Increased sitosterol absorption, decreased removal, and expanded body pools compensate for reduced cholesterol synthesis in sitosterolemia with xanthomatosis

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Abstract We measured the turnover and absorption of sitosterol and cholesterol, along with plasma sterol and lipoprotein concentrations, in one control and two subjects with sitosterolemia with xanthomatosis. All individuals consumed the same diet which contained approximately 500 mg/day of cholesterol and 250 mg/day of sitosterol. Sterol absorption was measured by the plasma dual-isotope ratio method and turnover by plasma isotope-kinetic analysis. In two sitosterolemic subjects, 28% and 63% of the sitosterol and 69% and 49% of the cholesterol were absorbed, respectively, compared to 4% of the sitosterol and 44% of the cholesterol in the control. As expected, plasma sitosterol specific activities decayed much more rapidly than cholesterol in the control subject. In contrast, plasma sitosterol and cholesterol specific activity-time curves were similar and decayed more slowly in the sitosterolemic subjects. In the control subject, the total sitotsterol pool was 290 mg and was linearly related to low absorption (18 mg/day); whereas the total sitosterol pool was 17 times (4800 mg) and 13 times (3500 mg) larger, respectively, in the sitosterolemic subjects and was expanded out of proportion to increased absorption because of decreased removal. Daily cholesterol turnover and synthesis were markedly reduced in the sitosterolemic subjects. In four sitosterolemic subjects, plasma concentrations of total sterols, low density lipoproteins, and apolipoprotein B were increased, while those of high density lipoproteins and apolipoprotein A-I were low to normal. The low density lipoproteins were very similar to those of normal control subjects in density distribution, peak flotation rate, sterol-to-protein (apolipoprotein B) ratio, particle size, and morphology. These results demonstrate in patients with sitosterolemia with xanthomatosis that: *1*) the absorption of sitosterol and cholesterol is enhanced; *2*) tissue recognition between cholesterol and sitosterol is lost; *3*) total exchangeable sitosterol pools are expanded out of proportion to absorption because of decreased excretion; *4*) plasma sterol and lipoprotein concentrations favor tissue deposition; and *5*) cholesterol synthesis is diminished. We postulate that the changes in sitosterol metabolism (increased absorption, loss of tissue sterol structural recognition, expanded pools, and hepatic retention) are a response to reduced cholesterol synthesis in these subjects. —**Salen, G., V. Shore, G. S. Tint, T. Forte, S. Shefer, I. Horak, E. Horak, B. Dayal, L. Nguyen, A. K. Batta, F. T. Lindgren, and P. O. Kwiterovich, Jr.** Increased sitosterol absorption, decreased removal, and expanded body pools compensate for reduced cholesterol synthesis in sitosterolemia with xanthomatosis. *J. Lipid Res.* 1989. **30:** 1319–1330.

Supplementary key words turnover • lipoproteins • apoB

The development of symptomatic atherosclerosis is a well-recognized early complication in patients with sitosterolemia with xanthomatosis (1–5). Other clinical features of this rare disorder, first described by Bhattacharyya and Connor (6), include tendon and tuberous xanthomas, recurrent arthritis, and arthralgias and hemolytic episodes (1,6,7). Although both sexes are affected prematurely with coronary atherosclerosis, males become symptomatic at a younger age than females. The mechanism for the accelerated atherosclerosis is not understood, but may be related to the plant sterols and 5α-saturated stanols that accumulate in the plas-

Abbreviations: GLC, gas-liquid chromatography; TLC, thin-layer chromatography; TMS, trimethylsilyl; HPLC, high performance liquid chromatography; LDL, low density lipoproteins; HDL, high density lipoproteins; ELISA, enzyme-linked immunosorbent assay; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A. The following systematic names are used for sterols and 5α-stanols referred to by trivial names: cholesterol, 5-cholesten-3β-ol; campesterol, 24-methyl-5-cholesten-3β-ol; sitosterol, 24-ethyl-5-cholesten-3β-ol; stigmasterol, 24-ethyl-5,22-cholestadien-3β-ol; avenosterol, 24-ethylidene-5-cholesten-3β-ol; cholestanol, 5α-cholestan-3β-ol; 5α-campestanol, 24-methyl-5α-cholestan-3β-ol; 5α-sitostanol, 24-ethyl-5α-cholestan-3β-ol.

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ma and tissues of affected subjects. In all sitosterolemic tissues except brain, approximately 16% of the sterols are unsaturated plant sterols, campesterol, stigmasterol, and sitosterol, and 3.5% are 5α-saturated stanols, cholestanol, 5α-campestanol, and 5α-sitostanol (8–11). The large amounts of unsaturated plant sterols arise from the diet since endogenous synthesis is nil (12,13). In distinction, 5α-saturated stanols are probably produced endogenously from the corresponding unsaturated sterols, because diets contain only small quantities of cholestanol and 5α-saturated plant sterol derivatives (10). Normally 5% or less of dietary sitosterol is absorbed from the intestine (12-15) as contrasted with about 40–60% absorption of dietary cholesterol (16,17). Apparently the ethyl substituent at C-24, which differentiates sitosterol from cholesterol, hinders intestinal absorption. However, because of structural similarity, both sterols are believed to share a common absorption pathway (18). For these reasons, plant sterol mixtures that contain predominantly sitosterol were used for many years to treat hypercholesterolemia by competitively blocking intestinal cholesterol absorption (19). Yet, despite feeding up to 7 g of sitosterol per day, plasma concentrations increased only marginally although plasma cholesterol levels declined about 22% (12).

In this report, we have compared the absorption and turnover of sitosterol with that of cholesterol in sitosterolemic and control subjects. In addition, plasma sterols, apolipoproteins A-I and B, and high density and low density lipoprotein concentrations were measured. Our objective was to seek relationships between sitosterol and cholesterol plasma concentrations, absorption, and cholesterol synthesis in these subjects.

METHODS

Clinical

Studies were conducted in four siblings in whom a diagnosis of sitosterolemia with xanthomatosis was established by clinical and biochemical criteria, and in one control subject. Complete clinical and biochemical descriptions have been presented elsewhere (1,2,10). There were three female subjects aged 29, 27, and 23 years, and one male subject who was 18 years old when he died of an acute myocardial infarction. All subjects ate regular meals and the food selections were supervised by the Metabolic Unit dietician. Weekly food diaries were reviewed and daily calorie and sterol intakes were calculated. A representative 24-h food sample was collected and the following sterol content was determined by capillary GLC: cholesterol, 529 mg/2000 calories and sitosterol 238 mg/2000 calories. Fat (30% soybean oil) made up 40% of the calories with a ratio of saturated to polyunsaturated fatty acids of 0.4. Caloric intake and weight remained constant throughout the study. The control subject for the absorption and turnover studies was the 35-year-old husband of the 29-year-old sitosterolemic subject and ate the same diet.

Blood was collected into tubes that contained solid EDTA (Becton Dickinson, Rutherford, NJ), after the subjects had fasted overnight for 12 h. Plasma was separated from erythrocytes by centrifugation at 5000 *g* for 10 min at 4°C. The research protocols were approved by the human studies committees of the University of Medicine and Dentistry of New Jersey-New Jersey Medical School, Newark, NJ and the Veterans Administration Medical Center, East Orange, NJ.

Experimental design

Plasma sterol concentrations were measured weekly for 10 weeks on fasting specimens by capillary GLC in the four affected and single control subjects. Plasma apolipoprotein A-I and apolipoprotein B concentrations were determined immunochemically on two to six separate specimens during this period. Preparative density gradient ultracentrifugation and analytical ultracentrifugation gave concentrations and density distribution profiles of low density lipoproteins and high density lipoproteins. Lipoprotein size and morphology were determined by electron microscopy.

Percent and mass absorption of exogenous cholesterol and sitosterol were measured by the plasma dual-isotope ratio method in two sitosterolemic subjects and the one control subject.

At the same time, the rates of turnover of sitosterol and cholesterol in the plasma were estimated by mathematical analysis of specific activity-time curves after simultaneous intravenous pulse-labeling with [^{3}H]sitosterol and [^{14}C]-cholesterol. Body pool sizes and turnover rates for sitosterol and cholesterol were calculated.

Cholesterol and sitosterol absorption measurements

The plasma dual-isotope ratio method originally described to study cholesterol absorption in rats by Zilversmit and Hughes (20) and modified for studies in humans by Samuel et al. (21,22), was used to measure cholesterol and, for the first time, sitosterol absorption. Radiolabeled [^{14}C]cholesterol and [^{3}H]sitosterol were injected intravenously and [^{3}H]cholesterol and [^{14}C]sitosterol were fed orally in the morning after a 14-h fasting period. Plasma was obtained 3,5,7,9, and 15 days after labeling, and cholesterol and sitosterol were isolated individually by HPLC, and their respective isotope ratios were determined. Mass absorption for each sterol was calculated from the dietary intakes of cholesterol and sitosterol (sterol content measured in a 24-h sample of food) multiplied by the mean percent absorption of cholesterol and sitosterol determined by the plasma dual-isotope ratio method.

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Sterol turnover

Cholesterol and sitosterol turnover were calculated according to the two-compartment model system (22–27). This model system was selected for sitosterol because it has been shown previously that sitosterol is not synthesized endogenously in humans, and plasma specific activities after isotopic labeling decay rapidly giving an excellent fit for two exponentials (12).

For cholesterol, other studies have shown that the decay of plasma cholesterol specific activity also can be described by two or three exponential functions. Using the three-pool model, total cholesterol pool sizes are larger than calculated from the two-pool model. However, since our goal was to compare cholesterol turnover with sitosterol turnover, we analyzed both sterol specific activity decay curves by the same two-pool model system. We recognize that quantitative estimates for cholesterol pool size might be underestimated, but turnover rates should be within 8–9% of those calculated by the three-pool model (24).

The method of calculation and the assumptions were essentially the same as in earlier studies (12,13), i.e., virtually no sitosterol is excreted from B, ($K_B = 0$), and sitosterol is not synthesized in either pool. Thus $S_B = 0$ and S_A represents the amount of sitosterol absorbed from the diet each day. For cholesterol turnover, excretion from and synthesis in pool B were assumed to be 0 ($K_B = 0$, $S_B = 0$. Thus, the calculated size of pool B for cholesterol represents a minimum as does the size of the total exchangeable body cholesterol pool.

Radiolabeled sterols

[4-^{14}C]Cholesterol (sp. act 57.5 mCi/mmol) and [1,2-^{3}H]cholesterol (sp act 45 Ci/mmol) were obtained from New England Nuclear Corp., Boston, MA. [4-^{14}C]Sitosterol (sp act 57.5 mCi/mmol) was purchased from Amersham Corporation, Arlington Heights, IL. [3α-^{3}H]Sitosterol was prepared from sitosterol by the method of Dayal et al. (28) with a final specific activity of 1 Ci/mmol. All labeled sterols were examined by HPLC (see below) prior to use, and were more than 98% pure.

One sitosterolemic subject (KCN) and one control subject received intravenously 2.8 μCi [3α-^{3}H]sitosterol and 3.0 μCi [4-^{14}C]cholesterol, and simultaneously were fed 0.8 μCi of [4-^{14}C]sitosterol and 12.0 μCi [1,2-^{3}H]cholesterol. Subject KeC (sitosterolemia) was injected intravenously with 3.2 μCi [4-^{14}C]cholesterol and 1.3 μCi [3α-^{3}H]sitosterol. Simultaneously she was fed 10.5 μCi [1,2-^{3}H]cholesterol and 3.4 μCi [4-^{14}C]sitosterol. The intravenously administered radioactive sterols were dissolved in 1.0 ml of absolute ethanol, suspended in 150 ml of physiologic saline, and infused immediately. The radiolabeled sterols given orally were dissolved in 1.0 ml of ethanol and suspended in 120 ml of milk.

Chemical studies

Plasma sterol concentrations were measured by capillary GLC (29). After saponifying 1.0-ml aliquots of plasma with 1 N NaOH at 70°C for 1 h, neutral sterols were extracted with hexane and 70 μg of 5α-cholestane was added as an internal standard. The solvent was evaporated, and TMS ether derivatives were prepared by the addition of 100 μl of Sil Prep (Analtech, Deerfield, IL). Excess pyridine was evaporated and the residue was dissolved in 100 μl of hexane; 1 μl was analyzed. Capillary GLC was carried out an a Hewlett-Packard model 5840 gas chromatograph (Hewlett-Packard, Palo Alto, CA) equipped with a flame ionization detector and fitted with an open tubular fused silica column (0.32 mm × 26 m) that was internally coated with a 0.21 μm film of CP Wax 52CB (Chrompack, Bridgewater, NJ). The following operating conditions were employed: column temperature (isothermal), 210°C; flame ionization detector, 295°C; carrier gas flow, 1.0 ml/min, and split ratio 9:1. The retention times relative to 5α-cholestane of the sterol and 5α-stanol TMS ether derivatives were: cholesterol, 1.86; cholestanol, 1.71; campesterol, 2.36; 5α-campestanol, 2.24; stigmasterol, 2.46; sitosterol, 2.86; 5α-sitostanol 2.72 and avenosterol 3.46.

Cholesterol and sitosterol were isolated by HPLC from plasma specimens to measure the ^{3}H/^{14}C ratios and their respective ^{3}H and ^{14}C specific activities. Three ml of plasma was saponified and the neutral sterols and stanols were extracted with hexane. The unsaturated sterols were separated as a group from 5α-stanols by argentation TLC (9), and the fractions were eluted individually with ethyl ether. The R_f values for unsaturated sterols and 5α-stanols were 0.35 and 0.42, respectively. The unsaturated sterol fraction was injected into a Waters Associates ALC 201 high performance liquid chromatography system (Milford, MA) that used a Model 401 refractive index detector, and a "Z" module radial compression system fitted with a 100 × 8 mm I. D. Nova-Pak C_{18} reversed-phase column (5μ particle size). The mobile phase consisted of methanol–water–chloroform 100:10:7.5 (v/v/v) and flowed at 2 ml/min. Cholesterol was eluted at 19.9 min, campesterol at 22.3 min, and sitosterol 25.3 min. A representative chromatograph revealed that cholesterol was entirely separated from sitosterol (**Fig. 1**). 5α-Cholestane was added as an internal standard to each eluted fraction; four-fifths was taken for radioactivity measurement and the remainder was quantitated as the TMS ether derivative by capillary GLC as described above.

Radioactivity assay

Measured portions of the purified sterols were dissolved in toluene phosphor (4.2% Liquiflor, New England Nuclear) and assayed for radioactivity in a Bechman Model

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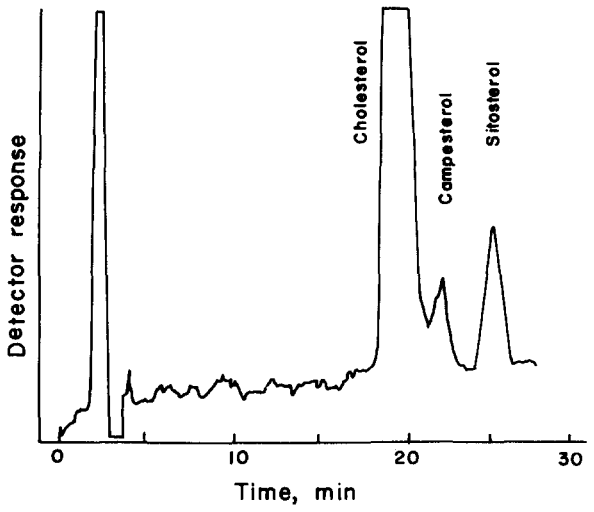

Fig. 1. Isolation of unsaturated sterols from the plasma of a sitosterolemic subject by HPLC. Although there was small overlap of campesterol with cholesterol, sitosterol was completely separated from cholesterol.

LS-250 liquid scintillation system (Beckman Instruments, Inc., Fullerton, CA). The efficiencies for counting ^{3}H and ^{14}C were 43% and 69%, respectively. The samples were counted for 10 to 100 min so that at least 100 counts above background were recorded.

Apolipoproteins and lipoproteins

Apolipoprotein B concentrations in plasma and isolated lipoproteins were quantitated by single radial immunodiffusion (29), and specific, competitive enzyme-linked immunosorbent assay (ELISA) similar to that described by Engvall (30) and Ishiguro et al. (31). The sensitivity range of the ELISA was 0.01 to 1.5 μg (in 100 μl per well) in microtiter plates. Assay conditions have been described previously (29). Concentrations of apolipoprotein A-I were determined by single radial immunodiffusion (32).

Preparative density gradient ultracentrifugation and analytical ultracentrifugation gave concentrations and density distribution profiles of low density lipoproteins and high density lipoproteins (33,34).

Total low density lipoproteins ($d < 1.063$ g/ml) and high density lipoproteins (d 1.063–1.20 g/ml) were isolated as described previously (33). These major classes were separated into 12 subfractions each by preparative density gradient ultracentrifugation in a swinging bucket rotor (34), and/or taken for analytical ultracentrifugation (33). Densities of isolated lipoprotein subfractions were determined with a Mettler/Paar calculating precision density meter (Anton Paar K. G., Graz, Austria). Peak flotation rates, corrected to zero concentration for the Johnston-Ogston effect, were determined from analytical ultracentrifugally generated schlieren patterns (33). Levels of HDL_{2b}, HDL_{2a}, and HDL_3 subfractions of high density lipoproteins were determined by computerized curve resolution of the schlieren patterns (35) and by quantitative analysis of the distribution of lipoprotein mass across the density range in the density gradient (36). Total protein (37) and total lipids, as well as apoA-I and/or apoB were determined for isolated lipoproteins. Total sterols, esterified and unesterified sterols, and triglycerides were quantitated using enzyme reagent kits (Worthington Biochemical Corp., Freehold, NJ and Boehringer Mannheim Diagnostics Inc., Houston, TX). Phospholipids were determined from the organic phosphorus content (38).

The structure and size of low and high density lipoproteins were determined by negative staining electron microscopy (39).

RESULTS

Plasma sterol and lipoprotein concentrations

Concentrations of plasma sterols and 5α-stanols are given in **Table 1**. In the sitosterolemic family, elevated levels of cholestanol, campesterol, sitosterol, and the respective 5α-saturated plant sterol derivatives remained relatively constant during the 10 weeks of observation. Although only subjects TC and RC showed increased plasma cholesterol levels, total plasma sterol concentrations were abnormally high in all four affected subjects when plant sterols and

TABLE 1. Plasma sterol[a] and 5α-stanol concentrations

Subject (Diagnosis)	Sex	Age	Cholesterol	Cholestanol	Campesterol	5α-Campesterol	Sitosterol	5α-Sitostanol
		yr			*mg/dl*			
VN(control) n = 10	M	35	187 ± 9	0.4 ± 0.2	ND	ND	0.4 ± 0.2	ND
KCN(sitosterolemia) n = 10	F	29	184 ± 25	2.1 ± 0.1	7.5 ± 1.1	1.3 ± 0.9	15 ± 2.0	2.9 ± 1.3
KeC(sitosterolemia) n = 10	F	23	202 ± 25	4.7 ± 1.0	8 ± 3.1	1.4 ± 0.2	14 ± 4.1	2.2 ± 0.7
TC(sitosterolemia) n = 10	F	27	233 ± 12	3.8 ± 1.4	10 ± 5	1.9 ± 1.0	21 ± 8.3	5.4 ± 2.5
RC(sitosterolemia) n = 10	M	18	249 ± 39	7.5 ± 2.4	13 ± 1.5	2.6 ± 0.9	20 ± 5.5	3.9 ± 1.1

Values given as mean ± SD; ND, not detected.
[a]Small amounts of stigmasterol and avenosterol were detected in the plasma of sitosterolemic family members.

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5α-saturated stanols were included in the measurements. The high plasma unsaturated plant sterol concentrations reflect the absorption of these sterols from the intestine since the food contained abundant plant sterols. However, in subjects on this diet, plasma sitosterol concentrations were 40 to 50 times lower in the control subject.

Plasma lipoprotein data in the sitosterolemic siblings are given in **Table 2**. The levels of low density and intermediate density lipoproteins in the three female and one male members were markedly elevated, as compared to previously reported analytical ultracentrifugal data (40) for age- and sex-matched control subjects. However, the distribution of low density lipoproteins in the preparative density gradient was similar to that of the controls. The major proportion of the total low density lipoproteins was isolated in the subfraction of d 1.034 g/ml in all samples of Table 2 [except the females from the Modesto study (40), whose lipoproteins were not separated in this manner]. The ratio of lipoprotein mass in adjacent subfractions (d 1.026 g/ml and d 1.044 g/ml) varied among subjects, but the sitosterolemic subjects did not differ consistently from the controls in this respect. This distribution is consistent with peak flotation rates ($S_f°$) within the normal range for all subjects (Table 2) and with a sterol-to-protein ratio of 1.47–1.71 in the major LDL subfraction of sitosterolemic subjects as compared to 1.495–1.64 for the controls. In spite of increased concentrations of plant sterols and 5α-stanols, the mean particle diameter, 25.7 ± 2.8 nm (n = 200), for sitosterolemic LDL was not unusual as determined by electron microscopy (39). The LDL were not distinguishable morphologically from normal LDL. The high density lipoprotein concentrations were below normal to normal in the sitosterolemic subjects. In RC, the distribution among HDL_{2b}, HDL_{2a}, and HDL_3 and the $F°_{1.20}$ rate were normal, but the total concentration was low; in TC, in comparison to normal young women (35,36,40), the proportion of less dense high density lipoproteins and $F°_{1.20}$ rate, as well as the concentration of total high density lipoprotein were low. Electron micrography of HDL from the male sitosterolemic subject RC showed that particles are round with a mean diameter of 8.5 ± 1.7 nm (n = 220), consistent with the predominance of small, dense HDL (39).

Plasma concentrations of apolipoproteins B and A-I for the sitosterolemic family are given in **Table 3**. Increased concentrations of apoB, in combination with low to normal levels of apoA-I were present in all sitosterolemic subjects. These apolipoprotein values reflect the increased LDL and low HDL concentrations, determined by analytical and preparative ultracentrifugation (Table 2). Apolipoprotein B concentrations from immunoassay of plasma and isolated lipoproteins were consistent with the total protein (from the modified Lowry procedure) of the isolated d < 1.063 g/ml lipoproteins from the same plasma.

TABLE 2. Plasma lipoprotein concentration, density distribution, and peak flotation rate in sitosterolemic and control subjects

Subject	Sex	VLDL	IDL + LDL	HDL	HDL_{2b}	HDL_{2a}	HDL_3
			mg/dl plasma				
RC	M	(*1*)[a] 53 (51-54)	594 (490-697)	195 (181-209)	28 (26-28)	38 (37-39)	129 (115-143)
		(*2*)[b] 38	549	219	42	80	97
		(*3*)[c]	7.42	2.23			
Controls (n = 20)	M	(*1*) 58 (26-103)	326 (241-406)	318 (288-356)	44 (22-72)	88 (61-119)	191 (156-214)
		(*2*) 44 (2-123)	323 (247-399)	338 (285-376)	34 (8-74)	127 (87-165)	160 (134-183)
		(*3*)	7.17 (6.40-8.17)	2.17 (1.95-2.60)			
KeC	F	(*1*) 85 (71-104)	432 (354-503)	295 (272-338)	50 (34-65)	88 (80-96)	158 (157-158)
KCN	F	(*1*) 90 (47-123)	574 (568-583)	294 (282-313)	76 (55-88)	73 (64-86)	146 (134-165)
		(*2*) 136	578	262	59	92	111
		(*3*)	6.97	2.02			
TC	F	(*1*) 128 (99-156)	538 (523-552)	268 (262-275)	21 (18-24)	51 (38-64)	197 (187-206)
		(*2*) 180	595	241	0	67	174
		(*3*)	6.53	1.82			
Controls	F	(*1*) 60; 77[d]	339; 240	347; 428	44; 85	103; 100	201; 243
		(*4*)[e] 47 ± 31	309 ± 64	342 ± 42	63 ± 39	138 ± 28	141 ± 26
		(*3*)	6.94 (6.21-7.85)	2.23 (1.81-2.52)			

[a](*1*): Protein and lipid mass recovered in fractions from preparative density gradient ultracentrifugation; VLDL, d < 1.0067 g/ml; IDL + LDL, d 1.0067–1.063 g/ml; HDL, d 1.063–1.20 g/ml; HDL_{2b}, d 1.063–1.100 g/ml; HDL_{2a}, d 1.100–1.125 g/ml; HDL_3, d 1.125–1.20 g/ml. Values for RC, KeC, KCN, and TC are the average (range) of three or four analyses over a 1–2 year period.

[b](*2*): From analytical ultracentrifugal analysis of one plasma sample per individual. Samples from male controls are the same as in (*1*).

[c](*3*): Peak flotation rates for LDL ($S_f°$) and HDL ($F°_{1.20}$) from analytical ultracentrifugation. Rates are expressed as Svedbergs (10^{-13} cm/sec per dyne per g) in a solution of density 1.063 g/ml at 26°C (1.748 molal NaCl). $F_{1.20}$ values are flotation rates expressed as Svedbergs in a solution of density 1.20 g/ml.

[d]Aged 18 and 28 yr, respectively.

[e](*4*): Analytical ultracentrifugation data for clinically normal females (n = 20), aged 27–34 yr, in the Modesto study (35, 40).

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TABLE 3. Plasma apolipoprotein concentrations in sitosterolemic and control subjects

Subject	ApoA-I	ApoB
	mg/dl	
RC	102	131
Controls; male, n = 6	147 (137–154)	69 (52–81)
KeC	141 (137–154)[a]	108 (99–144)[a]
TC	131 (124–135)[a]	131 (102–157)[a]
KCN	121 (108–130)[a]	110 (99–114)[a]
Controls; female,[b] n = 10	162 (148–184)	66 (52–81)

[a]Average (range) of four to five samples over a 3-yr period.
[b]Plasma from same two female subjects as in Table 2 (*1*) plus eight other age-matched female control subjects.

Cholesterol and sitosterol absorption

The absorption of cholesterol and sitosterol was measured by the plasma dual-isotope ratio method after simultaneous intravenous and oral isotope sterol dosing. The $^{3}H/^{14}C$ ratios of cholesterol and sitosterol isolated individually from the plasma were compared with the ideal isotope ratios for cholesterol and sitosterol. The ideal sterol isotope ratio represents the ratio that would have been obtained if absorption had been 100%, and is calculated by dividing the total radioactive oral dose by the total radioactive injected dose. The results (**Table 4**) show that 4% of dietary sitosterol (equivalent to 12 mg/day) was absorbed in the control subject, compared with 28% (70 mg/day) and 63% (158 mg/day) absorbed, respectively, in two sitosterolemic subjects. For cholesterol, 44% was absorbed in the control subject and 49% and 69%, respectively, in the two sitosterolemic subjects. The mean coefficient of variation was ± 5% for cholesterol absorption in the three subjects, which is in excellent agreement with previous measurements of cholesterol absorption by this method (16,22). The constancy of the $^{3}H/^{14}C$ ratio in plasma also argues against the in vivo loss of tritium from [1,2-^{3}H]cholesterol as reported by Davidson et al. (41). Also, we recognize that the plasma dual-isotope ratio method may rarely overestimate cholesterol absorption as reported in rhesus monkeys by Bhattacharyya and Eggen (42).

For percent sitosterol absorption, the mean coefficient of variation was similar to cholesterol (± 4%) in the control subject, but was higher in the two sitosterolemic subjects. The greater variability in percent sitosterol absorption in the sitosterolemic subjects might reflect delay in oral [^{14}C]sitosterol equilibrating with the larger plasma and hepatic sitosterol pools (13). Nevertheless, percent intestinal sitosterol absorption is small relative to cholesterol in the control subject, but rises to approach cholesterol in the sitosterolemic subjects. The finding is evidence for loss of intestinal recognition of the C-24 ethyl substituent on the sitosterol molecule, which is the sole structural difference between sitosterol (29 carbons) and cholesterol (27 carbons).

Sitosterol and cholesterol turnover

Figs. 2A and 2B show the normalized plasma specific activity-time curves for injected [^{3}H]sitosterol and [^{14}C]-cholesterol in the control and sitosterolemic subjects. Normalized values were computed to allow for direct comparisons of the two sterol specific activity decay curves. In the control subject, plasma [^{3}H]sitosterol specific activities decayed more rapidly than [^{14}C]cholesterol specific activities, a finding that has been noted previously in five control subjects (12). In contrast, the specific activi-

TABLE 4. Sitosterol and cholesterol absorption

Sterol	Subject (Diagnosis, Age) VN(Control, 35)	KCN(Sitosterolemia, 29)	KeC(Sitosterolemia, 23)
Cholesterol			
Intake (mg/day)	600	500	500
Absorption (%)	44	49	69
Mean coefficient of variation[a] (± %) n = 5	5	5	5
Absorption (mg/day)	264	245	345
Sitosterol			
Intake (mg/day)	300	250	250
Absorption (%)	4	63	28
Mean coefficient of variation[a] (± %) n = 5	4	23	11
Absorption (mg/day)	12	158	70

[a]Mean coefficient of variation = $\frac{SD}{mean} \times 100$.

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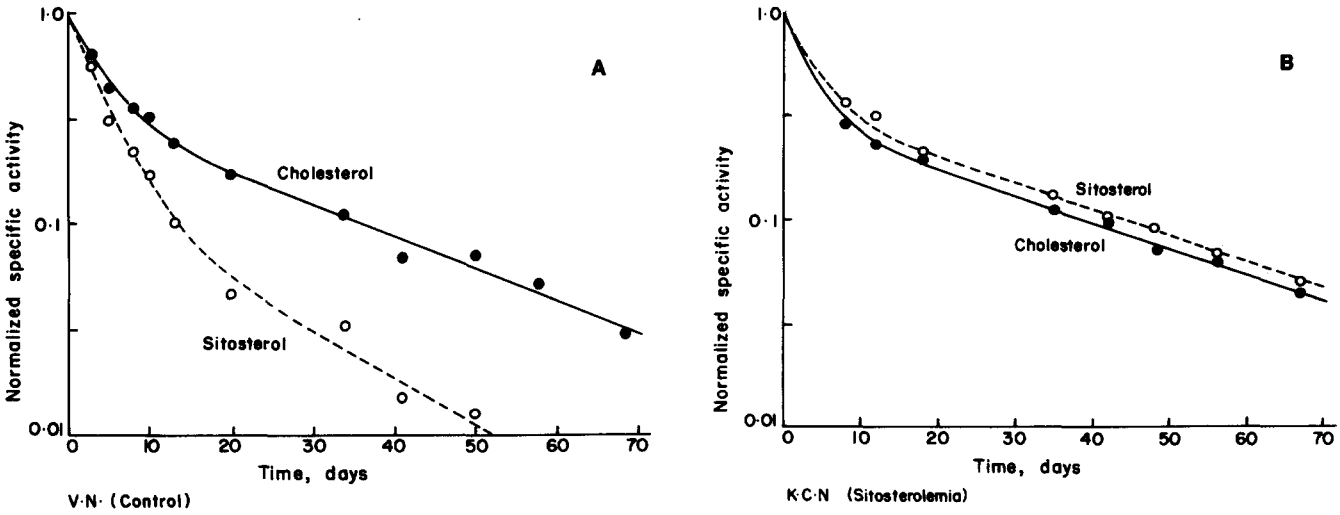

Fig. 2. A: Comparison of normalized specific activity-time curves for [^{3}H]sitosterol and [^{14}C]cholesterol in control subject after intravenous pulse-labeling. Normalized specific activities were calculated by dividing the sterol specific activity (dmp/mg) at any time point by the total radioactive dose administered multiplied by 100. [^{3}H]Sitosterol specific activities decayed more rapidly than [^{14}C]cholesterol activities. Both curves could be fitted to a two-pool model by the curve-peeling method (12, 13). B: Normalized specific activity-time curves of [^{3}H]sitosterol and [^{14}C]cholesterol in a sitosterolemic subject after intravenous pulse-labeling. The specific activity decay of [^{3}H]sitosterol was almost identical to [^{14}C]cholesterol.

ty decay of plasma [^{3}H]sitosterol was prolonged and was similar to that of [^{14}C]cholesterol in the sitosterolemic subjects. **Table 5 and Table 6** list the parameters of sitosterol and cholesterol turnover that were derived from mathematical analysis of the specific activity-time curves. In addition to the longer half-life for the second sitosterol exponential, the excretion coefficient from pool A (K_A) was slower than for cholesterol in both sitosterolemic subjects. The results indicate that plasma sitosterol and cholesterol equilibrate similarly with their respective tissue pools in the sitosterolemic subjects, and the rate of excretion of both sterols from the body was much slower than in the control subject.

Calculation of the sizes of sitosterol pools A and B in the control subject, according to the two-pool model, revealed a total exchangeable pool of 290 mg. In distinction, the total exchangeable sitosterol pool amounted to 4800 mg in sitosterolemic subject KCN and 3500 mg in KeC. These values were 17 and 13 times larger, respectively, than that observed in the control individual. The turnover rates for sitosterol were 162 mg/day and 52 mg/day in the sitosterolemic subjects compared to 18 mg/day in the control. Since sitosterol is not synthesized in mammalian tissues (12,13), the turnover rate is equivalent to absorption. Thus, on the same diet, the sitosterolemic subjects absorbed 9 and 3 times more sitosterol than the control subject.

TABLE 5. Sitosterol turnover and distribution parameters

Parameter	VN(Control)	KCN(Sitosterolemia)	KeC(Sitosterolemia)
$t_{1/2A}$, Half-life of first exponential (days)	2.8	2.7	11
$t_{1/2B}$, Half-life of second exponential (days)	14	24	112
K_A, Rate constant for excretion from pool A (days^{-1})	0.139	0.067	0.014
K_{AB}, Rate of transfer from A to B (days^{-1})	0.08	0.110	0.28
K_{BA}, Rate of transfer from B to A (days^{-1})	0.066	0.113	0.27
K_{AA}, Fractional loss from pool A (days^{-1})	−0.219	−0.177	−0.043
M_A, Size of pool A (mg)	130	2400	1700
M_B, Size of pool B (mg)	160	2400	1800
$M_A + M_B$, Total exchangeable body sitosterol pool (mg)	290	4800	3500
PR_A, Turnover (absorption) rate (mg/day)	18	162	52

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TABLE 6. Cholesterol turnover and distribution parameters

Parameter	VN(Control)	KCN(Sitosterolemia)	KeC(Sitosterolemia)
$t_{1/2A}$, Half-life of first exponential (days)	2.8	2.4	5
$t_{1/2B}$, Half-life of second exponential (days)	20	24	74
K_A, Rate constant for excretion from pool A ($days^{-1}$)	0.079	0.077	0.024
K_{AB}, Rate of transfer from A to B ($days^{-1}$)	0.097	0.130	0.076
K_{BA}, Rate of transfer from B to A ($days^{-1}$)	0.111	0.108	0.038
K_{AA}, Fractional loss from pool A ($days^{-1}$)	−0.176	−0.207	−0.110
M_A, Size of pool A (grams)	26	11	20.5
M_B, Size of pool B (grams)	23	13	40.8
$M_A + M_B$, Total exchangeable body cholesterol pool (grams)	49	24	61.3
PR_A, Turnover rate (grams/day)	2.1	0.86	0.71
Weight (kg)	100	65	62
Synthesis (mg/kg/day) = PR_A − absorption/kg	18.4	9.5	5.9

A linear relationship (**Fig. 3**) was noted when total sitosterol body pool size was plotted against daily absorption in the control subject and six other normal subjects (eight experiments) who were studied earlier (12,13). However, for the sitosterolemic subjects, the total exchangeable sitosterol pools[1] were 1.7 and 2.8 times larger, respectively, than would have been predicted based on the plotted relationship of pool size to absorption. Thus, the total sitosterol pool was expanded out of proportion though absorption was enhanced. The enlargement of the sitosterol pool beyond that expected from increased absorption alone probably resulted from diminished excretion of sitosterol from the body as reflected by the reduced rate constant (K_A) (Table 5).

For cholesterol, the sizes of both exchangeable pools (M_A and M_B) were much smaller in KCN and of similar size in KeC compared to the control subject. However, it should be emphasized that sitosterolemic subjects exhibit large xanthomas and that cholesterol in the xanthomas equilibrates very slowly with the plasma cholesterol pool (5,13). For this reason, the calculated value for the total exchangeable cholesterol pool probably underestimates the actual size of the cholesterol pool in the sitosterolemic subjects.

Cholesterol turnover rates in the sitosterolemic subjects were reduced 59% and 66%, respectively, compared to the control subject (Table 6). Since turnover consists of both absorbed plus synthesized cholesterol, daily synthesis also can be estimated by subtracting absorbed cholesterol (Table 4) from turnover (Table 6). Computed in this way, cholesterol synthesis amounted to 560 mg/day or 9.5 mg/kg per day and 365 mg/day or 5.9 mg/kg per day, respectively, in the sitosterolemic subjects, and was substantially less than 1800 mg/day or 18.4 mg/kg per day in the control subject. Thus, cholesterol synthesis was much smaller in the sitosterolemic subjects.

DISCUSSION

The results of this investigation clearly demonstrate significant differences in the metabolism of cholesterol and sitosterol in control and sitosterolemic subjects.

Sterols and lipoproteins

Because of increased amounts of plant sterols and 5α-stanols, total plasma sterol concentrations are generally elevated in sitosterolemic subjects as demonstrated in Table 1. However, plasma cholesterol levels vary and both normal and high concentrations have been reported in the same subject at different times and in other sitosterolemic subjects (2–8). Since absorbed sterols are major constituents of the plasma and tissue sterol pools, variations in diet are probably responsible for the swings in plasma sterol concentrations in the subjects. Plasma LDL and apolipoprotein B concentrations were elevated, and probably reflect the increased transport of plasma sterols and 5α-stanols. Therefore, it was noteworthy that the ratio of sterol-to-protein (apoB) in the major LDL, the density distribution of the LDL, and lipoprotein particle size and morphology were normal. The increased content of plant sterols and 5α-stanols apparently was incorporated into plasma lipoproteins without affecting structure or chemical relationships. As a consequence, the appearance of si-

[1]Exchangeable is used to denote tissue sterols that equilibrate with their plasma radioactive sterol pool. Thus, the exchangeable pool comprised kinetic pools A(M_A) and B(M_B). The tissues that comprise pool A are plasma, liver, intestine, and erythrocytes, while pool B tissues consist mostly of muscle and adipose tissue. Brain and nervous system sterols do not equilibrate with the plasma sterol compartment and do not contribute to the mass of kinetic pools A and B. Xanthoma sterols equilibrate very slowly with plasma sterols (13) and their mass is only partially included in the calculation of the exchangeable sterol pool.

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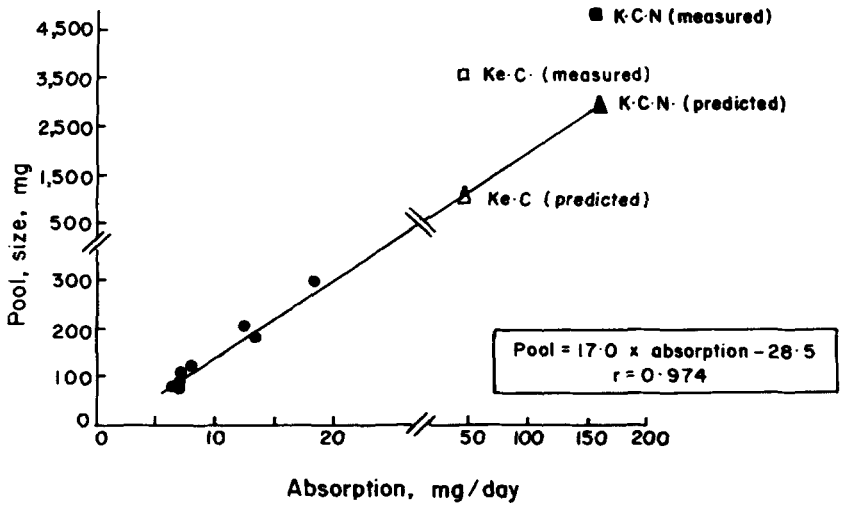

Fig. 3. Relationship between total body exchangeable[1] sitosterol pools (mg) and the daily absorption (mg/day) of sitosterol. This relationship was linear in seven control subjects (eight studies) including VN in this study. However, in sitosterolemic subjects, KCN and KeC, the calculated sitosterol pools were substantially larger than the predicted pool sizes.

tosterolemic LDL was not distinguishable from control LDL. Even higher concentrations of apolipoprotein B were reported in sitosterolemic subjects by Kwiterovich et al. (4). Thus, atherosclerosis and xanthoma formation in sitosterolemia probably relate to elevated concentrations of normal LDL and apolipoprotein B. In contrast, HDL and apolipoprotein A-I concentrations tended to be low in the sitosterolemic subjects.

Sitosterol absorption

Although enhanced sitosterol absorption has been reported in sitosterolemic subjects (3,6–8), the sterol-balance method (dietary sitosterol – fecal sitosterol = absorption) used for these determinations has been shown to be inaccurate and overestimates sitosterol absorption in control subjects (12). Also, the earlier sitosterol absorption studies did not report absorption measurements in control subjects. Therefore, in this investigation, sitosterol absorption was assessed by two independent methods in both control and sitosterolemic subjects. The plasma dual-isotope ratio method compares the ratio of fed to injected labeled sitosterol in the plasma, with the ideal radioactive ratio that would be obtained when 100% of the fed dose was absorbed. In the control subject, 4% or 12 mg/day of dietary sitosterol was absorbed, compared with 63% and 28% or 158 mg/day and 70 mg/day, respectively, in two sitosterolemic subjects. The second method is based on the fact that sitosterol is not synthesized endogenously in humans including sitosterolemic subjects (12,13). Therefore, the turnover rate as calculated by mathematical analysis of sitosterol specific activity-time curves is equivalent to daily absorption and has been validated in five subjects in an earlier study (12). In the control subject, 18 mg/day (6%) of dietary sitosterol was absorbed compared with 162 mg/day (65%) and 52 mg/day (21%), respectively, in the sitosterolemic subjects. The agreement of sitosterol absorption measurements by these two independent methods, in both control and sitosterolemic subjects, lends confidence to their validity. More important, the results indicate that percent sitosterol absorption approaches that of cholesterol in the sitosterolemic subjects, but in controls, cholesterol is absorbed 10 times more efficiently than sitosterol. Although solubility of sitosterol in intestinal micelles (43,44), intestinal sitosterol ester formation (45), or sitosterol enterocyte transport (46) have been suggested as factors limiting absorption, the enhancement of these mechanisms seems unlikely to explain the increased absorption of sitosterol in sitosterolemic subjects.

Cholesterol absorption

An important new observation in this study is that percent cholesterol absorption was increased in the sitosterolemic subjects. Thus, sitosterol and other structurally similar sterols, i.e., shellfish sterols (14), are absorbed more efficiently along with more cholesterol in sitosterolemic subjects.

It was also noteworthy that enhanced sitosterol absorption did not interfere with cholesterol absorption in these subjects. This finding argues against differences in micellar sterol solubility as the determining mechanism for sterol absorption, since it would be unlikely that sitosterol would be 10 times more soluble in sitosterolemic than in control intestinal micelles.

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Sitosterol and cholesterol turnover

We have demonstrated again that the turnover of labeled sitosterol is more rapid than cholesterol in the control subject and is similar to results published earlier for six control subjects (12,13). Two mechanisms are responsible: the liver secretes sitosterol more rapidly than cholesterol in the bile, and the intestine absorbs sitosterol much less well than cholesterol. Thus, as compared with cholesterol, injected isotopic sitosterol is preferentially secreted into the bile and poorly reabsorbed from the intestine, which explains its speedy removal from the body and rapid plasma decay in control subjects (Fig. 2A). In contrast, the specific activity decay of plasma sitosterol was almost identical to that of plasma cholesterol and both sterols were retained longer in the two sitosterolemic subjects (Fig. 2B). This indicates that the liver, like the intestine, fails to differentiate sitosterol from cholesterol, and hence sitosterol is excreted at about the same rate as cholesterol into the bile. Bhattacharyya and Connor (6), Miettinen (3) and Lin et al. (13) have all reported previously that plant sterols (sitosterol) were not preferentially secreted into the bile of sitosterolemic subjects as they were in normal subjects. Also bile from sitosterolemic subjects was undersaturated with cholesterol (3,13). Therefore, hepatic sterol secretion appears to be diminished in this condition.

The total sitosterol pool calculated by mathematical analysis of specific activity decay curves was 13 and 17 times larger in the two sitosterolemic subjects than in control subjects. Although a linear relationship exists between absorption and pool size in the control subjects, the calculated total pool size was almost two to four times larger than the predicted values in the sitosterolemic subjects (Fig. 3). Since the rate constant, K_A, which governs the removal of sitosterol from pool A, is reduced, the larger than expected size of the sitosterol pool can be explained by decreased hepatic secretion.

An important new biochemical mechanism discovered in the sitosterolemic subjects is reduced cholesterol turnover. Isotope kinetic calculations revealed that cholesterol turnover was 59% and 66% smaller in our two patients (Table 6), and about 50% lower in the patient studied by Lin et al. (13), than similarly fed control subjects. Moreover, since cholesterol absorption was greater in the sitosterolemic subjects than control (Table 4), the difference in daily turnover must result from decreased cholesterol synthesis (Table 6). In support, Miettinen (3) found cholesterol synthesis 50% and 80% lower in a sitosterolemic subject than similarly fed control subjects when studied by the sterol balance method 4 years apart. Further, we have recently demonstrated significantly reduced cholesterol synthesis associated with a deficiency of total microsomal HMG-CoA reductase activity in freshly isolated mononuclear leukocytes from sitosterolemic subjects (L. Nguyen, G. Salen, S. Shefer, G. Ness and V. Shore, unpublished observations). The latter finding points to an abnormality in the rate-controlling enzyme for cholesterol biosynthesis as responsible for decreased cholesterol synthesis in this disease.

Biochemical defect

We postulate that the principal abnormality in sitosterolemia with xanthomatosis is reduced cholesterol synthesis which presumably is inherited. Although cholesterol absorption may increase slightly, cholesterol input cannot meet tissue needs and tissue cholesterol concentrations decline (2). As a consequence, the following compensatory changes may result. *1*) Intestinal sterol structure discrimination is lost so that plant sterol (sitosterol) absorption rises. Because of their abundance in the diet, more plant sterols are absorbed and become available to substitute for cholesterol and expand tissue sterol pools. *2*) Hepatic sterol secretion decreases which helps to conserve sterols in the body. Plant sterols are no longer preferentially excreted into the bile and biliary cholesterol concentrations are reduced (3,6,13). *3*) Plasma sterol and LDL concentrations increase and, coupled with low HDL concentrations (Tables 1 and 2), favor tissue sterol deposition.

In some tissues, the cholesterol-sitosterol combination does not produce obvious functional or morphologic impairment, as noted by microscopic examination of liver, lung, muscle (3), and lipoprotein ultrastructure. However sitosterolemic erythrocytes are more prone to destruction, and Wang et al. (5) have reported that hemolytic episodes occurred in a sitosterolemic subject. Also Shefer et al. (47) found that sitosterol competitively blocks hepatic cholesterol 7α-hydroxylase, the rate-determining enzyme in bile acid synthesis (47) which may allow the diversion of cholesterol and plant sterols into their respective 5α-saturated products (10). Further, sitosterolemic monocytes take up and degrade LDL more actively than control monocytes (48). Apparently, sitosterolemic mononuclear cells express increased numbers of LDL receptors, either because plant sterols and 5α-stanols do not inhibit receptor formation, or because the cells need cholesterol since synthesis is reduced.

With regard to treatment, cholestyramine, a drug that binds intestinal bile acids and interrupts the enterohepatic circulation, lowers plasma sterol concentrations greater than 25% (3,8,10,14) in sitosterolemic subjects. When added to low cholesterol and plant sterol diets this combination appears to be the most effective therapeutic choice. In contrast, lovastatin, an HMG-CoA reductase inhibitor would not appear to be as beneficial in sitosterolemia if cholesterol synthesis is already suppressed.

Finally, we recognize that our results may be interpreted differently. We have considered the alternative possibility that increased intestinal sitosterol absorption can result from an unknown mechanism. Thus, increased plant sterol ab-

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sorption may lead directly to tissue accumulation and possibly induce feedback inhibition of cholesterol synthesis. The reduction in cholesterol synthesis in sitosterolemic subjects then could result from the accumulated plant sterols and 5α-stanols. However, Shefer et al. (49) reported that cholestanol, the 5α-dihydro derivative of cholesterol, actually increased hepatic cholesterol synthesis and HMG-CoA reductase activity when fed to rats. Moreover, Brown and Goldstein (50) found that sitosterol was much less inhibitory than cholesterol in suppressing cholesterol synthesis (HMG-CoA reductase activity) in fibroblasts. Lastly, Boberg, Åkerlund and Björkhem (51) noted that sitosterol administered intravenously to rats accumulated in the liver, but failed to suppress HMG-CoA reductase activity. Therefore, structurally similar sterols are apparently less potent regulators of cholesterol synthesis than cholesterol.

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