

Sitosterolemia

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It is almost 18 years since the first reports on sitosterolemia appeared (1). Two sisters with tendon xanthomas and normal plasma cholesterol levels were found to have elevated plant sterol concentrations in the plasma. A high percentage of dietary sitosterol was absorbed from the intestine, as measured by the sterol balance technique, and was believed to account for the plant sterol accumulation. Since the original report, 27 patients from 16 families have been detected (1–14). The clinical presentation includes tendon xanthomas, accelerated atherosclerosis particularly affecting males at a young age, hemolytic episodes, and arthritis and arthralgias. The risk of premature atherosclerosis was observed in several young male subjects who died because of acute myocardial infarctions associated with extensive coronary and aortic arteriosclerosis. The youngest was a 13-year-old Amish male who had four other homozygous siblings (3). In addition, a 17-year-old male, personally followed by the authors, developed angina pectoris, showed an abnormal cardiac stress test with decreased coronary artery perfusion, and died suddenly of an acute myocardial infarction while exercising (15). Examination of his coronary arteries at post mortem revealed 60% occlusion of the left anterior descending coronary artery (Fig. 1). However, multiple microinfarctions were noted in the myocardium which suggested that the atherosclerotic process had begun earlier and was chronic and progressive.

Sitosterolemia is inherited as a recessive trait (14). Heterozygotes are clinically and biochemically normal, although plasma sitosterol levels of some heterozygous subjects may be slightly but significantly increased over controls. These values still differ quantitatively from homozygotes by 10- to 20-fold (9, 12). Of interest is the high degree of inheritance of the homozygous state. In two unrelated families, homozygous sitosterolemia was present in 4/4 and 2/4 siblings respectively, from each family.

Biochemical features

The hallmark biochemical feature of the disease is the demonstration of elevated concentrations of sitosterol

(24-ethyl cholesterol) in the plasma (1, 16). Actually all dietary sterols are found in plasma (17), but since sitosterol is usually the most abundant in the diet, proportionately greater quantities are present in plasma and tissues (Fig. 2). For this reason, the condition has been named sitosterolemia (17). In addition, the respective 5 α -dihydro derivative of cholesterol (cholestanol) and the 5 α -dihydro plant sterol derivatives, 5 α -campestanol and 5 α -sitostanol, are present in increased amounts in plasma and tissues (Fig. 3) (18, 19). As diets contain only small amounts of cholestanol, 5 α -campestanol, and 5 α -sitostanol, the 5 α -dihydro derivatives probably are produced endogenously in larger amounts (19).

The diagnosis of sitosterolemia is established by demonstrating increased amounts of plant sterols (campesterol, sitosterol, stigmasterol, and avenasterol) and 5 α -stanols in plasma and tissues (1, 15, 19). The usual colorimetric assay that depends on a double bond between carbons 5 and 6, or enzymatic method that detects the 3 β -hydroxy group do not distinguish sitosterol from cholesterol. Therefore, to find plant sterols and 5 α -stanols and establish the diagnosis, gas-liquid chromatography using a capillary column is necessary (Fig. 4), although high performance liquid chromatography can also be applied (12). In one family with four homozygous siblings, the unsaturated sterols represented about 16% of the total plasma sterols with cholestanol and 5 α -dihydro plant stanols making up about 4% (2, 8). In other families, plant sterols and 5 α -stanols may account for as little as 11% to as much as 25% of the plasma sterols (2, 3, 19). Thus, cholesterol represents only about 80% of the total plasma sterols in sitosterolemic homozygotes. The concentration and distribution of sterols and stanols from a number of sitosterolemic homozygotes from five families and their heterozygous relations are presented in Table 1.

Abbreviations: VLDL, very low density lipoprotein; LDL, low density lipoprotein; IDL, intermediate density lipoprotein; HDL, high density lipoprotein; CTX, cerebrotendinous xanthomatosis; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A.

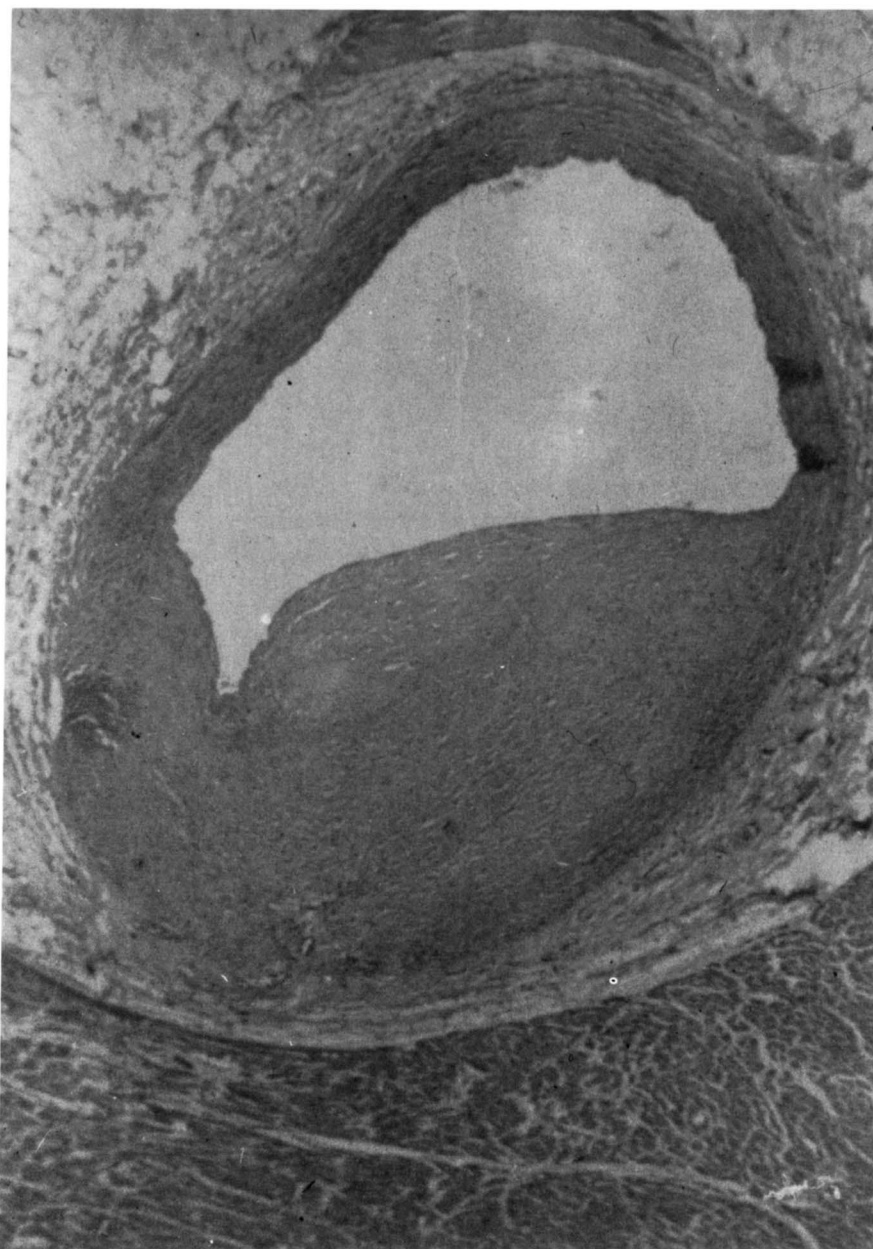


Fig. 1. Section of coronary artery from a 17-year-old sitosterolemic male who died of an acute myocardial infarction. Sixty percent occlusion of the vessel lumen by atherosclerotic thickening of the vessel wall.

For controls, values from 20 healthy subjects are given; they show that cholesterol normally represents 99.6% of the total sterols with about 0.2% cholestanol and 0.2% plant sterols. In some heterozygotes, cholestanol and sitosterol levels are similar to controls (20–22). However, in several obligate heterozygotes, cholestanol and sitosterol levels were slightly but significantly higher than the control means, but still substantially less than those found in their homozygous offsprings (9, 12). Plasma cholesterol concentrations may also vary considerably in homo-

zygotes. As illustrated in Table 1, cholesterol levels may be low but are usually increased over age-matched controls. However, some subjects (LBU) show extremely high cholesterol concentrations that resemble the levels found in LDL receptor-deficient hypercholesterolemic subjects.

Plasma lipoproteins have been measured in homozygous sitosterolemic subjects and the increased amounts of unsaturated plant sterols and saturated 5α -stanols are distributed in about the same proportion among the various lipoprotein fractions (HDL, LDL, IDL and VLDL)

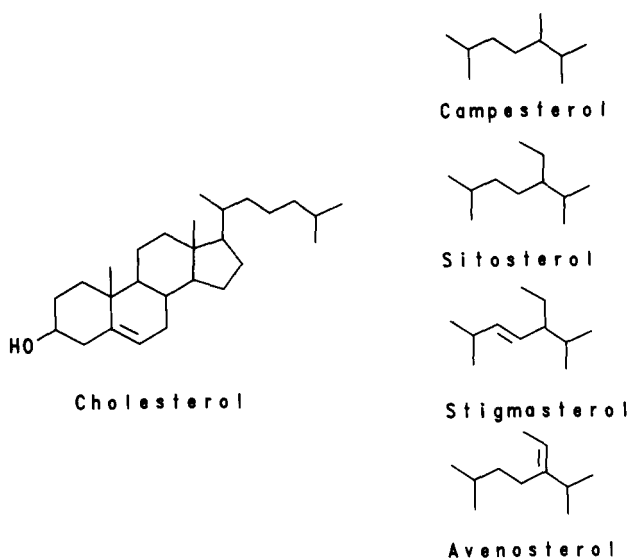


Fig. 2. Structure of plant sterols found in plasma of sitosterolemic subjects. These sterols have the same ring nucleus as cholesterol, but differ by the addition of substituents on the side chain at carbon 24, the presence of a double bond at carbon 22 in stigmasterol, and a double bond between carbons 24 and 28 in avenosterol.

(1, 6, 23). However, low density lipoprotein (LDL) concentrations tend to be elevated, reflecting higher total sterol concentrations as compared to age- and sex-matched controls. Despite the incorporation of increased amounts of plant sterols and 5α -saturated stanols, preparative density gradients for each lipoprotein class were similar to that of controls (23). The major proportion of the total low density lipoproteins was isolated in the subfraction of d 1.034 g/ml. The mean particle diameter, 25.7 ± 2.8 nm, for sitosterolemic LDL was not unusual as determined by electron microscopy, and the sitosterolemic LDL was not distinguishable morphologi-

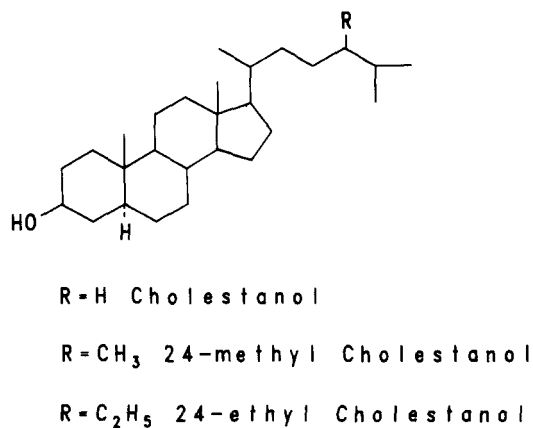


Fig. 3. Structures of 5α -saturated stanols found in plasma of sitosterolemic subjects. Cholestanol is the 5α -dihydro derivative of cholesterol; 5α -campestanol and 5α -sitostanol are the 5α -dihydro derivatives of campesterol and sitosterol, respectively.

cally from normal LDL. High density lipoprotein (HDL) concentrations tended to be normal or low in the homozygous sitosterolemic subjects (23). Electron microscopy of HDL from a male sitosterolemic subject with severe symptomatic atherosclerosis showed that the particles were round with a mean diameter of 8.5 ± 1.7 nm, consistent with the predominance of small, dense HDL (23).

Plasma concentrations of apolipoprotein B are usually increased and apolipoprotein A-I decreased for sitosterolemic homozygotes (23). However, normal plasma apoB and A-I levels were present in heterozygotes (20, 24). Thus, apolipoprotein values reflect the increased LDL and usually low HDL concentrations detected in these patients as determined by analytical and preparative ultracentrifugation (23).

Tissue sterol concentrations were measured in a 17-year-old sitosterolemic homozygote male who died unexpectedly of an acute myocardial infarction, and showed about 16% plant sterols and 5α -stanols in plasma (15). The total sterol concentrations in red blood cells, liver, lung, and heart were not different from control, but the cholesterol concentrations in these tissues were reduced and offset by the increased amounts of plant sterols and 5α -saturated stanols. Of importance, the individual plant sterols and 5α -saturated stanols were deposited in the tissues in about the same proportion that they were present in LDL. This suggested that the tissue sterols originated

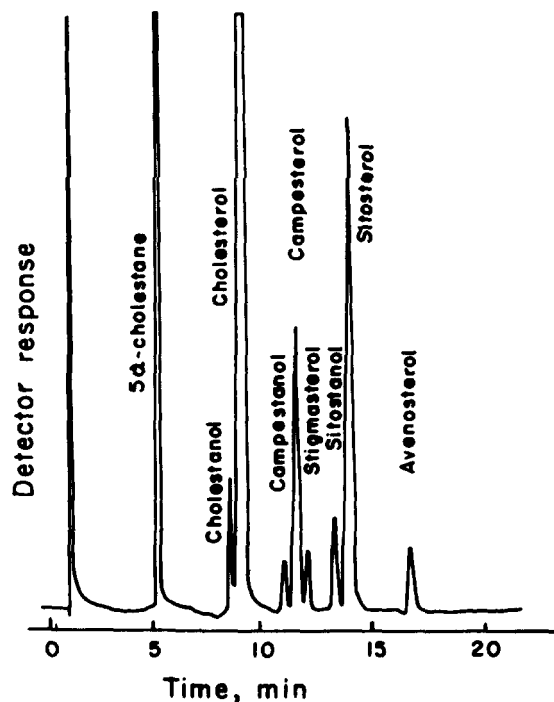


Fig. 4. Capillary gas-liquid chromatogram of plasma sterols and 5α -stanols from a homozygous sitosterolemic subject. In normal plasma only cholesterol and trace amounts ($< 1\%$) of cholestanol and sitosterol are detected. 5α -Cholestane is added as an internal standard. Separation was performed on Chrompack CPWax-57CB capillary column.

TABLE 1. Plasma sterols and stanols

Patients	Age	Cholesterol	Sitosterol	Campesterol	5 α -Cholestanol	5 α -Sitostanol	5 α -Campestanol
	<i>yr</i>	<i>mg/dl</i>					
Homozygotes							
KCN ^a (10) ^b	29	184 \pm 25	15 \pm 2	7.5 \pm 1.1	2.1 \pm 1.0	2.9 \pm 1.0	1.3 \pm 0.9
KC ^a (10)	23	202 \pm 25	14 \pm 4.1	8 \pm 3.1	4.7 \pm 1.0	2.2 \pm 1	1.4 \pm 0.2
TC ^a (10)	27	233 \pm 12	21 \pm 8.3	10 \pm 0.5	3.8 \pm 1.4	5.4 \pm 1.2	1.9 \pm 1.0
RC ^a (10)	18	249 \pm 39	20 \pm 5.5	13 \pm 1.5	7.5 \pm 2.4	3.9 \pm 1.0	2.6 \pm 0.9
GB ^c (10)	28	292 \pm 8	13 \pm 0.9	8 \pm 0.2	1.8 \pm 0.1	1.9 \pm 1.0	0.8 \pm 0.4
CL	42	134	27	13	1.6	3.0	2.6
JBR ^d	14	256	30	14.5	4.0	2.7	2.5
LBU	24	482	56	24.0	11.0	6.0	4.0
Heterozygotes							
AC ^a (5)	50	210 \pm 26	0.95 \pm 0.17	ND ^e	0.65 \pm 0.21	ND	ND
VC ^a (5)	56	194 \pm 14	0.36 \pm 0.09	ND	0.34 \pm 0.19	ND	ND
DB ^c (3)	25	204 \pm 27	0.66 \pm 0.05	ND	0.34 \pm 0.13	ND	ND
RBR ^d	48	254	0.8	ND	1.2	ND	ND
Controls, n = 20	17-62	180 \pm 5	0.22 \pm 0.20	ND	0.20 \pm 0.20	ND	ND

^aC family, includes heterozygous parents.

^bNumber of samples analyzed in parentheses.

^cB family, includes heterozygous sister.

^dR family, includes heterozygous mother.

^eND, not detected.

from plasma. In contrast, brain sterols in the sitosterolemic subject were composed almost entirely of cholesterol (15). Thus, despite the presence of large amounts of plant sterols and 5 α -stanols, the blood-brain barrier in sitosterolemia remains intact and is not permeable to circulating LDL. This is in contradistinction to cerebrotendinous xanthomatosis (CTX), a lipid storage disease, where increased amounts of cholestanol deposit in the brain and suggests that the blood brain-barrier is damaged and more permeable to circulating LDL (2, 15). Interestingly, atheromas in the aorta of this sitosterolemic subject contained increased amounts of esterified sterols, about 50% of the cholesterol and sitosterol were esterified as compared with only 10% esterified sterols in visceral organs (15).

Sterol composition in bile is different from controls in sitosterolemia. Not only is less cholesterol secreted into the bile, but sitosterol appears in the same or lower proportion relative to cholesterol in bile as compared in plasma (1, 2, 5, 6, 25). Normally the liver preferentially secretes sitosterol into bile so there is a 3-fold enrichment of sitosterol relative to cholesterol as compared to blood in control subjects (26). Biliary bile acids include cholic acid, deoxycholic acid, and lesser amounts of chenodeoxycholic acid and are secreted into bile in amounts adequate to prevent steatorrhea (1, 2, 4, 5, 27). No unusual biliary bile acids were detected, although it has not been established whether sitosterol and other plant sterols can be converted to primary bile acids in homozygotes. Recently, Bhattacharyya et al. (25) reported radioactive bile acids derived from [¹⁴C]sitosterol in the feces of three sitosterolemic subjects, but the precise identification of these

compounds was not carried out (28, 29). However, it was noted that the large quantities of sitosterol and cholestanol in sitosterolemic liver competitively inhibited cholesterol 7 α -hydroxylase, the rate-determining enzyme for bile acid synthesis, which may eventually lead to decreased bile acid production and deficient pool size (27). Thus, sitosterolemic liver has lost both the capacity to recognize sitosterol and the ability to preferentially secrete the 24-ethyl sterol into the bile. In addition, cholesterol secretion into bile is markedly diminished. Also, since biliary cholesterol secretion (lithogenicity) relative to bile acids and phospholipids is decreased (4), gallstones have not been detected in sitosterolemic subjects. (G. Salen, unpublished observation).

Monocyte (mononuclear leukocytes) sterol composition has been measured in four sitosterolemic homozygotes (30). The sterols and stanols are similar in composition (sitosterol, campesterol, cholestanol, 5 α -campestanol, and 5 α -sitostanol) as found in LDL indicating that the plant sterols and stanols originate from plasma. However, total sterol concentrations in monocytes from the sitosterolemic homozygotes were 2 to 3-times larger than in control monocytes. Thus, monocytes, which are precursors to foam cells, contain increased quantities of cholesterol, plant sterols, and 5 α -stanols that may contribute to the accelerated atherosclerotic process in this disease.

Sitosterol metabolism

It has long been known that sitosterol is poorly absorbed from the intestine (26, 31). The low plasma concentrations found in animals and humans fed large amounts of dietary plant sterols attest to restricted intesti-

nal absorption (26). However, early sitosterol balance studies, where fecal outputs were measured and subtracted from dietary inputs, gave confusing high values (between 30 to 50% of intake) for absorption (26). To overcome potential errors in the balance technique, sitosterol absorption has been measured by two independent isotopic methods. The isotope kinetic method estimates absorption by mathematical analysis of specific activity decay curves after intravenous pulse-labeling with a tracer dose of radioactive sitosterol (26, 32–34). In normal and hyperlipidemic subjects, the plasma specific activity decay of sitosterol is much more rapid than the specific activity decay of cholesterol when both isotopic sterols were injected intravenously (Fig. 5) (5, 23–26). These decay curves can be divided into two exponentials and analyzed mathematically according to the two-pool model. Table 2 lists values for controls and three homozygous sitosterolemic subjects from two unrelated families. Since sitosterol is not synthesized endogenously in normal humans and sitosterolemic subjects (5, 26), the production rate is equivalent to absorption and in the control subjects amounted less than 10 mg per day or about 5% of daily intake. Mean total sitosterol body pool size was also calculated and amounted to about 130 mg in controls. In contrast, sitosterol turnover in the three homozygous sitosterolemic subjects was much slower as compared to controls. Sitosterol production rates were 5 to 10 times larger than the control mean, confirming the enhanced absorption found in homozygotes with this disease. Total body pools were also tremendously enlarged and ranged from 3500 to 9500 mg. Although absorption was in-

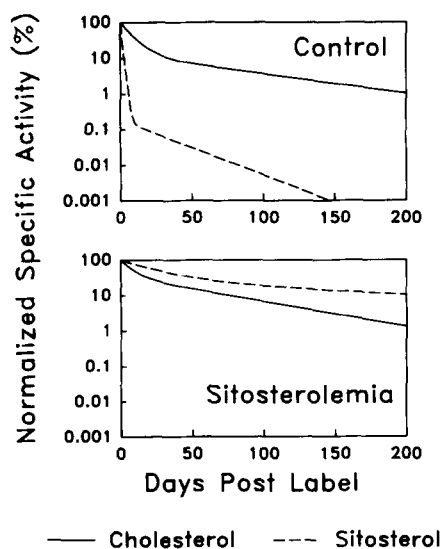


Fig. 5. The normalized specific activity versus time curves for cholesterol and sitosterol are illustrated for a control and homozygous sitosterolemic subjects. In the control subject, sitosterol decays more rapidly than cholesterol as contrasted with slower decay of sitosterol than cholesterol in the homozygote.

TABLE 2. Sitosterol turnover

	Controls (n=5)	Sitosterolemic Homozygotes ^a		
		CL	KCN	KEC
$t_{1/2A}$ (days)	3.8 ± 0.2	7.0	2.7	.11
$t_{1/2B}$ (days)	13.8 ± 2.4	91	24	112
K_A (day ⁻¹)	0.17 ± 0.04	0.015	0.067	0.014
M_A (mg)	80 ± 36	5100	2400	1700
M_B (mg)	46 ± 22	4400	2400	1800
$M_A + M_B$ (mg)	126 ± 32	9500	4800	3500
PR_A (mg/day)	7.9 ± 2.3	80	162	52

Results from references 5, 23, 26.

^aPatients KCN and KEC are sisters and unrelated to CL.

creased in these subjects, the extraordinary body pool size did not relate linearly to absorption (23, 24). The discrepancy could be explained by the fact that the elimination constant from pool A (K_A) was 2 to 10 times more rapid in control subjects than in sitosterolemic homozygotes. Thus, sitosterolemic homozygotes hyperabsorb sitosterol from the intestine but also retain the plant sterol in body tissues (5, 23, 24). This finding of very slow sitosterol turnover associated with increased absorption and very delayed removal has recently been noted in three additional sitosterolemic subjects from two unrelated families (25) who received radioactive sitosterol intravenously. Body pool sizes were extraordinarily expanded as noted previously. Sitosterol turnover has also been studied in two sitosterolemic heterozygotes (parents of homozygotes). The results show that sitosterol absorption was increased 2 to 3 times over controls but body pool sizes were not increased because sitosterol removal was rapid (24). Thus, heterozygotes still retain the ability to excrete sitosterol normally.

Enhanced sitosterol absorption in homozygotes and heterozygotes has been confirmed independently by absorption measurements obtained by adapting the plasma dual-isotope ratio method used to study cholesterol absorption (Table 3) (23, 24). In this technique, [¹⁴C]sitosterol is fed and [³H]sitosterol is administered intravenously at the same time. The ³H/¹⁴C ratio is then determined in plasma sitosterol and compared to the ideal

TABLE 3. Sitosterol and cholesterol absorption^a

	Controls		Sitosterolemic Homozygotes	
	1	2	KCN	KEC
	%		%	
Sitosterol absorption	4	5	63	28
Cholesterol absorption	44	48	49	69

^aPlasma dual-isotope ratio method, data from reference 23.

ratio which is calculated by dividing the total oral dose by total injected dose and is equivalent to 100% absorption (23). In two homozygotes, 28% and 63% of dietary sitosterol were absorbed which is in good agreement with the absorption values calculated by the independent isotope kinetic method (Table 2). Two healthy control subjects who consumed the same diet absorbed 4% and 5% of dietary sitosterol, respectively, while two obligate heterozygotes absorbed 15% and 17% of dietary sitosterol, respectively. Thus, sitosterol absorption is enhanced in homozygotes.

Cholesterol absorption and turnover

Cholesterol absorption as measured by the plasma dual-isotope ratio method tended to be at the high end of the normal range (49% and 69% of intake) in sitosterolemic homozygotes as reported in Table 3 (23). Thus, increased sitosterol absorption does not interfere with cholesterol absorption in sitosterolemic homozygotes although it is believed that sitosterol and cholesterol share the same intestinal absorption pathway. Also, it is important to realize that cholesterol is absorbed about 10 times more efficiently than sitosterol in healthy control subjects, but that percent sitosterol absorption approaches cholesterol absorption in some sitosterolemic homozygotes (Table 3) (23). There was no difference in cholesterol absorption between controls and heterozygotes (24). Although it has been suggested that the limited absorption of sitosterol compared to cholesterol in normal subjects may relate to greater affinity of sitosterol for intestinal bile acid micelles (35), diminished intestinal sitosterol esterification (36), and reduced sitosterol enterocyte transport (37), the up-regulation of these mechanisms seems unlikely to explain the increased absorption of sitosterol in sitosterolemic homozygotes.

An important, new biochemical finding observed in the sitosterolemic subjects is reduced cholesterol turnover (Table 4) (4, 5, 23). Not only is the plasma specific ac-

tivity decay of cholesterol much slower in homozygotes than control subjects, but turnover ($PR_A = \text{synthesis plus absorbed cholesterol}$) is markedly reduced. Calculations of cholesterol turnover by the isotope kinetic method revealed values 50–70% smaller in homozygotes than similarly fed controls (5, 23–25). Moreover, since cholesterol absorption tended to be large in sitosterolemic subjects, the diminished daily production must result from decreased cholesterol synthesis. When turnover values were corrected by subtracting absorbed cholesterol, average cholesterol synthesis was about 50% lower in sitosterolemic homozygotes than in healthy controls (23–25). In support, Miettinen (4) found cholesterol synthesis as measured by the sterol balance technique 50% and 80% lower in a homozygous sitosterolemic subject than in similarly fed control subjects when studied on two occasions 4 years apart. In contrast, cholesterol turnover and synthesis in sitosterolemic heterozygotes resembled control subjects and was not decreased (24).

Mechanism of reduced cholesterol synthesis

A major discovery from balance and isotopic turnover studies was that cholesterol synthesis in sitosterolemic homozygotes was extremely low (Table 4) (4, 5, 23, 25). In order to better understand this observation, HMG-CoA reductase, the rate-controlling enzyme for cholesterol biosynthesis, was measured in liver microsomes from two sitosterolemic homozygotes (38). For comparison, liver specimens were obtained from 11 liver transplant donors whose livers became available when appropriate recipients could not be located. In the control livers (Table 5), mean HMG-CoA reductase activity was 5.3 and 8.2 times greater, respectively, than the values from the two sitosterolemic liver specimens. About 72% of the HMG-CoA reductase was expressed (active) in the sitosterolemic livers compared to 49% in the controls.

HMG-CoA reductase protein concentrations were determined in these same microsomal specimens by immunoblotting and densitometric scanning (Table 5). In the control liver microsomes, the mean relative mass of HMG-CoA reductase per mg of microsomal protein was 6.8 and 8.9 times larger, respectively, than the values for the two sitosterolemic livers. Thus, markedly reduced HMG-CoA reductase activity and enzyme protein characterize sitosterolemic liver. However, when the catalytic efficiency of HMG-CoA reductase (activity per unit protein) was calculated by dividing the enzyme specific activity by the enzyme mass, no difference was detected between control and sitosterolemic livers. This suggests that although reduced quantities of HMG-CoA reductase are produced by the sitosterolemic livers, catalytic function of the enzyme is normal. To further explore the severe deficiency of HMG-CoA reductase, poly A⁺ RNA was isolated from liver specimens obtained from two control and one homozygous sitosterolemic subjects and hybridized

TABLE 4. Cholesterol turnover^a

	Controls (n = 4)	Sitosterolemic Homozygotes		
		KL	KCN	KEC
t _{1/2A} , days	6.7 ± 0.6	8.6	2.4 ^c	5.0
t _{1/2B} , days	53 ± 6	81	24	74
K _A , day ⁻¹	0.045 ± 0.006	0.023	0.077	0.024
M _A , g	29 ± 8	31	11	21
M _B , g	48 ± 16	34	13	41
M _A + M _B , g	77 ± 9	65	24	65
PR _A , mg/day	1450 ± 560	670	860	710
Synthesis, mg/kg/day	14.6 ± 6.0 ^c	^b	9.5 ^c	5.9 ^c

^aData from references 4, 5, 23.

^bNot available.

^cSynthesis estimated by subtracting absorbed cholesterol (Table 3) from turnover (PR_A).

TABLE 5. Hepatic microsomal HMG-CoA reductase activity and mass^a

Subjects	Activity	HMG-CoA Reductase Mass	Catalytic Efficiency
	<i>pmol/mg/min</i>	<i>peak area/mg</i>	<i>pmol/min/peak area</i>
Controls (n = 11)	98.1 ± 28.8	1.4 ± 0.14	68.6
Sitosterolemic homozygotes			
KCN	11.9	0.16	74.3
TC	18.4	0.21	76.2

^aFrom reference 38.

with pRED 227 and pHRED 102, which are full-length sequence cDNA probes for hamster and human HMG-CoA reductase, respectively, and pCAT 10, a probe for human catalase mRNA. The Northern blots (Fig. 6, A and B) showed virtually no signals for sitosterolemic HMG-CoA reductase mRNA, as contrasted with signals from the HMG-CoA reductase mRNA from the control specimens. Both control and sitosterolemic specimens gave signals for catalase mRNA that indicated that the RNA isolated from control and sitosterolemic livers was intact. Thus, the deficiency of microsomal HMG-CoA reductase in sitosterolemic livers can be attributed to the very low levels of HMG-CoA reductase mRNA that are available for enzyme translation (38).

LDL receptor binding was also measured in twelve control and two sitosterolemic liver membrane preparations. Total binding (assayed in the absence of unlabeled LDL) was 54% and 80% higher, respectively, in the two sitosterolemic liver membrane preparations than the mean for the control measurements (Table 6). Similarly, high affinity, receptor-mediated LDL binding recorded as the difference between total binding and nonspecific binding (assayed in the presence of abundant unlabeled LDL) was 2.2 and 3.3 times greater, respectively, in the sitosterolemic than in the control livers. Therefore, sitosterolemic livers express increased numbers of LDL receptors, so that a much higher proportion of LDL was receptor-bound and more circulating LDL was taken up than by control liver membranes (38).

In a separate experiment, Biel et al. (39) measured in vivo LDL turnover and found greater production associated with rapid catabolism consistent with the expression of more LDL receptors in a sitosterolemia subject as compared with three matched controls.

In two sitosterolemic liver specimens, lipofuscin-like pigment was distributed in the liver cytosol (38). The nature of this pigment has not been determined at this time (Fig. 7).

Treatment

Bile acid malabsorption produced by either binding resins (cholestyramine or colestipol) or ileal bypass surgery is an effective treatment of sitosterolemia (2, 4, 7, 11, 19). Plasma cholesterol concentrations decline dramati-

cally (decrease 25% to 50%) and the percent reduction in plasma sterol concentrations obtained with these drugs or surgery is greater than similarly treated hypercholesterolemic subjects. In most sitosterolemic patients, plant sterols usually decrease proportionally to cholesterol, and



Fig. 6. Northern blot analysis of sitosterolemic hepatic mRNA. Northern blots of liver poly A⁺ RNA from a sitosterolemic homozygote (TC) and two control subjects that were probed with pRED 227 (A) and pHRED 102 for HMG-CoA reductase mRNA and pCAT 10 for catalase mRNA (B). Virtually no signal from sitosterolemic HMG-CoA reductase mRNA was detected (38).

TABLE 6. Hepatic LDL receptor binding^a

Subjects	Total	Receptor-Mediated
Control, n = 12	204.0 ± 10.0	95 ± 8.2
Sitosterolemic homozygotes		
TC	315.3	193.2
KCN	336.8	312.8

^aFrom reference 38.

cholestanol and 5 α -saturated stanols were virtually eliminated from the blood (19). However, it is important to realize that not all patients respond similarly, and cholestyramine treatment produced less reduction of plasma plant sterols than cholesterol in a Japanese sitosterolemic family (12). Of note, clinical improvement including disappearance of xanthomas, elimination of aortic stenosis murmur, and decreased frequency in angina pectoris and arthritic attacks have been noted in several subjects treated with either cholestyramine or ileal bypass surgery (7, 11, 20).

Lovastatin, a competitive inhibitor of cholesterol biosynthesis that is widely used in the treatment of hyper-

cholesterolemia has been tried but has not been an effective treatment in sitosterolemia. Plasma cholesterol, plant sterols, or 5 α -saturated stanols were not reduced in two homozygous sitosterolemic subjects (20, 22).

The effect of the various treatments can be explained by examining cholesterol biosynthesis and LDL receptor function in freshly isolated peripheral mononuclear leukocytes (monocytes). These cells synthesize cholesterol and express HMG-CoA reductase activity and LDL receptors in parallel to the liver. In five homozygous sitosterolemic subjects from three unrelated families, mononuclear leukocyte cholesterol synthesis as measured by the conversion of acetate to cholesterol was 30–70% below the mean value from 16 healthy control subjects (20, 22). Subnormal monocyte cholesterol synthesis in the sitosterolemic subjects was supported by measurements of HMG-CoA reductase activity which were 50–70% lower in the homozygotes than the control mean. In contrast, LDL receptor function in monocytes from four of the five homozygous patients was increased 60% over the control mean. Thus, sitosterolemic mononuclear leukocytes manifest the same defect in cholesterol biosynthesis as the liver and compensate by the increased expression of LDL receptors.

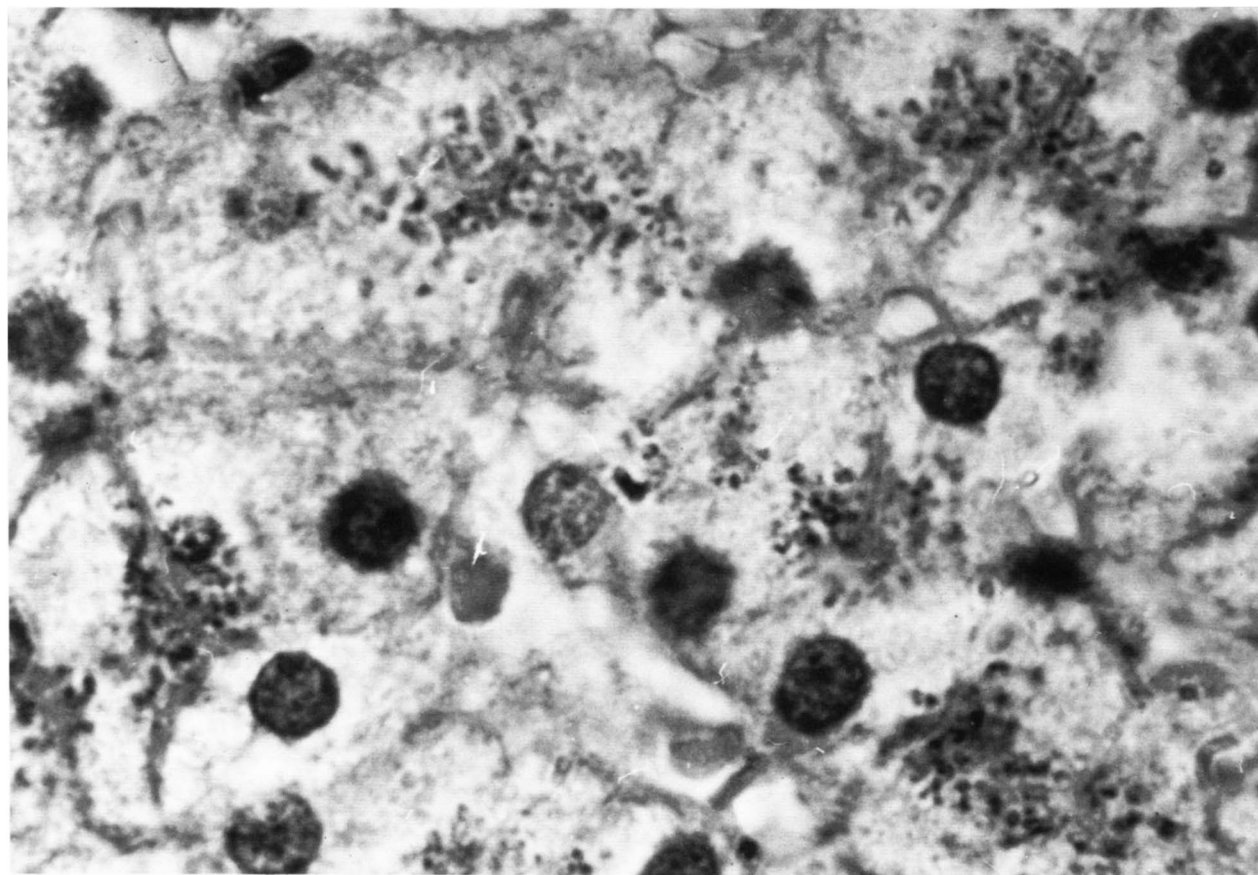


Fig. 7. Light microscopy of liver from sitosterolemic homozygote that shows lipofuscin-like granules deposited randomly in cytosol of hepatocyte. Hematoxylin and eosin \times 250 (38).

When the enterohepatic circulation of bile acids is interrupted, reducing the hepatic bile acid flux, HMG-CoA reductase activity increased 13% and LDL receptor function rose 40% in freshly isolated monocytes from healthy control subjects (20). In contrast, monocytes from four homozygous sitosterolemic subjects (from three unrelated families) failed to up-regulate either cholesterol synthesis (conversion of acetate to cholesterol) or HMG-CoA reductase activity when treated similarly (20, 22). In fact, HMG-CoA reductase activity paradoxically declined. Monocyte LDL receptor function responded normally to bile acid malabsorption by increasing between 20% and 30% in the sitosterolemic mononuclear cells (20, 22, 40, 41).

Lovastatin treatment produced no change in plasma sterol concentrations in two unrelated sitosterolemic homozygous subjects and caused only a small rise in monocyte HMG-CoA reductase activity compared with a 28% reduction in plasma sterol concentrations and a 38% increase in monocyte HMG-CoA reductase activity in control and hypercholesterolemic heterozygous subjects (20, 22). Although lovastatin competitively inhibits mevalonic acid synthesis, HMG-CoA reductase activity normally increases. Apparently, the block in cholesterol production produces gene expression for the synthesis of HMG-CoA reductase. LDL receptor function also did not change in the homozygous sitosterolemic monocytes as compared to a 41% increase in receptor-mediated LDL binding in control cells from subjects treated with lovastatin (20, 22).

These results indicate a major abnormality in cholesterol homeostasis in sitosterolemic subjects. Depressed cholesterol biosynthesis is due to a pronounced deficiency of the rate-controlling enzyme, HMG-CoA reductase, caused by virtual absence of HMG-CoA reductase mRNA. Interruption of the enterohepatic circulation of bile acids reduces the hepatic bile acid flux and, normally stimulates bile acid synthesis (42), but fails to increase cholesterol production in sitosterolemic homozygotes. Cholesterol 7 α -hydroxylase activity (rate-controlling for bile acid synthesis) rises in response to bile acid malabsorption (27) so that more bile acids are formed (4). Cholesterol biosynthesis (HMG-CoA reductase activity) should increase and more LDL receptors expressed to provide additional cholesterol as substrate for bile acid synthesis. In normal subjects, the decrease in plasma cholesterol reflects the balance between input of new cholesterol (synthesis) and the removal and catabolism of LDL. Because sitosterolemic subjects cannot up-regulate HMG-CoA reductase, the demand for more substrate for bile acid synthesis can only be met by the catabolism of LDL. Thus, there is a greater than expected fall in plasma sterol concentrations (cholesterol and plant sterols) in sitosterolemic subjects.

Lovastatin, which normally lowers plasma cholesterol by competitively inhibiting HMG-CoA reductase activity and in turn stimulates expression of LDL receptors, was

ineffective treatment for sitosterolemia (20, 22). Apparently, sitosterolemic cholesterol synthesis is so low that further inhibition of HMG-CoA reductase does not increase LDL receptor function. With this in mind, hypercholesterolemic patients who do not respond to therapeutic doses of lovastatin should have their plasma sterols tested by gas-liquid chromatography as the failure to respond to lovastatin may indicate sitosterolemia (20, 22).

Inherited abnormality

At the present time, the principal inherited defect has not been established with certainty. However, three abnormal mechanisms, hyperabsorption of sitosterol, decreased sitosterol elimination, and reduced cholesterol synthesis, have been linked to the pathogenesis of sitosterolemia and predisposition to atherosclerosis. The hyperabsorption of sitosterol and other dietary sterols from the intestine is well documented, but by itself will not cause the enormous sitosterol pools in this disease. Sitosterolemic heterozygotes also hyperabsorb sitosterol but do not accumulate the plant sterol (24). Not until the intestinal pathway for cholesterol absorption is elucidated will the mechanism for sitosterol hyperabsorption be understood.

To date, all sitosterolemic homozygotes show diminished hepatic secretion of sitosterol and cholesterol. Bile contains reduced amounts of both sitosterol and cholesterol, and biliary sterol excretion is further decreased when dietary intake is restricted (25). Clearly, the combination of decreased removal with increased absorption accounts for the gigantic sitosterol and other plant sterol pools.

A third key feature of the disease is abnormal regulation of cholesterol biosynthesis. We have demonstrated that reduced cholesterol synthesis results from a deficiency of HMG-CoA reductase in the liver and mononuclear cells of sitosterolemic subjects (38, 43), and that HMG-CoA reductase mRNA is barely detected in the liver. LDL receptor function is enhanced in most sitosterolemic homozygotes to provide cellular sterols. Attempts to stimulate cholesterol synthesis (HMG-CoA reductase) by inducing bile acid malabsorption (cholestyramine or ileal bypass surgery) or a low sterol diet did not increase HMG-CoA reductase activity in freshly isolated mononuclear cells (20, 22). Thus, the up-regulation of cholesterol synthesis is prevented in sitosterolemia.

Moreover, it is important to emphasize that HMG-CoA reductase activity and the expression of LDL receptors are normally regulated in the same direction. In other words, factors stimulating HMG-CoA reductase increase the number of LDL receptors (41, 43). In contrast, sitosterolemic mononuclear cells and liver show diminished HMG-CoA reductase activity and enzyme mass in combination with increased LDL receptor expression (20, 22, 38, 43). These observations lead us to believe that the

inherited defect in sitosterolemia involves an abnormality of the HMG-CoA reductase gene.

However, at this time it is still not possible to establish which mechanism is primary. It still is possible that enhanced sitosterol absorption and accumulation are primary events. Therefore, low cholesterol synthesis and enhanced receptor function may conceivably relate to the accumulated sterols and stanols and/or an oxygenated derivative. However, Boberg, Åkerlund, and Björkhem (44) have reported that sitosterol is not an effective feedback inhibitor of HMG-CoA reductase, and Shefer et al. (45) noted that cholestanol feeding actually increases HMG-CoA reductase activity in rat liver. Thus, neither cholestanol nor sitosterol are down-regulators of cholesterol biosynthesis.

Summary

Sitosterolemia is a rare inherited lipid storage disease characterized chemically by the accumulation of plant sterols and 5α -saturated stanols in plasma and tissues. Very low cholesterol synthesis due to a deficiency of HMG-CoA reductase associated with increased intestinal plant sterol absorption and slow hepatic sterol removal are major biochemical features. Because cholesterol synthesis cannot up-regulate, bile acid malabsorption mobilizes body sterols for bile acid synthesis and dramatically lowers plasma and monocyte sterol concentrations and may halt the progression of the atherosclerotic process. ■

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