Hyperabsorption and retention of campestanol in a sitosterolemic homozygote: comparison with her mother and three control subjects

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Abstract We measured the percent absorption, turnover, and distribution of campestanol (24-methyl-5α-cholestan-3β-ol) in a sitosterolemic homozygote, her obligate heterozygous mother, and three healthy human control subjects. For reasons relating to sterol hyperabsorption, the homozygote consumed a diet low in plant sterols that contained campestanol at about 2 mg/day. The heterozygote and three control subjects were fed a diet supplemented with a spread that contained campestanol at 540 mg/day and sitostanol (24-ethyl-5α-cholestan-3β-ol) at 1.9 g/day as fatty acid esters. Plasma campestanol concentrations determined by capillary gas-liquid chromatography were 0.72 ± 0.03 mg/dl in the homozygote, 0.09 ± 0.04 mg/dl in the heterozygote, and 0.05 ± 0.03 mg/dl for the control mean. After simultaneous pulse labeling with [3α-^{3}H]campestanol intravenously and [23-^{14}C]campestanol orally, the maximum percent absorption measured by the plasma dual-isotope ratio method as a single time point was 80% in the homozygote, 14.3% in the heterozygote, and 5.5 ± 4.3% as the mean for three control subjects. Turnover (pool size) values estimated by mathematical analysis of the specific activity versus time [3α-^{3}H]campestanol decay curves were as follows: 261 mg in the homozygote, 27.3 mg in the heterozygote, and 12.8 ± 7.6 mg in the three control subjects (homogygote vs. controls, $P < 0.001$). The calculated production rate (mg/24 h) equivalent to actual absorption in the presence of dietary sterols and stanols was 0.67 mg/day or 31% of intake in the homozygote, 2.1 mg/day or 0.3% of intake in the heterozygote, and 0.7 ± 0.3 mg/day or 0.1% of intake in the three control subjects. However, the excretion constant from pool A (K_A) was prolonged markedly in the homozygote, but was 100 times more rapid in the heterozygote and three control subjects. Thus, campestanol, like other noncholesterol sterols, is hyperabsorbed and retained in sitosterolemic homozygotes. However, campestanol absorption was only slightly increased in the sitosterolemic heterozygote and removal was as rapid as in control subjects.—Salen, G., G. Xu, G. S. Tint, A. K. Batta, and S. Shefer. **Hyperabsorption and retention of campestanol in a sitosterolemic homozygote: comparison with her mother and three control subjects.** ***J. Lipid Res.*** **2000.** 41: **1883–1889.**

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Plant sterols are widely distributed in vegetables present in the diets that are consumed daily by most humans. The most common dietary plant sterols are 24-methyl-5-cholesten-3β-ol (campesterol) and 24-ethyl-5-cholesten-3β-ol (sitosterol), the respective 24-methyl and 24-ethyl derivatives of 5-cholesten-3β-ol (cholesterol) (**Fig. 1**). Between 200 and 300 mg/day are ingested in the diet each day. However, because plant sterols contain extra methyl and ethyl substituents at the C-24 position of the cholesterol side chain, absorption is limited, hepatic removal is rapid, and plasma concentrations in most humans are low (1–3).

The 5α-dihydro derivatives of the most common plant sterols have been prepared by catalytic hydrogenation of the respective Δ^5-unsaturated precursors, campesterol and sitosterol (Fig. 1). These 5α-stanol derivatives are more resistant to autooxidation and when esterified with fatty acids and incorporated into foods such as margarine and mayonnaise, allow the released free stanols easier entry into the micellar phase of intestinal contents after enzymatic hydrolysis (4). Miettinen, Puska, and Gylling (4) fed stanol fatty esters at 1.8 to 2.6 g/day to mildly hypercholesterolemic subjects, and found that plasma cholesterol concentrations were reduced 10–15%.

Depending on the vegetable oil source, between 10% and 30% of the stanols in the hydrogenated mixture is campestanol and 70% to 90% is 24-ethyl-5α-cholestan-3β-ol (sitostanol). Although intestinal absorption of sitostanol is almost nil (5–7), little information is available about the absorption of campestanol in humans. Some years ago, Lees et al. reported that campesterol, the Δ^5-unsaturated precursor, was much better absorbed than sitosterol and reached substantial plasma levels of about 20

Abbreviations: ACAT, acyl-coenzyme A:cholesterol acyltransferase; campestanol, 24-methyl-5α-cholestan-3β-ol; campesterol, 24-methyl-5-cholesten-3β-ol; cholesterol, 5-cholesten-3β-ol; coprostanol, 5β-cholestan-3β-ol; GLC, gas-liquid chromatography; HMG, 3-hydroxy-3-methylglutaryl; sitostanol, 24-ethyl-5α-cholestan-3β-ol; sitosterol, 24-ethyl-5-cholesten-3β-ol.

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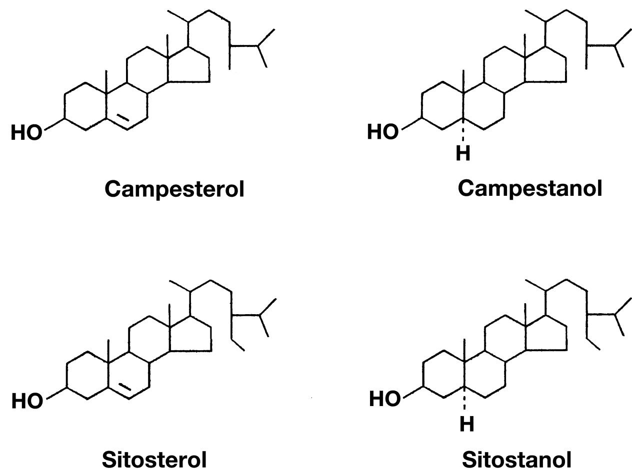

Fig. 1. Structures of the two most common dietary plant sterols and their respective 5α-dihydro derivatives present in a stanol fatty acid ester mixture. Campestanol has a methyl substitutent at C-24 and sitosterol has an ethyl group at C-24 in the side chain.

mg/dl in patients fed a plant sterol mixture derived from soybean oil that contained 30% campesterol and 70% sitosterol (8, 9).

Sitosterolemia is a rare, recessively inherited lipid storage disease that was first described by Bhattacharyya and Connor in 1974 (10). The clinical phenotype includes tuberous and tendon xanthomas, accelerated arteriosclerosis, hemolytic episodes, thrombocytopenia, arthritis, and arthralgias and is associated with elevated plasma and tissue plant sterol and stanol concentrations (11). Obligate heterozygotes appear clinically normal and do not show elevated tissue or plasma plant sterol and stanol concentrations (12). At least two mechanisms seem responsible for the biochemical findings in homozygotes: plant sterols and their 5α-dihydro derivatives are hyperabsorbed from the intestine, coupled with hepatic secretion that is markedly depressed (13–15). Another important finding is that the cholesterol biosynthetic pathway, including rate-controlling and other key enzyme activities (i.e., 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase, HMG-CoA synthase, acetoacetyl thiolase, squalene synthase, and 7-dehydrocholesterol Δ^7-reductase), is inhibited and discordantly downregulated with increased expression of low density lipoprotein receptors in liver and monocytes (16, 17). As a result, monocyte sterol and stanol concentrations including cholesterol, plant sterols, and their respective 5α-dihydro derivatives are increased such that these cells resemble foam cells (18, 19). However, reduced cholesterol biosynthesis could not be related to specific defects in the genes encoding key enzymes of the cholesterol biosynthesis pathway (20). Nevertheless, the abnormal gene involved was mapped to chromosome 2p21 (21).

In this article, we measured the absorption, distribution, and turnover of 24-methyl-5α-cholestan-3β-ol (campestanol), in a sitosterolemic homozygote, her heterozygous mother, and three unrelated adult subjects who served as controls. Because of the risks of sterol hyperabsorption and excessive tissue accumulation, the homozygote was fed a diet low in plant sterols and stanols, whereas the heterozygote and controls consumed a spread enriched with campestanol (540 mg/day) and sitostanol (1.9 g/day). In contrast to the three controls and heterozygote, who absorbed little campestanol, and excreted what was absorbed rapidly, substantial absorption and retention of campestanol occurred in the homozygote.

MATERIALS AND METHODS

Materials

Cholesterol, 5β-cholestan-3β-ol (coprostanol), and 5α-cholestane were purchased from Sigma (St. Louis, MO) and used as standards for the measurement of sterols and stanols by capillary gas-liquid chromatography (GLC).

Clinical

Studies were conducted in a 41-year-old female sitosterolemic homozygote who underwent ileal bypass surgery in 1986 to halt accelerated atherosclerosis and has since noted the disappearance of tuberous xanthomas, aortic stenosis murmur, and symptomatic angina pectoris. Prior to surgery, her 17-year-old brother, who was similarly affected, died of an acute myocardial infarction with extensive coronary artery atherosclerosis. Detailed descriptions have been presented elsewhere (11, 12). Her 62-year-old mother is an obligate sitosterolemic heterozygote without clinical atherosclerosis (13). Three healthy male volunteers, aged 38, 45, and 50 years, also participated.

Experimental

The sitosterolemic homozygote consumed a low sterol diet because of concerns about hyperabsorption and excessive tissue retention. The diet contained the following (amounts are approximate): cholesterol (225 mg/day), sitosterol (150 mg/day), campesterol (20 mg/day), sitostanol (20 mg/day), and campestanol (2 mg/day). To obtain these measurements, a 24-h pool of food was homogenized and an aliquot was analyzed by GLC. The sitosterolemic heterozygote and three control subjects ate regular food supplemented with spread that contained campestanol (540 mg/day) and sitostanol (1.9 g/day) as fatty acid esters for 4 weeks. The stanol fatty acid ester-enriched spread was formulated by the Benecol Division (Raisio Group, Raisio, Finland). No untoward side effects were observed. After 1 week on the diets, each subject received simultaneously [3α-^{3}H]campestanol intravenously and [23-^{14}C]campestanol orally suspended in milk. The percent absorption of exogenous [^{14}C]campestanol was measured by the plasma dual-isotope ratio method.

After the pulse of intravenously injected [3α-^{3}H]campestanol, specific activity-versus-time curves were plotted and turnover rates were estimated by mathematical analysis of the radioactive decay curves. Body pool sizes, production rates, and kinetic parameters for campestanol were also calculated. The research protocol was approved by the Human Studies Committee at the University of Medicine and Dentistry of New Jersey (UMDNJ)-New Jersey Medical School (Newark, NJ) with the restriction that the sitosterolemic homozygote consume a low sterol diet. Signed informed consent was obtained from each participating subject.

Campestanol absorption

The plasma dual-isotope ratio method, originally described by Zilversmit and Hughes (22) for the study of cholesterol absorption in rats and modified by Samuel, Crouse, and Ahrens (23) for studies of humans and by Salen et al. (12, 13) for studies of sitoster-

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olemic homozygotes and heterozygotes, was used to measure campestanol absorption. This method was applied to measure campestanol absorption in rabbits (24). [3α-^{3}H]campestanol was injected intravenously and [23-^{14}C]campestanol was fed in the morning after a 12-h fasting period. Plasma was obtained 2, 5, and 7 days after oral and intravenous pulse labeling, and the campestanol ^{14}C/^{3}H ratios were determined in the plasma postlabeling samples. The percent absorption was calculated by dividing the mean plasma ^{14}C/^{3}H ratio for the 3 days by the ideal ratio of the total administered ^{14}C and ^{3}H multiplied by 100. The ideal ratio is the theoretical ratio that would be found if absorption of the tracer dose of [^{14}C]campestanol was 100%. It was recognized that because the oral dose of [23-^{14}C]campestanol was administered after a 12-h fast, absorption occurred in the absence of competing intestinal dietary sterols and stanols, and would be considered maximum.

Turnover

Campestanol turnover was calculated according to the two-compartment model system (1, 12–15, 24). This model system was selected for campestanol because plant sterols and stanols are not synthesized endogenously, plasma-specific activities decay rapidly after isotopic pulse labeling, and the resulting plasma-specific activity-versus-time curves usually provide an excellent fit for two exponentials. The plotted specific activity-versus-time curves were analyzed with PK Analyst software (MicroMath, Salt Lake City, UT).

The methods of calculation and assumptions for pharmacokinetic data analysis were essentially the same as used for campestanol turnover reported in prior studies, that is, virtually no campestanol is excreted from pool B (K_B = zero), and campestanol originates solely from the diet and is not synthesized endogenously in either pool A or pool B (24). Thus, S_B = zero, and S_A represents the amount of campestanol absorbed from the diet each day.

Radiolabeled campestanol

[23-^{14}C]campestanol (specific activity, 50 μCi/μmol) was custom synthesized by Amersham Life Science (Arlington Heights, IL) and provided by the Benecol Division (Raisio Group). [3α-^{3}H]campestanol was prepared from a sample of greater than 99% pure campestanol (Benecol Division, Raisio Group) by the method of Dayal, Salen, and Tint (25), with a final specific activity of 91 μCi/μmol. The labeled campestanol was examined by silica gel G thin-layer chromatography [developing solvent, chloroform–acetone 97:3 (v/v), R_f = 0.35] and was approximately 98% pure. Each subject received 0.5 μCi of [23-^{14}C]-campestanol orally and 2.0 μCi of [3α-^{3}H]campestanol intravenously. The orally administered [23-^{14}C]campestanol was dissolved in 0.5 ml of absolute ethanol suspended in 10 ml of milk, and the intravenously administered [3α-^{3}H]campestanol was dissolved in 100 μl of absolute ethanol suspended in 50 ml of physiologic saline and injected via a 22-gauge butterfly needle infusion set into an antecubital vein.

Chemical studies

Plasma dietary sterol concentrations were measured by GLC. After saponification of 0.5 ml of plasma or 2 ml of homogenized diet in 1 N NaOH at 70°C for 1 h, neutral sterols and stanols were extracted with hexane. Seventy micrograms of 5α-cholestane and 70 μg of coprostanol were added prior to extraction as internal standards. The solvent was evaporated, and trimethylsilyl (TMS) ether derivatives were prepared by the addition of 50 μl of Sil Prep (Analtech, Deerfield, IL). After being allowed to stand for 30 min, pyridine was evaporated and the residue was dissolved in 50 μl of hexane; 1 μl was analyzed by GLC in the splitless mode. Capillary GLC was performed on a Hewlett-Packard (Palo Alto, CA) model 5840 gas chromatograph equipped with a flame ionization detector and fitted with an open tubular fused silica column (0.32 mm × 26 m) internally coated with a 0.21-μm film of CP Wax 52 CB (Chrompack, Bridgewater, NJ). Operating conditions were as follows: column temperature (isothermal) 210°C, flame ionization detector 295°C, and helium carrier gas flow 1.0 ml/min. The retention times relative to 5α-cholestane for TMS ether derivatives were as follows: cholesterol 1.86, campesterol 2.36, campestanol 2.24, stigmasterol 2.46, sitosterol 2.86, sitostanol 2.72, and avenosterol 3.46.

Radioactive campestanol (^{3}H and ^{14}C) was determined in 0.5-ml specimens of plasma. After saponification, hexane extractions, and solvent evaporation, the neutral sterol-stanol fraction was redissolved in 5.0 ml of ethyl acetate. Four-fifths were taken for radioactivity measurements dissolved in Eculum (New England Nuclear, Boston, MA). Radioactivity was assayed in a Beckman (Fullerton, CA) LS-6500 liquid scintillation system. The efficiency for counting ^{3}H and ^{14}C was 43% and 69%, respectively. The samples were counted for 10 to 100 min so that at least 100 counts above background were recorded.

Statistics

Data are reported as means ± SD. The statistical significance of differences between the groups was estimated by Student's *t*-test (unpaired), and significance was accepted at a P level less than 0.05.

RESULTS

In **Table 1** are presented plasma sterol and stanol concentrations for the five study subjects. As expected, plasma from the sitosterolemic homozygote contained large amounts of Δ^5-unsaturated plant sterols campesterol and sitosterol, and their respective 5α-dihydro derivatives campestanol and sitostanol. In particular, levels of campestanol and other plant sterols in the homozygote were about 10 to 40 times higher than the mean value (0.05 ± 0.03 mg/dl) for the three controls and her heterozygous mother. This difference was especially noteworthy,

TABLE 1. Plasma sterol and stanol concentrations[a]

Patient	Cholesterol	Campestanol	Campesterol	Sitostanol	Sitosterol
			mg/dl ± SD		
Homozygote	118 ± 10	0.72 ± 0.06	6.67 ± 0.74	5.45 ± 0.55	18.5 ± 2.0
Heterozygote	203 ± 10	0.09 ± 0.04	0.76 ± 0.11	0.19 ± 0.05	0.67 ± 0.13
Control 1	208 ± 11	0.07 ± 0.04	0.44 ± 0.05	0.14 ± 0.02	0.46 ± 0.13
Control 2	148 ± 10	0.06 ± 0.06	0.60 ± 0.37	0.12 ± 0.37	0.69 ± 0.37
Control 3	188 ± 14	0.02 ± 0.01	0.27 ± 0.16	0.005 ± 0.002	0.03 ± 0.02

[a] Mean of three determinations, using plasma specimens obtained 2, 5, and 7 days after isotopic pulse labeling.

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TABLE 2. Percent absorption of campestanol

Patient	$^{14}C/^{3}H \pm SD$[a] (n = 3)	Coefficient of Variation[b]	Ideal $^{14}C/^{3}H$ Ratio	Maximal Percent Absorption
		%		%
Homozygote	0.20 ± 0.08	40	0.25	80
Heterozygote	0.035 ± 0.002	5.7	0.25	14.3
Control 1	0.018 ± 0.006	33	0.25	7.4
Control 2	0.014 ± 0.004	29	0.25	5.8
Control 3	0.009 ± 0.003	33	0.25	3.5

[a] Mean $^{14}C/^{3}H$ ratio from three plasma samples obtained 2, 5, and 7 days after isotopic pulse labeling with [23-^{14}C]campestanol and [3α-^{3}H]campestanol.

[b] SD divided by mean, multiplied by 100.

as the control and heterozygous individuals consumed a diet enriched with campestanol (540 mg/day) and sitostanol (1.9 g/day) for 4 weeks. Thus, the trace plasma concentrations of Δ^5-unsaturated plant sterols and their 5α-dihydro derivatives were indicative of markedly reduced plant sterol and stanol absorption despite the large dietary intakes. Moreover, the sitosterolemic homozygote had undergone ileal bypass surgery 10 years earlier in an effort to halt the progression of atherosclerosis. Cholesterol concentrations had declined substantially (12), but plant sterols and their respective 5α-stanol derivative levels, although reduced, still remained elevated.

In **Table 2** are listed the mean $^{14}C/^{3}H$ ratios found in plasma, after simultaneous pulse labeling with [23-^{14}C] campestanol given orally and [3α-^{3}H]campestanol administered intravenously. This fraction represents the mean of three ratio determinations for blood obtained from each subject 2, 5, and 7 days after simultaneous oral and intravenous pulse labeling. Significantly higher $^{14}C/^{3}H$ ratios were observed in the sitosterolemic homozygote than for her heterozygous mother or for the three control subjects. The $^{14}C/^{3}H$ ratios were then converted to percent absorption by dividing the mean plasma value by the ideal isotope ratio. The calculated percent absorption was 80% in the homozygote, 14.3% in her mother, and 5.6 ± 4.3% as the mean for the three control subjects. However, it is important to recognize that the [^{14}C]- and [^{3}H]campestanol were administered in the morning at 8 AM after the subjects had not eaten for 12 h, such that absorption of the tracer dose of [23-^{14}C]campestanol was measured in the absence of competing intestinal dietary sterols and stanols. Thus, percent absorption represents the maximum intestinal absorption.

Figure 2 illustrates the decay curves of [^{3}H]campestanol specific activity versus time in the sitosterolemic homozygote, heterozygote, and a control subject. The specific activity of [^{3}H]campestanol decayed rapidly in the control and heterozygous subjects, especially over the initial 7 days after pulse labeling, and reflected fast turnover in pool A such that virtually all radioactivity was eliminated from plasma. In contrast, the initial campestanol specific activities in the homozygote were almost 1/100 of controls and the decay curve for [^{3}H]campestanol was almost flat.

In **Table 3** are presented values for campestanol turnover derived by mathematical analysis of the specific activity decay curve of [3α-^{3}H]campestanol after intravenous pulse labeling. In the sitosterolemic homozygote, heterozygote, and two of the three control subjects, the resulting campestanol specific activity-versus-time decay curves could be divided into two exponentials by the curve peeling technique and analyzed according to equations used previously for the estimation of turnover of cholesterol and sitosterol. In one control subject (control 3), the curve for the specific activity decay of [^{3}H] campestanol best fit a single-pool model and was analyzed accordingly.

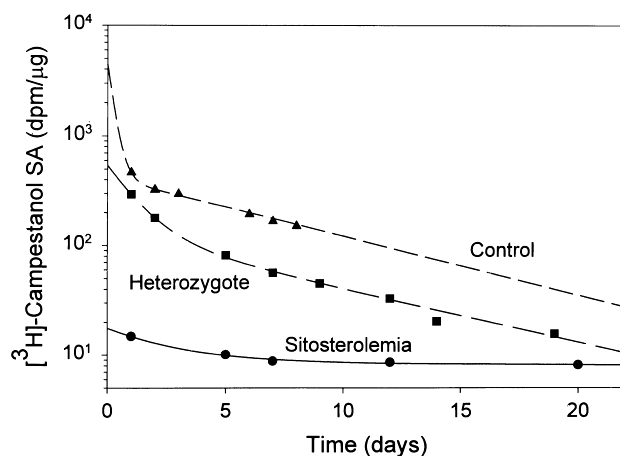

Fig. 2. Specific activity versus time curves. After [3α-^{3}H]-campestanol was administered intravenously, the specific activity decayed rapidly in control subjects and the sitosterolemic heterozygote, but was much slower in the homozygote.

As expected, the half-lives of [3α-^{3}H]campestanol were markedly prolonged in the fast (M_A) and slow (M_B) turning over compartments in the homozygote, but more rapid and comparable in the heterozygote and two controls.

The production rate (PR_A), which is the average 24-h input of campestanol into pool A, varied from 0.61 mg/day in the sitosterolemic homozygote, who consumed a diet that contained campestanol at about 2 mg/day, to 2.1 mg/day in the heterozygote, and 1.4, 0.5, and 0.2 mg/day, respectively, in the three control subjects fed the diet enriched with campestanol at 540 mg/day. Because campestanol is not synthesized endogenously, the production rate is equivalent to the actual absorption averaged over 24 h and represented 31% of intake (2 mg/day) in the homozygote, 0.3% of intake (540 mg/day) in the heterozygote, and 0.1% of the intake (540 mg/day) in the three control subjects.

As expected, the campestanol pool size was substantially larger in the sitosterolemic homozygote (261 mg) than the mean for the three controls (9.4 ± 7.6 mg) ($P < 0.001$). However, the campestanol pool size in the sitosterolemic heterozygote was not significantly different (27.3 mg) from the mean value for the three controls.

The removal constant K_A, which governs the elimination of campestanol from the body through pool A, was nearly 100 times more rapid in the three control subjects than in the homozygote. This fact indicated that the large campestanol pool in the sitosterolemic homozygote also

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TABLE 3. Distribution and physical constants for [3α-^{3}H]campestanol[a]

	Patients				
	Homozygote	Heterozygote	Control 1	Control 2	Control 3
$t_A^{1/2}$ (days)	1.9	0.8	0.8	0.83	3
$t_B^{1/2}$ (days)	290.0	5.6	5.7	6.2	—
PR_A (mg/day)	0.61	2.1	1.4	0.5	0.2
M_A (mg)[b]	125	4.8	4.1	0.5	4.2
M_B (mg)[b]	136	22.5	14.7	4.7	—
$M_A + M_B$ (mg)	261	27.3	18.8	5.2	4.2
K_A (day $^{-1}$	−0.0049	−0.43	−0.34	−0.99	−0.05
K_{AB} (day $^{-1}$)	0.19	1.18	1.00	4.38	—
K_{BA} (day $^{-1}$)	0.18	0.25	0.27	0.46	—

[a] Specific activity = A exp(−at) + B exp(−bt). The input into pool A (M_A) equals absorption, PR_A (mg/day), because there is no endogenous synthesis of campestanol. K_A is the removal constant from pool A, and K_{AB} and K_{BA} are the equilibrium constants between the two pools. Assume no synthesis in pool B (M_B) and the uptake or excretion of campestanol from pool B except through pool A.

[b] Pool A (M_A) is composed of campestanol in plasma, liver, erythrocytes, and intestine, where equilibrium between tissues is rapid as compared with pool B (M_B) made up of campestanol in skeletal muscle with slower equilibrium.

resulted from delayed excretion or retention of campestanol. In contrast, removal constant K_A was as rapid in the heterozygote as the mean value for the three control subjects. Thus, diminished absorption and rapid removal accounted for small body pools ($M_A + M_B$) of campestanol in the three controls. Further, despite greater absorption of campestanol in the sitosterolemic heterozygote, rapid removal prevented enlargement of the campestanol pool.

DISCUSSION

The results of this investigation demonstrate that campestanol, like other plant sterols (sitosterol) and shellfish sterols, is hyperabsorbed from the intestine of sitosterolemic homozygotes, but is poorly absorbed from the intestine of normal individuals. In comparison, the sitosterolemic homzoygote absorbed 14.6 times more campestanol than the mean value for three control subjects and 5.6 times more campestanol than her mother, who is an obligate heterozygote for this condition. Moreover, the excess accumulation of campestanol in the body of the homozygote as evidenced by the elevated plasma concentration (Table 1) and increased body pool (Table 3) also reflected the slow elimination of campestanol from the body. The removal constant, K_A, which measured the excretion of campestanol from the body through pool A, was 100 times more rapid in the three control subjects than the homozygote. Further, there was little difference in the removal constant K_A between the obligate heterozygote and the three controls. Thus, the small campestanol body pool and plasma concentration in the heterozygote reflected rapid excretion of campestanol even though percent absorption was almost three times greater than the mean value for the control subjects.

These studies also emphasize the role of the intestinal sterol and stanol pools, which include both dietary and biliary cholesterol and dietary plant sterols and stanols, in limiting absorption. Percent absorption in the sitosterolemic heterozygote as measured by the plasma dual-isotope ratio method was 15 times greater than absorption calculated as the production rate by the isotope kinetic method. This difference reflected the fact that the plasma dual-isotope ratio measured maximum absorption of a tracer dose of radioactive campestanol at a single time point after a 12-h fast, when the intestine was virtually free of other competing dietary sterols and stanols, whereas the isotope kinetic method estimated average campestanol absorption (mg/day) over 24 h. Because the three controls and heterozygote consumed large amounts of campestanol (540 mg/day) and sitostanol (1.9 g/day) in their diets, actual campestanol absorption was reduced to 0.4% and 0.1%, respectively, of their dietary intakes. Thus, not only was campestanol poorly absorbed at the enterocyte, but displacement of campestanol from the intestinal micelle pool by the large mass of dietary sitostanol likely occurred. It has been noted previously that sitostanol, which is more hydrophobic than campestanol, has a greater affinity but lower capacity to saturate mixed micelles (26–28). At each meal, during which stanol fatty acid esters were consumed in large amounts, the intestinal sterol-stanol pool was composed mainly of dietary sitostanol, which preferentially displaced campestanol and cholesterol from the intestinal mixed micelles to further reduce their absorption.

Another mechanism limiting plant sterol-stanol absorption has been proposed by Child and Kuksis (29), who suggested that the enterocyte brush border membrane selectively excludes sitosterol as compared with cholesterol. Thus, the enterocyte brush border membrane recognizes sterols with extra methyl or ethyl substituents at C-24 on the apolar side chain and does not allow them to enter the intestinal cell, while facilitating the entry of cholesterol.

It is also important to consider that esterification of cholesterol catalyzed by acyl-coenzyme A:cholesterol acyltransferase (ACAT) located in the enterocyte may also limit the absorption of plant sterols and stanols. Earlier studies by Kuksis and Huang (30) of dogs with lymph fistula showed that virtually all cholesterol that was recovered in the lymph chylomicrons was esterified as compared with sitosterol, which was present mainly in the free form. Apparently, the extra methyl and ethyl

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substituents located on the apolar side chain may hinder ACAT, further reducing the intestinal esterification of the plant sterols, which further slows absorption. It had also been noticed previously that in serum, sitosterol was much more slowly esterified than cholesterol by lecithin:cholesterol acyltransferase (1). Interestingly, this mechanism is bypassed in sitosterolemic homozygotes, where the absorption of sitosterol approaches that of cholesterol and both are equally esterified in plasma (12).

An important implication of these results relates to the use of plant sterol-stanol mixtures to block intestine cholesterol absorption to lower plasma concentrations and to decrease the risk of atherosclerosis (4, 31). Although Δ^5-unsaturated plant sterol mixtures (campesterol plus sitosterol) found in vegetable oils were used previously to treat hypercholesterolemia (8, 9, 32), the therapeutic mixture was discontinued from the United States market in the 1980s because a large volume had to be taken with every meal, which proved cumbersome and often resulted in poor patient compliance. The 5α-dihydro plant sterol derivatives have been prepared and esterified with rapeseed oil fatty acids to make stanol fatty acid esters (4). These 5α-stanol mixtures are composed chiefly of 5α-sitostanol with varying proportions of 5α-campestanol and are more resistant to oxidative destruction. After hydrolysis, the increased hydrophobicity of the stanols facilitates preferential entry in the micellar phase of intestinal contents to displace cholesterol. When fed as either a spread or mayonnaise to hypercholesterolemic subjects, plasma cholesterol concentrations declined 10–15% (4). No untoward side effects were reported. Because the stanol fatty acid ester mixtures contained about 10–30% campestanol and 70–90% sitostanol depending on the vegetable oil source, it was important to show that campestanol, like sitostanol, was poorly absorbed (33). Previous studies have shown that almost no sitostanol is absorbed (33). However, because Lees and colleagues in earlier studies (8, 9) found substantial absorption of the Δ^5-unsaturated precursor, campesterol, the percent absorption and pool size of campestanol, the 5α-dihydro derivative, had to be determined. When stanol fatty acid ester mixtures were fed to New Zealand White rabbits, campestanol was barely detected in plasma, but there was significant absorption (11.6%) of tracer doses of campestanol in the absence of intestinal sterols (24). However, when a campestonal-sitostanol mixture (stanol fatty acid esters) was fed, and actual absorption was determined by the isotope kinetic method, percent campestanol absorption declined to only 0.3% of the dietary campestanol intake in the rabbits. Similarly, most humans show only trace plasma concentrations, whereas a sitosterolemic heterozygote had greater absorption, but did not accumulate campestanol or sitostanol when fed the mixture at almost 2.5 g/day (Tables 1 and 3). In contrast, her sitosterolemic homozygous daughter showed substantial baseline plasma campestanol concentrations with markedly increased absorption and tissue pools. However, even in the homozygote, the large intestinal dietary sterol/stanol pool markedly reduced actual campestanol absorption, presumably by displacing campestanol from the mixed micelle phase.

In summary, the results of this investigation confirm that campestanol in a campestanol-sitostanol mixture is poorly absorbed in control subjects and is rapidly excreted from the body. Thus, campestanol-sitostanol mixtures can be fed safely to hypercholesterolemic humans to decrease plasma cholesterol concentrations without concern that the plant stanols (campestanol or sitostanol) will be absorbed and accumulate. In contrast, campestanol was hyperabsorbed in a sitosterolemic homozygote but was not retained in her heterozygous mother because removal was as rapid as in control subjects. In this regard, it would seem unwise for sitosterolemic homozygotes to consume foods containing plant sterols or their 5α-dihydroderivatives. The hyperabsorption and retention of these noncholesterol sterols may play a role in the accelerated atherosclerosis seen in this disease.

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