Campestanol (24-methyl-5α-cholestan-3β-ol) Absorption and Distribution in New Zealand White Rabbits: Effect of Dietary Sitostanol

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Campestanol (24-methyl-5α-cholestan-3β-ol) is a naturally occurring plant stanol, structurally similar to cholesterol (5-cholesten-3β-ol) and widely distributed in vegetable oils consumed in human diets. We measured the absorption and turnover of campestanol by the plasma dual-isotope ratio method and mathematical analysis of specific activity versus time decay curves after simultaneous oral and intravenous pulse-labeling with [3α-^{3}H]- and [23-^{14}C]-labeled campestanol, respectively, in New Zealand White (NZW) rabbits: six fed chow and six fed chow with 125 mg/d campestanol and 175 mg/d sitostanol (24-ethyl-5α-cholestan-3β-ol). Plasma concentrations increased insignificantly from 0.08 ± 0.01 to 0.09 ± 0.01 mg/dL with dietary stanols. The percent campestanol absorption measured by the plasma dual-isotope ratio method after the rabbits were fasted for 6 hours yielded the percent absorption in the absence of competing intestinal sterols and stanols and declined insignificantly from 11.6% ± 3.5% in controls to 8.1% ± 3.7% in the treated rabbit groups. In contrast, the turnover, which measured actual absorption averaged over 24 hours, increased from 0.12 ± 0.05 to 0.37 ± 0.05 mg/d ($P < .05$) with campestanol and sitostanol added to the diet. However, the actual percent absorption declined from 3% to 0.3% of dietary intake with the campestanol and sitostanol–enriched diet. Campestanol pool sizes, although remaining small, increased slightly from 1.1 ± 0.4 to 2.5 ± 1.5 mg. The removal constant (K_A) from pool A (M_A) did not change significantly with added dietary campestanol and sitostanol ($K_{A=}$ − 0.040 ± 0.005 *v* −0.037 ± 0.007 d^{-1}). The results demonstrate small campestanol plasma concentrations and body pools even when the rabbits consumed substantial amounts because (1) intestinal absorption was limited and (2) was further reduced by competing dietary sitostanol, and (3) campestanol was removed rapidly from the body. Thus, campestanol, which shares the same basic structure and intestinal absorption pathway with cholesterol, does not accumulate when fed, and may be incorporated into the diet to block cholesterol absorption.

PLANT STEROLS, campesterol (24-methyl-5-cholesten-3β-ol) and sitosterol (24-ethyl-5-cholesten-3β-ol), are widely distributed in vegetable oils consumed in human diets. They are structurally similar to cholesterol, differing only by the addition of a methyl or ethyl group at C-24 on the apolar side chain (Fig 1). Because of the extra side chain substituents, campesterol and sitosterol are more poorly absorbed from the intestine than cholesterol.[1-3] However, as a consequence of their similar structures and reduced intestinal absorption, plant sterol mixtures have been used to block intestinal cholesterol absorption and decrease plasma concentrations.[4-10]

The respective 5α-dihydro derivatives of campesterol (campestanol) and sitosterol (sitostanol) have been prepared by catalytic reduction of the double bond at C-5,6 (Fig 1). These 5α-dihydrosterol derivatives are more stable to autooxidation than their Δ^5-unsaturated precursors and are less well absorbed from the intestine. Recently, 5α-stanol mixtures have been esterified with rapeseed oil fatty acids and the stanol fatty acid esters incorporated into foods (margarine and mayonnaise).[11] These stanol fatty acid esters mix easily with the oil phase of intestinal contents and interfere with cholesterol absorption and decrease plasma cholesterol concentrations.[11,12] Although there is some information about the absorption of the most abundant plant stanol in the mixture, sitostanol, virtually nothing is known about the absorption and metabolism of campestanol, which may constitute about 30% of the stanols in fatty acid esters.[13-16]

We therefore studied the absorption and distribution of radioactive campestanol fed orally and injected intravenously in New Zealand White (NZW) rabbits who consumed regular chow containing small amounts of campestanol and sitostanol and chow supplemented with 125 mg/d campestanol and 175 mg/d sitostanol. Our results indicate a small but finite absorption of campestanol, which was further reduced when fed together with sitostanol.

MATERIALS AND METHODS

Materials

Cholesterol (5-cholesten-3β-ol) and 5α-cholestane were obtained from Sigma Chemical (St Louis, MO) and used as standards for the measurement of sterols and stanols by capillary gas-liquid chromatography (GLC). Basic rabbit chow (Purina Mills, St Louis, MO) contained campestanol 0.027 mg/g and sitostanol 0.16 mg/g. Stanol fatty acid esters (Benecol Division, Raisio Group, Raisio, Finland) were added to the basic rabbit chow so that each gram of chow contained 0.83 mg campestanol and 1.17 mg sitostanol.

Experimental Plan

The experiments were performed in 12 NZW rabbits (Hazleton Laboratories, Denver, PA) that weighed 2.5 to 3.2 kg. Each rabbit consumed about 150 g chow/d for 4 weeks. Six rabbits were fed the basic chow diet, and six rabbits were fed the basic chow diet supplemented with 500 mg/d stanol fatty acid esters equivalent to 125 mg/d campestanol and 175 mg/d sitostanol. After 1 week on the diet, each rabbit received simultaneously [3α-^{3}H]campestanol intravenously and [23-^{14}C]campestanol intragastrically, and the percent absorption of exogenous [^{14}C]campestanol was measured by the plasma dual-isotope ratio method.[17-21]

Using the intravenously injected [3α-^{3}H]campestanol, campestanol turnover rates were estimated by mathematical analysis of specific

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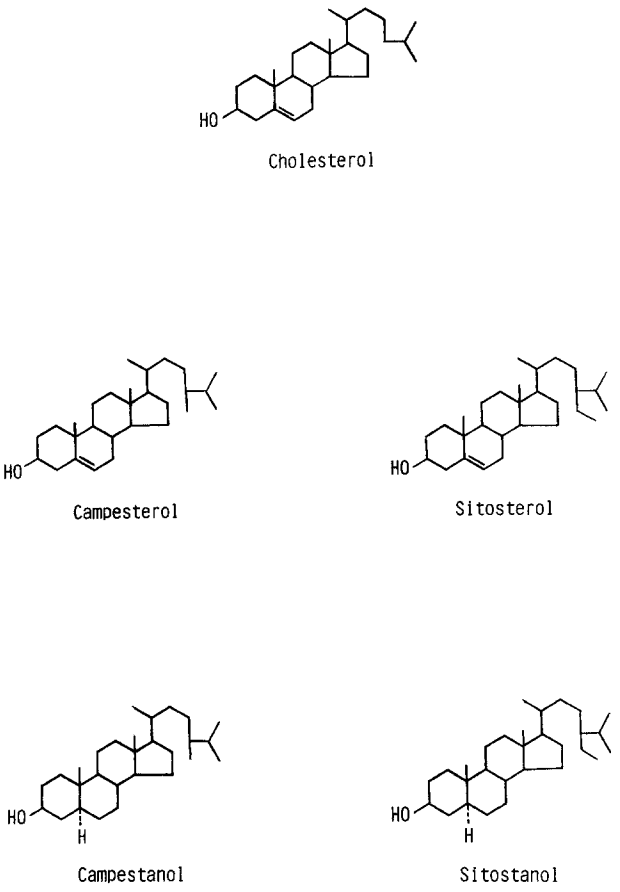

Fig 1. Structure of cholesterol and plant sterols and stanols. Campesterol contains a methyl substituent at C-24 and campestanol is its 5α-dihydro derivative. Sitosterol contains an ethyl substituent at C-24 and sitostanol is its 5α-dihydro derivative.

activity–time curves. Body pool sizes, production rates, and kinetic parameters for campestanol were also calculated.[1,21-24]

Campestanol Absorption

The plasma dual-isotope ratio method, originally described to study cholesterol absorption in rats by Zilversmit and Hughes[17] and modified for studies in humans by Samuel et al[18] and in sitosterolemic homozygotes and heterozygotes by Salen et al,[20,21] was used to measure campestanol absorption in NZW rabbits. [3α-^{3}H]campestanol was injected intravenously and [23-^{14}C]campestanol intragastrically in the morning after a 6-hour fasting period. Plasma was obtained 2, 3, and 5 or 6 days after intragastric and intravenous pulse-labeling, and the campestanol $^{14}C/^3H$ ratio was determined. The percent absorption was obtained by dividing the mean plasma $^{14}C/^3H$ isotope ratio for the 3 days by the ideal ratio of the total administered ^{14}C and ^{3}H multiplied by 100. It was recognized that since the oral dose of [23-^{14}C]campestanol was administered after a 6-hour fast, absorption occurred in the absence of competing intestinal dietary sterols and stanols.

Turnover

Campestanol turnover was calculated according to the two-compartment model system.[1,20,21] This model system was selected for campestanol because plant sterols and stanols, ie, sitosterol, are not synthesized endogenously, plasma specific activity after isotopic pulse-labeling decays rapidly, and the resulting plasma specific activity versus time curve provides an excellent fit for two exponentials. The plots of specific activity versus time were analyzed by 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 sitosterol turnover reported in prior studies,[1,20,21] ie, 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. Thus, S_B = zero, and S_A represents the amount of campestanol absorbed from the diet each day (Fig 2).

Radiolabeled Campestanol

[23-^{14}C]campestanol (specific activity, 50 μCi/μmol) was custom-synthesized by Amersham Life Science and provided by the Benecol Division, Raisio Group (Raisio, Finland). [3α-^{3}H]campestanol was prepared from a sample of greater than 99% pure campestanol (Benecol Division, Raisio Group) by the method of Dayal et al[25] with a final specific activity of 91 μCi/μmol. The labeled campestanols were examined by silica gel G thin-layer chromatography (developing solvent, chloroform:acetone 97:3 vol/vol, Rf = 0.35) and were approximately 98% pure. Each rabbit received 0.5 μCi [23-^{14}C]campestanol intragastrically and 1.0 μCi [3α-^{3}H]campestanol intravenously. The intragastrically administered [23-^{14}C]campestanol was dissolved in 0.5 mL absolute ethanol suspended in 1 mL milk, and the intravenously administered [3α-^{3}H]campestanol was dissolved in 100 μL absolute ethanol suspended in 2 mL physiologic saline injected via a 22-gauge butterfly needle infusion into the femoral vein. The rabbits were lightly sedated with a small dose of ketamine, Acepromazine, and Xylazine mixture and did not begin to eat for 2 to 3 hours after injection.

Chemical Studies

Plasma sterol concentrations were measured by capillary gas-liquid chromatography (GLC). After saponification of 100 μL plasma in 1N NaOH at 70°C for 1 hour, neutral sterols and stanols were extracted with hexane. Seventy micrograms of 5α-cholestane and 70 μg coprostanol were added prior to extraction as internal standards. The solvent was

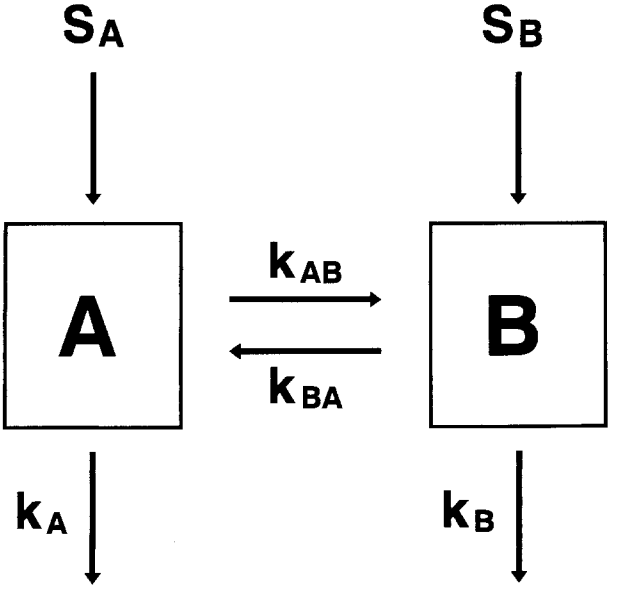

Fig 2. Two-pool model for the turnover of [3α-^{3}H]campestanol. S_A is the input into pool A and equals absorption, PR_A (mg/d), because there is no endogenous synthesis of campestanol. k_A is the removal constant from pool A, and k_{AB} and k_{BA} are the equilibrium constants between the 2 pools. There is no removal of campestanol from pool B (K_B = zero). A and B represent pools M_A and M_B, respectively.

evaporated, and trimethylsilyl (TMS) ether derivatives were prepared by the addition of 50 μL Sil Prep (Analtech, Deerfield, IL). After standing for 30 minutes, pyridine was evaporated and the residue was dissolved in 50 μL hexane; 1 μL was analyzed by GLC in the splitless mode. Capillary GLC was performed on 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) 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 time relative to 5α-cholestane for TMS-ether derivatives was 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 (^{3}H and ^{14}C) campestanol was determined in 300-μL specimens of plasma. After saponification, hexane extraction, and solvent evaporation, the neutral sterol-stanol fraction was redissolved in 5.0 mL ethyl acetate. Four fifths were taken for radioactivity measurements dissolved in Eculum (New England Nuclear, Boston, MA). Radioactivity was assayed in a Beckman LS-6500 liquid scintillation system (Beckman Instruments, Fullerton, CA). The efficiency for counting ^{3}H and ^{14}C was 43% and 69%, respectively. The samples were counted for 10 to 100 minutes so that at least 100 counts above background were recorded.

Statistics

Data are reported as the mean ± SD. The statistical significance of differences between the two rabbit groups was estimated by Student's *t* test (unpaired), and significance was accepted at a *P* level less than .05.

RESULTS

Table 1 lists the plasma sterol concentrations. Plasma cholesterol levels did not decline when the campestanol-sitostanol–enriched chow diet was fed. In contrast, only small amounts of campestanol were detected in plasma on the basic chow diet that contained approximately 4 mg/d campestanol, and the plasma concentration increased slightly but insignificantly from 0.08 ± 0.01 to 0.09 ± 0.01 mg/dL when 125 mg/d campestanol was added to the chow diet. Sitostanol was also present in very small amounts in plasma when the basic chow was fed with a daily intake of 24 mg/d sitostanol, and increased insignificantly when the chow diet contained 175 mg/d sitostanol. Interestingly, there were low plasma concentrations of the Δ^5-unsaturated plant sterol, sitosterol, which decreased significantly in rabbits fed chow enriched with campestanol and sitostanol.

The percent campestanol absorption determined by the plasma dual-isotope ratio method after simultaneous pulse-labeling with [23-^{14}C]campestanol orally and [3α-^{3}H]campestanol intravenously is presented in Table 2. The ^{14}C/^{3}H ratio of campestanol was reported for three specimens of blood obtained from each rabbit on days 2, 3, and 5 or 6 after pulse-labeling. No [^{14}C] radioactivity could be detected at day 9. The mean values for 18 determinations (three per rabbit) for the six rabbits in each group were then compared against the administered isotope ratio. The latter fraction represents the ^{14}C/^{3}H ratio that would be obtained if campestanol absorption was 100%, and is calculated by dividing the total [^{14}C] oral dose by the total [^{3}H] intravenous dose.

Table 1. Plasma Sterol Levels (mg/dL) in the Two Rabbit Groups

Diet	Cholesterol	Campestanol	Sitostanol	Sitosterol
Basic chow (n = 6)	41 ± 14	0.08 ± 0.01	0.06 ± 0.04	0.60 ± 0.06
Basic chow + campestanol + sitostanol (n = 6)	42 ± 13	0.09 ± 0.01	0.09 ± 0.03	0.43 ± 0.16*

*$P < .05$ *v* chow.

Table 2. Parameters of Campestanol Absorption in the Two Rabbit Groups

Parameter	Chow (n = 18)*	Chow + Campestanol + Sitostanol (n = 18)*
^{14}C/^{3}H ratio	0.058 ± 0.018	0.040 ± 0.018
Coefficient of variation (%)†	31	45
Administered ^{14}C/^{3}H ratio	0.50	0.50
Absorption (%)	11.6 ± 3.5	8.1 ± 3.7

*18 determinations (3 separate blood samples each from 6 rabbits).
†SD ÷ mean × 100.

The plasma ^{14}C/^{3}H ratio was higher when rabbits consumed the basic chow diet that contained only 4 mg/d campestanol and 24 mg/d sitostanol compared with the basic rabbit chow containing added campestanol (125 mg/d) and sitostanol (175 mg/d). However, the difference between groups was not statistically significant, as the coefficients of variation for the ratios were similarly large. When the plasma ^{14}C/^{3}H ratio was converted to percent absorption by dividing the plasma ratio by the administered ratio multiplied by 100, the percent campestanol absorption was 11.6% in chow-fed rabbits and declined insignificantly to 8.1% in rabbits fed chow supplemented with the campestanol-sitostanol fatty acid ester mixture. Since the basic chow contained about 4 mg/d campestanol, only about 0.5 mg/d would be absorbed on the basic chow diet, compared with 10.1 mg/d as the maximum campestanol absorption for rabbits fed basic chow enriched with 125 mg/d campestanol and 175 mg/d sitostanol. However, it is important to emphasize that the rabbits were fasted for 6 hours before radioactive campestanol was administered and did not eat again until 2 hours (sedated) after isotope injection, and thus the percent absorption by the dual-isotope ratio method was determined in the absence of intestinal dietary sterols and stanols.

Figure 3 illustrates the plasma specific activity–time decay curve for intravenously injected [3α-^{3}H]campestanol in six NZW rabbits fed basic chow that contained small amounts of campestanol (4 mg/d) and sitostanol (24 mg/d) and six NZW rabbits fed basic chow supplemented with 125 mg/d campestanol and 175 mg/d sitostanol. The intravenously administered [3α-^{3}H]campestanol specific activity decayed almost identically in both groups, which indicates that the turnover of campestanol in the rabbits was not greatly influenced by the larger intake of dietary campestanol and sitostanol (Fig 3). Further, the rapid elimination of [3α-^{3}H]campestanol was evidenced by the almost complete disappearance of [^{3}H]campestanol from the plasma 9 days after intravenous injection.

It is also evident that the constancy of the ^{14}C/^{3}H ratio over days 2, 3, and 5 or 6 (Table 2) corresponds to the bulk of the [^{3}H]campestanol-time curve (Fig 3). This finding suggests that the intragastrically administered [^{14}C]campestanol decayed in parallel to the intravenously injected [^{3}H]campestanol.

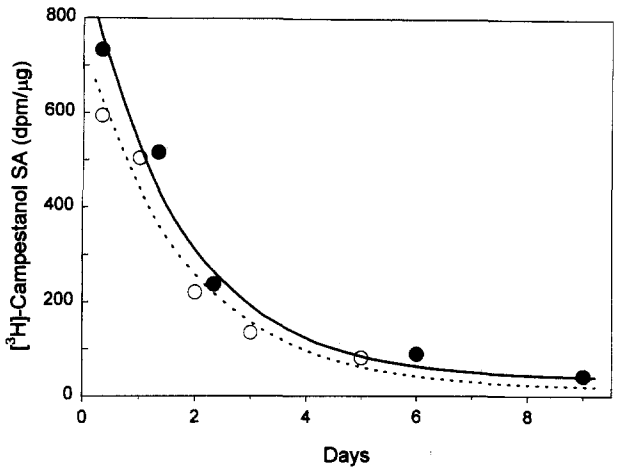

Fig 3. Specific activity versus time curve for [3α-^{3}H]campestanol in rabbits fed basic chow that contained 4 mg/d campestanol and 24 mg/d sitostanol (●) and rabbits fed basic chow supplemented with 125 mg/d campestanol and 175 mg/d sitostanol (○). The decay of [3α-^{3}H]campestanol is almost identical in the 2 rabbit groups.

Mathematical analysis[1,20,21] showed that each decay curve could be divided into two exponentials that reflect two kinetic pools of campestanol (Fig 2). Pool A is designated as the rapid-turnover pool composed of campestanol in the plasma, erythrocytes, liver, and intestine, and pool B is designated as the slow-turnover pool composed of campestanol in muscle.[1] Table 3 lists key parameters for campestanol turnover and distribution assuming that [3α-^{3}H]campestanol entered and was eliminated from the body solely through pool A.

The half-life of [^{3}H]campestanol in pool A ($t_{1/2\ A}$) for both rabbit groups was rapid and about equal. Similarly, the half-life for pool B ($t_{1/2\ B}$), although much slower than ($t_{1/2A}$), was also about the same magnitude for both rabbit groups. Rate constants (Table 3) reflecting the distribution and equilibrium of [3α-^{3}H]campestanol between the two pools were about the same, confirming the similar decay of plasma [3α-^{3}H]campestanol observed for the curves in Fig 3. In particular, K_A, the rate constant for the removal of campestanol from pool A (M_A), was fast and did not differ significantly between the two rabbit groups. Thus, campestanol was eliminated speedily from the body via pool A, and the rate constant (K_A) for the two treatment groups was not significantly different despite the much larger intake of dietary campestanol and sitostanol. The size of pool A (M_A) calculated by mathematical analysis of the two-pool model was 1.1 ± 0.4 mg in rabbits fed chow, and increased 55% to 2.4 ± 1.3 mg ($P < .05$) in rabbits fed the chow diet enriched with campestanol and sitostanol. Pool B (M_B), which has a slower turnover and contains campestanol entirely derived from pool A, increased from 0.012 ± 0.006 to 0.090 ± 0.137 mg (P = NS) with added dietary campestanol and sitostanol. Thus, the total campestanol body pool increased 56% and was 2.3-fold larger with the extra campestanol and sitostanol mixture added to the diet.

Table 3. Parameters of Campestanol Turnover and Distribution in the Two Rabbit Groups

Parameter	Basic Chow (n = 6)	Basic Chow + Campestanol + Sitostanol (n = 6)
$t_{1/2A}$ (d)	1.3 ± 0.2	1.1 ± 0.1
$t_{1/2B}$ (d)	17.2 ± 2.2	14.9 ± 2.3
PR_A (mg/d)	0.12 ± 0.05	0.38 ± 0.25*
M_A (mg)	1.1 ± 0.4	2.4 ± 1.3*
M_B (mg)	0.012 ± 0.006	0.09 ± 0.137
$M_A + M_B$ (mg)	1.12 ± 0.4	2.49 ± 1.5*
K_A (d^{-1})	−0.040 ± 0.005	−0.037 ± 0.007
K_{AA} (d^{-1})	−0.0033 ± 0.0003	−0.00030 ± 0.00012
K_{BB} (d^{-1})	−0.56 ± 0.08	−0.73 ± 0.12
K_{AB} (d^{-1})	0.043 ± 0.025	0.037 ± 0.07

NOTE. Specific activity = A exp (−at) + B exp (−bt).[1,20,21] Assume no synthesis in pool B and no input or excretion of campestanol from pool B except through pool A.

*$P < .05$ *v* basic chow.

The production rate (PR_A) measures the entry of campestanol into the body through pool A, and in the case of campestanol, it equals the averaged amount absorbed (milligrams per day) because there is no endogenous synthesis. In rabbits fed basic chow, this amounted to 0.12 ± 0.05 mg/d, and increased to 0.38 ± 0.25 mg/d ($P < .05$) in rabbits fed basic chow supplemented with 125 mg/d campestanol and 175 mg/d sitostanol. Since about 4 mg/d campestanol was fed with the basic chow diet in control rabbits, the actual percent campestanol absorption was 0.12/4 × 100, or 3% of the intake, whereas in rabbits fed chow supplemented with campestanol (125 mg/d) and sitostanol (175 mg/d), the actual percent campestanol absorption equaled 0.38/125 × 100, or 0.3% of the dietary intake. Thus, although the mass of absorbed campestanol increased slightly, the actual percent absorption declined drastically when a mixture of campestanol and sitostanol was added to the diet.

DISCUSSION

The results of this investigation demonstrate that small amounts of dietary campestanol were absorbed from the intestine of NZW rabbits and circulated in the plasma. Usually, campestanol can be found with its Δ^5-unsaturated plant sterol precursor, campesterol, in virtually all natural vegetable oils, eg, corn oil and soybean oil. These vegetable oils are widely consumed in American diets. In a sampling of corn oil, campestanol which is 5α-saturated, comprised about 3% of the total sterol fraction, along with its more abundant Δ^5-unsaturated precursor, campesterol, 27%, and other Δ^5-unsaturated plant sterol derivatives, sitosterol 60%, stigmasterol 6%, and sitostanol 4% (G. Salen, unpublished observations, May 1998). However, the stanol preparation used in chow-fed rabbits in these experiments was specially prepared by catalytic hydrogenation of vegetable oil Δ^5-unsaturated plant sterols to produce a mixture that contained only campestanol (42%) and sitostanol (58%), which were then esterified with rapeseed oil fatty acids to produce a stanol fatty acid ester mixture that would easily dissolve into the oil phase of intestinal contents.

Cholesterol and plant sterols and their 5α-stanol derivatives contain the same cyclopentanoperhydrophenanthrene ring structure and 8-carbon side chain as cholesterol, but differ from the cholesterol molecule by the addition of extra methyl or ethyl

substituents at C-24 on the apolar side chain (Fig 1). These additional substituents are recognized by the brush border of enterocytes such that intestinal absorption of plant sterols is markedly reduced compared with cholesterol.[26] For example, in one control subject fed regular food, 44% of cholesterol was absorbed, compared with only 4% of dietary sitosterol.[20] Moreover, 5α-saturation of the double bond at C-5,6 to form 5α-stanols further diminishes the percent intestinal absorption such that 5α-sitostanol is virtually unabsorbed.[13-15] However, because plant sterols and stanols are structurally similar to cholesterol (Fig 1), they share the same intestinal absorption pathway, and thus when present together with cholesterol in the intestine, poorly absorbed campestanol and sitostanol will hinder the absorption of cholesterol.[4,14,16]

Two methods were used to assess campestanol absorption. The plasma dual-isotope ratio method measures the plasma ratio of $^{14}C/^{3}H$ campestanol after simultaneous pulse-labeling with [23-^{14}C]campestanol administered orally and [3α-^{3}H]campestanol intravenously. The percent absorbed is calculated by dividing the observed plasma $^{14}C/^{3}H$ ratio by the ideal $^{14}C/^{3}H$ ratio, which would be the ratio if 100% of [23-^{14}C]campestanol administered orally was absorbed, multiplied by 100. Thus, the campestanol $^{14}C/^{3}H$ ratio represents absorption at a single time point. The percent campestanol absorption was similar in chow-fed rabbits (11%) and rabbits fed chow supplemented with campestanol and sitostanol (8.6%). However, these rabbits were fasted for 6 hours before [4-^{14}C]campestanol was administered orally. Thus, the percent campestanol absorption as determined by the plasma dual-isotope ratio method represents campestanol absorption when competing dietary sterols and stanols are absent from the intestine. In contrast, campestanol absorption measured by mathematical analysis of the [^{3}H]campestanol specific activity versus time decay curves revealed the input or production rate (PR_A) of campestanol that entered the rapid-turnover pool A over a 24-hour period as milligrams per day. Assuming that campestanol, like other plant sterols, is not synthesized endogenously, the daily production rate (PR_A) equals absorption (milligrams per day). When rabbits were fed the basic chow diet that contained small amounts of campestanol (4 mg/d) and sitostanol (24 mg/d), 0.12 mg/d campestanol was absorbed, equivalent to 3% of the dietary campestanol intake. In comparison, 0.38 mg/d campestanol was absorbed when the basic chow diet was supplemented with 125 mg/d campestanol and 175 mg/d sitostanol. Although the mass campestanol absorption increased 69% ($P < .05$), the actual percent absorption declined markedly to 0.3% of dietary intake. When these measurements for percent campestanol absorption derived by the isotope kinetic method and plasma dual-isotope ratio method are compared, the percent absorption determined by the plasma dual-isotope ratio method (Table 2) was higher in rabbits after fasting when dietary sterols and stanols were absent from the intestine. However, when large amounts of campestanol and sitostanol were fed, only 0.3% of intestinal campestanol was absorbed as measured over 24 hours by the isotope kinetic method. The explanation for this difference seems to be that the large amounts of sitostanol present in the diet and intestine competed with and reduced campestanol absorption. However, it is important to emphasize that the precise mechanism for intestinal absorption of cholesterol and plant sterols and stanols is not completely known.

At least four possible explanations could account for the reduced campestanol absorption when the plant stanol mixture was added to the diet: (1) The large dietary pool of sitostanol fatty acid esters displaced campestanol fatty esters from the intestinal bile salt micelles,[27] which reduced the content of campestanol fatty acid esters available for hydrolysis by pancreatic ester hydroxylase. This would diminish the release of free campestanol and maintain campestanol as a fatty acid ester, which cannot be absorbed intact from the intestine. (2) Sitostanol fatty acid esters may compete directly with campestanol fatty acid esters for pancreatic ester hydrolase. (3) Free sitostanol may directly interfere with the uptake of free campestanol by the enterocyte after hydrolysis of the fatty acid esters.[5] (4) Once in the enterocyte, sitostanol may competitively block campestanol esterification by intestinal acylcholesterol acyltransferase (ACAT), which is a necessary step for the incorporation of campestanol into chylomicrons.[1] Importantly, the same processes that block campestanol absorption would also operate to interfere and diminish the absorption of dietary and biliary cholesterol in the intestine. Indeed, stanol fatty esters have been used clinically in hypercholesterolemic humans to reduce cholesterol absorption and decrease the elevated plasma cholesterol levels.[11,12] The stanol fatty acid esters used in these studies were composed mostly of sitostanol derived from tall oil (pine tree sterols). However, the results of this investigation indicate that since dietary campestanol is also poorly absorbed from the intestine, a greater proportion of campestanol can be included in the stanol fatty acid ester mixture. Because of the limited absorption of campestanol and the combination with sitostanol, the mixture would be effective to reduce cholesterol absorption.

We realize that the percent absorption of campestanol as measured by the plasma dual-isotope ratio method was higher than determined by the isotope kinetic method. However, we believe the isotope kinetic method more accurately reflected campestanol absorption over a 24-hour period and would be more strongly influenced in the intestine by dietary sitostanol, as shown. In this regard, the constancy of the $^{14}C/^{3}H$ ratio over days 2, 3, and 5 or 6 suggests parallel decay of absorbed [23-^{14}C]campestanol with intravenously administered [3α-^{3}H]campestanol.

This finding of reduced campestanol absorption is important to consider since campesterol, the Δ^5-unsaturated precursor of campestanol, was absorbed more efficiently than Δ^5-sitosterol when fed to hypercholesterolemic subjects such that substantial amounts of campesterol were present in the plasma of some patients.[7,8] Also of significance is the fact that accelerated atherosclerosis is part of the clinical picture of patients with sitosterolemia. In this rare recessive lipid disorder, large amounts of plant sterols and stanols including campestanol and sitostanol accumulate in the plasma and tissues because of hyperabsorption and diminished sterol removal.[22] Thus, the low absorption and small body pools of campestanol in the rabbits fed large amounts of campestanol and sitostanol reinforces the proposition that campestanol would not accumulate during stanol fatty ester treatment.

In summary, we have examined the absorption of campesta-

nol by two methods in rabbits fed chow containing little campestanol and sitostanol and rabbits fed chow supplemented with campestanol 125 mg/d and sitostanol 175 mg/d. Higher campestanol absorption was observed in fasted rabbits in the absence of intestinal sterols, but the actual absorption was reduced markedly to 0.3% of intake when measured over 24 hours when large amounts of sitostanol were also present in the diet.

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REFERENCES

1. Salen G, Ahrens EH Jr, Grundy SM: Metabolism of β-sitosterol in man. J Clin Invest 49:952-967, 1970

2. Sylven C, Borgstrom B: Absorption and lymphatic transport of cholesterol and sitosterol in the rat. J Lipid Res 10:179-182, 1969

3. Wilson MD, Rudel LL: Review of cholesterol absorption with emphasis on dietary and biliary cholesterol. J Lipid Res 35:943-955, 1994

4. Tilvis RS, Miettinen TA: Serum plant sterols and their relation to cholesterol absorption. Am J Clin Nutr 43:92-97, 1986

5. Heinemann TO, Kullack-Ublick GA, Bietruck B, et al: Mechanism of action of plant sterols on inhibition of cholesterol absorption. Eur J Clin Pharmacol 40:59-63, 1991

6. Mattson FH, Grundy SM, Crouse JR: Optimizing the effect of plant sterols on cholesterol absorption in man. Am J Clin Nutr 35:697-700, 1982

7. Lees RS, Lees AM: Effects of sitosterol therapy on plasma lipid and lipoprotein concentrations, in Greten H (ed): Lipoprotein Metabolism. Berlin, Germany, Springer, 1976, pp 20-124

8. Lees AM, Mok HYI, Lees RS, et al: Plant sterols as cholesterol lowering agents: Clinical trials in patients with hypercholesterolemia and studies of sterol balance. Atherosclerosis 28:325-338, 1977

9. Kudchodkar BJ, Horlick L, Sodhi HS: Effects of plant sterols on cholesterol metabolism in man. Atherosclerosis 23:239-248, 1976

10. Ikeda I, Tanaka K, Sugano M, et al: Inhibition of cholesterol absorption in rats by plant sterols. J Lipid Res 29:1573-1582, 1988

11. Vanhanen HT, Blomqoist S, Ehnholm C, et al: Serum cholesterol precursors and plant sterols in hypercholesterolemic subjects with different apo E phenotypes during dietary sitostanol ester treatment. J Lipid Res 34:1535-1544, 1993

12. Miettinen TA, Puska P, Gylling H, et al: Reduction of serum cholesterol with sitostanol-ester margarine in a mildly hypercholesterolemic population. N Engl J Med 333:1308-1312, 1995

13. Lutjohan D, Meese CO, Crouse JR, et al: Evaluation of deuterated cholesterol and deuterated sitostanol for measurement of cholesterol absorption in humans. J Lipid Res 34:1039-1046, 1993

14. Hassan AS, Rampone AJ: Intestinal absorption and lymphatic transport of cholesterol and β-sitostanol in the rat. J Lipid Res 20:646-653, 1979

15. Ikeda I, Sugama M: Comparison and absorption of beta sitosterol and beta sitostanol in rats. Arteriosclerosis 30:227-237, 1978

16. Heinemann TO, Leiss O, von Bergmann K: Effect of low dose sitostanol on serum cholesterol in patients with hypercholesterolemia. Atherosclerosis 61:219-223, 1986

17. Zilversmit DB, Hughes LB: Validation of a dual isotope plasma ratio method for measurement of cholesterol absorption in rats. J Lipid Res 15:465-473, 1974

18. Samuel P, Crouse JR, Ahrens ER Jr: Evaluation of an isotope ratio method for measurement of cholesterol absorption in man. J Lipid Res 19:82-93, 1978

19. Samuel P, McNamara DJ, Ahrens EH Jr, et al: Further validation of the plasma isotope ratio method for measurement of cholesterol absorption in man. J Lipid Res 23:480-489, 1982

20. Salen G, Shore V, Tint GS, et al: Increased sitosterol absorption, decreased removal and expanded body pools compensate for reduced cholesterol synthesis in sitosterolemia with xanthomatosis. J Lipid Res 30:1319-1330, 1989

21. Salen G, Tint GS, Shefer S, et al: Increased sitosterol absorption is offset by rapid elimination to prevent accumulation in heterozygotes with sitosterolemia. Arterioscler Thomb 12:563-568, 1992

22. Salen G, Shefer S, Nguyen L, et al: Sitosterolemia. J Lipid Res 33:945-955, 1992

23. Lin H-J, Wang C, Salen G, et al: Sitosterol and cholesterol metabolism in a patient with coexisting phytosterolemia and cholestanolosis. Metabolism 32:126-133, 1983

24. Bhattacharyya AK, Connor WE, Lin DS, et al: Sluggish sitosterol turnover and hepatic failure to excrete sitosterol into bile cause expansion of body pool of sitosterol in patients with sitosterolemia and xanthomatosis. Atheroscler Thromb 11:1287-1294, 1991

25. Dayal B, Salen G, Tint GS, et al: A facile synthesis of [3α-^{3}H]sitosterol. Steroids 42:635-640, 1983

26. Child P, Kuksis A: Uptake of 7-dehydro derivatives of cholesterol, campesterol, and β-sitosterol by rat erythrocytes, jejunal villus cells, and brush border membranes. J Lipid Res 24:552-565, 1983

27. Ikeda I, Tanabe Y, Sugano M: Effects of sitosterol and sitostanol on micellar solubility of cholesterol. J Nutr Sci Vitaminol 29:1573-1582, 1988