol synthesis with cholestyramine treatment not only potentiates the cholesterol-lowering effect but also highlights an abnormality in the regulation of cholesterol biosynthesis in sitosterolemia. In contrast, lovastatin, which is a potent competitive inhibitor of HMG-CoA reductase and normally produces a 20–30% increase in cellular LDL receptor function and a proportional decline in plasma cholesterol concentrations (14), did not reduce plasma cholesterol and plant sterols in homozygous sitosterolemic patients (14) (Table 1). Apparently, subnormal cholesterol biosynthesis is already compensated by an increase in LDL receptor function and LDL receptors do not increase further during inhibition of cholesterol biosynthesis.

The therapies with cholestyramine and lovastatin not only support our contention of the fundamental abnormality in the regulation of cholesterol biosynthesis that underlies sitosterolemia, but the response to these therapies can also be used to detect sitosterolemia. Many hypercholesterolemic patients are treated with cholestyramine and lovastatin without having their plasma sterol composition analyzed by high performance or gas–liquid chromatography. Therefore, the failure to respond to lovastatin and a substantial response to cholestyramine may suggest sitosterolemia and the need for analysis of plasma sterol composition.

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