Plant Stanol Fatty Acid Esters Inhibit Cholesterol Absorption and Hepatic Hydroxymethyl Glutaryl Coenzyme A Reductase Activity to Reduce Plasma Levels in Rabbits

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The aim of this study was to study the inhibitory effect of dietary stanols (campestanol and sitostanol) fatty acid esters (SE) on intestinal cholesterol absorption. New Zealand white rabbits were fed regular chow alone or enriched with 0.2% cholesterol, 0.33% SE + cholesterol, 0.66% SE + cholesterol, 1.2% SE + cholesterol, 2.4% SE + cholesterol, and 1.2% SE alone. After 2 weeks, plasma cholesterol levels increased 3.6 times in the cholesterol group and did not decrease after addition of 0.33% or 0.66% SE to the cholesterol-enriched diets. However, after addition of 1.2% SE to the cholesterol diet, plasma cholesterol concentration decreased 50% ($P < .001$), but it did not decrease further after doubling of SE to 2.4%. Percent cholesterol absorption measured by the plasma dual-isotope ratio method was 73.0% ± 8.1 % in the cholesterol group, which was similar to untreated baseline control. The percent absorption of cholesterol did not decrease significantly after addition of 0.33% or 0.66% SE to the cholesterol diet but decreased 43.8% ($P < .001$) in the 1.2% SE + cholesterol group, a finding similar to those in rabbits fed 1.2% SE alone. Increasing SE to 2.4% in the cholesterol diet did not further decrease absorption. Hepatic hydroxymethyl glutaryl coenzyme A (HMG-CoA) reductase activity reflecting cholesterol synthesis and low-density lipoprotein receptor-mediated binding unexpectedly decreased 67% ($P < .01$) and 57% ($P < .05$) in rabbits fed 1.2% SE alone. Increasing dietary SE intake to 1.2% reduced cholesterol absorption and plasma levels. Dietary SE intake below 1.2% was ineffective and above 2.4% did not further decrease percent absorption or plasma cholesterol levels. These results support the hypothesis that dietary SEs competitively displace cholesterol from intestinal micelles to reduce cholesterol absorption and decrease plasma cholesterol levels.

SITOSTEROL (24-ethyl-5-cholesten-3β-ol) and campesterol (24-methyl-5-cholesten-3β-ol) are common plant sterols that are widely distributed in vegetable oils consumed in most human diets. The structure of these plant sterols is similar to that of cholesterol (Fig 1), differing only by the addition of a methyl or ethyl group at C-24 on the apolar side chain. Because of the extra side chain substituents, campesterol and sitosterol are poorly absorbed from the intestine,[1,2] and plant sterol mixtures have been used therapeutically to reduce intestinal cholesterol absorption[3] to decrease plasma concentrations.[4] Among the suggested mechanisms, dietary plant sterols may compete and displace cholesterol from the intestinal bile acid micelles[5-7] and competitively block enterocyte transport.

The 5α-dihydro derivatives of sitosterol (sitostanol) and campesterol (campestanol) are even more difficult to absorb from the intestine and may be more effective hypocholesterolemic agents.[8-10] Recently, 5α-stanol mixtures (sitostanol and campestanol) have been prepared by catalytic hydrogenation of Δ^5-unsaturated sterols and esterified with rapeseed oil fatty acids. The stanol fatty acid esters (SE) mix easily with the oil phase of intestinal contents. We reported[11] that in rabbits fed SE, little campestanol was accumulated in the body because of diminished intestinal absorption and rapid hepatic clearance.

In this study, we investigated the effect of increasing dietary SE on percent intestinal cholesterol absorption from the intestine and its consequent effect on plasma cholesterol levels and hepatic cholesterol and bile acid syntheses. The study showed that a critical amount of dietary SE inhibited intestinal cholesterol absorption and reduced plasma cholesterol levels significantly. Further increases of SE intake did not produce a greater reduction in cholesterol absorption or plasma levels, and insufficient SE did not affect cholesterol absorption or plasma levels. Moreover, feeding 1.2% SE alone inhibited cholesterol absorption and unexpectedly depressed hepatic hydroxymethyl glutaryl coenzyme A (HMG-CoA) reductase activity in rabbits.

MATERIALS AND METHODS

Materials

Cholesterol (5-cholesten-3β-ol) and coprostanol (5β-cholestan-3β-ol) 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. [^{3}H] and [^{14}C] cholesterol were produced from New England Nuclear (Boston, MA). Stanol fatty acid esters (SE) were provided by Benecol Division, Raisio Group (Raisio, Finland). The SE contained 60% (wt/wt) stanols (free stanols). There was 58% sitostanol and 42% campestanol in the stanols. The SE were prepared by catalytic hydrogenation of vegetable oil Δ^5-unsaturated plant sterols to produce a mixture that contained only campestanol and sitostanol esterified with rapeseed oil fatty acids. SE were added to the basic rabbit chow or the rabbit chow containing 0.2% cholesterol (Ch diet).

Animal Experiment

Dietary treatment. The experiment was divided into 2 studies. Male New Zealand white (NZW) rabbits weighing approximately 5 lb were used.

Study 1 was designed to determine the most effective dose of SE that

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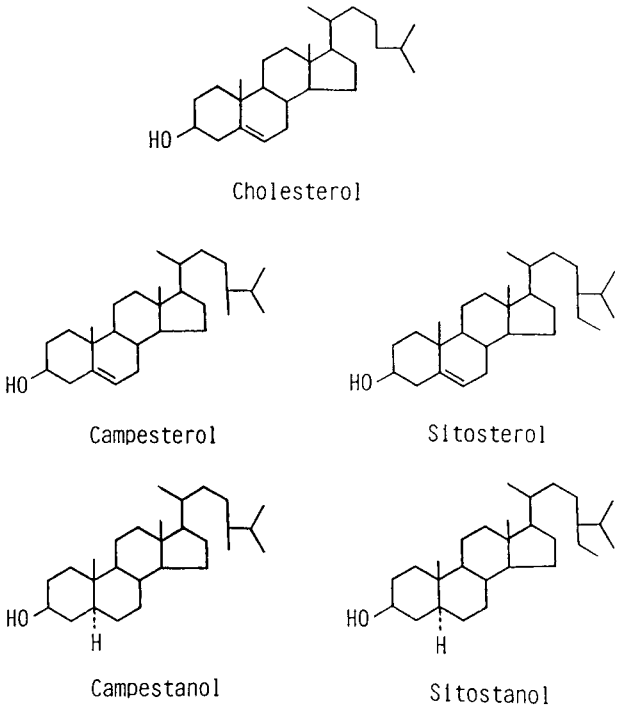

Fig 1. Structures of cholesterol and plant sterols and stanols. Campesterol has an addition methyl group at C-24 and sitosterol, an additional ethyl group on the side chain. The respective 5α-dihydro derivatives are campestanol and sitostanol.

would significantly reduce intestinal cholesterol absorption and plasma levels when fed to rabbits in combination with 0.2% cholesterol. In this study, NZW rabbits were fed regular rabbit chow (n = 6); chow enriched with 0.2% cholesterol (Ch diet; 0.3mg cholesterol/d; n = 10); chow enriched with cholesterol (0.3 g cholesterol/d) and 0.33% SE (0.5 g SE or 0.3g free stanols/day; n = 10); chow enriched with cholesterol (0.3 g cholesterol/d) and 0.66% SE (1.0 g SE or 0.6 g free stanols/d; n=11); chow enriched with cholesterol (0.3 g cholesterol/d) and 1.2% SE (1.8 g SE or 1.1 g free stanols/d; n = 3); or chow enriched with cholesterol (0.3 g cholesterol/d) and 2.4% SE (3.6 g SE or 2.2 g free stanols/d; n = 4). After 2 weeks of treatment, the percent absorption of cholesterol was determined in 4 control, 3 Ch + 0.33%SE, 6 Ch + 0.66% SE, 3 Ch + 1.2% SE, and 4 Ch + 2.4% SE by the plasma dual-isotope ratio method. At completion of the study, the animals were killed, and liver specimens were collected for measurement of activities of the rate-limiting enzymes for cholesterol and bile acid synthesis.

Study 2 was designed to investigate the effect of Ch + 1.2% SE feeding on percent cholesterol absorption, metabolism, and plasma cholesterol levels. NZW rabbits were fed chow alone (n = 6), 0.2% Ch (0.3 g cholesterol/d; n = 7), 0.2% Ch (0.3 g cholesterol/d) + 1.2% SE (1.8 g SE or 1.1 g free stanols/d; n = 7), or 1.2% SE (1.8 g SE or 1.1 g free stanols/d) alone (n = 6) for 4 weeks. Percent cholesterol absorption was measured in 6 rabbits from each group by the plasma dual-isotope ratio method.

After completion, 3 animals each from the control, Ch, Ch + 1.2% SE, and1.2% SE alone groups were operated on to construct bile fistulas for measurement of the bile acid pool size using the method described previously.[12] The remaining rabbits from each group were killed and liver specimens were collected for assays of cholesterol and activities of the rate-limiting enzymes for cholesterol and bile acid synthesis.

The project for rabbit experiments was approved by Animal Studies Committees at VA Medical Center, East Orange, NJ, and UMDNJ-New Jersey Medical School, Newark, NJ.

Isotope labeling for study of cholesterol absorption. The plasma dual-isotope ratio method, originally described to study cholesterol absorption in rats by Zilversmit and Hughes[13] and modified for studies in humans by Samuel et al[14] and in sitosterolemic homozygotes and heterozygotes by Salen et al,[15,16] was used to measure cholesterol absorption in NZW rabbits. After 2 weeks of feeding, the rabbits were given 1.0 μCi of [^{14}C]cholesterol in 2 mL of 10% Liposyn solution (Abbott Laboratories, North Chicago, IL) by gavage and 2.0 μCi of [^{3}H]cholesterol in 1 mL of 2.5% Liposyn solution via ear vein. The rabbits were lightly sedated with a small dose (0.2 mL) of ketamine, acepromazine, and xylazine mixture (50 mg, 0.5mg, and 5 mg/mL). Blood samples (1 mL each time) were taken 1, 2, 3, 7, 10, and 14 days after labeling while the animals continued to consume the experimental diets. Plasma cholesterol concentrations were measured, and the radioactivity (dpm) of [^{3}H] and [^{14}C] was counted. Percent absorption of dietary cholesterol was obtained by dividing the mean plasma ^{14}C/^{3}H ratio of radio activity (dpm) by the theoretical ratio of the total administrated [^{14}C] and [^{3}H] multiplied by 100. The data from samples obtained on days 2, 3, and 7 were used to calculate the mean plasma ^{14}C/^{3}H ratio because [^{14}C] radio activity was insufficient in samples after day 10.

Chemical Analysis

Assays for bile acids and sterols. Bile acids were quantitated by capillary gas chromatography as previously described.[12]

In the presence of an internal standard, coprostanol, cholesterol in plasma (100 μL) or bile (500 μL) was quantitated by capillary gas-liquid chromatography as previously described.[17] For hepatic sterols, 0.5 g of liver tissue with 200 μg coprostanol (internal standard) was homogenized, and its neutral sterols were extracted with chloroform. After evaporation, the residue was dissolved in 10 mL of ethyl acetate. Three milliliters of the solution was hydrolyzed, and the released free cholesterol was quantitated by gas chromatography as mentioned previously.[17]

Hepatic microsomal cholesterol 7α-hydroxylase and HMG-CoA reductase and mitochondrial cholesterol 27-hydroxylase activities. Hepatic microsomes and mitochondria were prepared by differential ultracentrifugation,[18] and the protein level was determined according to the method of Lowry et al.[19]

Hepatic microsomes were used for determination the activities of HMG-CoA reductase and cholesterol 7α-hydroxylase. The assay for HMG-CoA reductase activity was as described previously[12] and was mainly based on the method by George et al.[20] Cholesterol 7α-hydroxylase activity was measured in hepatic microsomes after removal of endogenous lipid by acetone and reconstitution of the microsomal protein with cholesterol and optimal amounts of cofactors by the isotope incorporation method of Shefer et al.[18] Mitochondrial sterol 27-hydroxylase activity was assayed by an isotope incorporation method as described previously by Shefer et al.[21]

Receptor-mediated low-density lipoprotein binding. Receptor-mediated low-density lipoprotein (LDL) binding to rabbit liver membranes was assayed by the method described previously by Nguyen et al[22] and Kovanen et al.[23] Receptor-mediated LDL binding to the liver membranes was determined as the difference between total binding of [^{125}I]LDL (assayed in the absence of unlabeled LDL) and nonspecific binding (assayed in the presence of 40-fold excess unlabeled LDL).

Statistical Study

Data are shown as means ± SD and were compared statistically by use of the Student *t* test (unpaired). The BMDP Statistical Software

(BMDP Statistical Software, Los Angeles, CA) was used for statistical evaluations. $P < .05$ was considered statistically significant.

RESULTS

Study 1: To Determine the Effect of Increasing Doses of Dietary Stanols Fatty Acid Esters (SE) on Plasma Cholesterol Levels in Cholesterol-fed Rabbits

In this study, NZW rabbits were fed either chow or chow enriched with 0.2% Ch, Ch + 0.33% SE, Ch + 0.66% SE, Ch + 1.2% SE, or Ch + 2.4% SE.

After 2 weeks, plasma cholesterol levels increased 4-fold and did not decrease when either 0.33% or 0.66% SE was incorporated into the Ch diet (Fig 2). However, after addition of 1.2% SE to Ch diet, plasma cholesterol concentrations were reduced 58% ($P < .01$) to 69 ± 22 mg/dL. Doubling the dietary SE intake to 2.4% did not further reduce plasma cholesterol levels (Fig 2).

Percent cholesterol absorption measured by the plasma dual-isotope ratio method was similar in controls and Ch-fed rabbits (Fig 3) and did not decrease after addition of 0.33% or 0.66% SE to the Ch-enriched rabbit chow. However, percent cholesterol absorption decreased 46% ($P < .01$) after 1.2% SE was incorporated to the Ch diet and did not significantly decrease further after doubling of the amount of SE (2.4%) in the Ch diet (Fig.3).

Study 2: Effect of 1.2% SE on Intestinal Cholesterol Absorption, Plasma Cholesterol Levels, and Metabolism in Ch-fed Rabbits.

After 2 weeks of feeding, plasma cholesterol levels increased 3.6 times in the Ch group (from 37.1 ± 5.4 mg/dL to 146.1 ± 21.5 mg/dL, $P < .001$) but decreased 50% ($P < .001$) from this level when 1.2% SE was added to the Ch diet (72.4 ± 23.3 mg/dL). Feeding of 1.2% SE alone reduced plasma cholesterol concentrations16% (31.1 ± 5.8 mg/dL; NS) compared with untreated baseline control. After 4 weeks of feeding, plasma cholesterol concentrations in the Ch + 1.2% SE group increased insignificantly to 99.6 ± 54.6 mg/dL but remained significantly lower ($P < .001$) than the group fed Ch alone for 4 weeks (263.0 ± 34.1 mg/dL).

Figure 4 shows that plasma stanol (campestanol and sitostanol) concentrations did not increase after feeding of either 1.2% SE alone or 1.2% SE + Ch. Similarly, levels of the unsaturated plant sterols campesterol and sitosterol in the plasma, which were 0.41 ± 0.35 mg/dL and 0.42 ± 0.12 mg/dL, respectively, at baseline, did not change significantly after feeding of either 1.2% SE (0.21 ± 0.22 mg/dL and 0.20 ± 0.06 mg/dL) or 1.2% SE + Ch (0.17 ± 0.15 mg/dL and 0.21 ± 0.20 mg/dL).

Table 1 shows changes in biliary stanol and sterol concentrations (μg /mL) and output (μg/h) in rabbits after feeding of

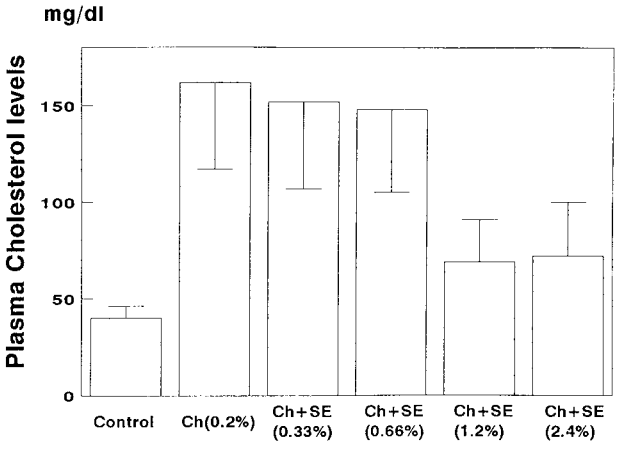

Fig 2. Plasma cholesterol levels in rabbits with different treatments: 0.2% Ch and 0.33%, 0.66%, 1.2%, and 2.4% SE added to 0.2% Ch. Data are presented as means ± SD. Plasma cholesterol levels increase significantly in rabbits fed 0.2% Ch alone and 0.2% Ch + 0.33% or 0.66% SE compared with the baseline values, but decrease significantly compared with those fed 0.2% Ch alone after addition of 1.2% or 2.4% SE to the cholesterol diet.

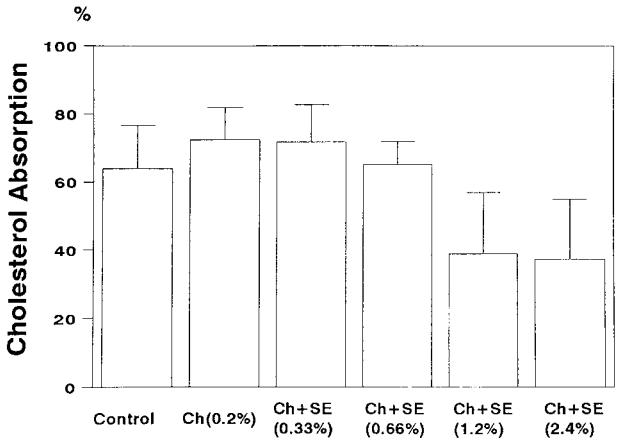

Fig 3. Intestinal cholesterol absorption (presented as percent dietary cholesterol absorbed) in rabbits with different treatments: 0.2% Ch and 0.33%, 0.66%, 1.2%, and 2.4% SE added to 0.2% Ch. Data are presented as means ± SD. The absorption rate is decreased significantly compared with those fed 0.2% Ch alone after addition 1.2% or 2.4% SE to the cholesterol diet.

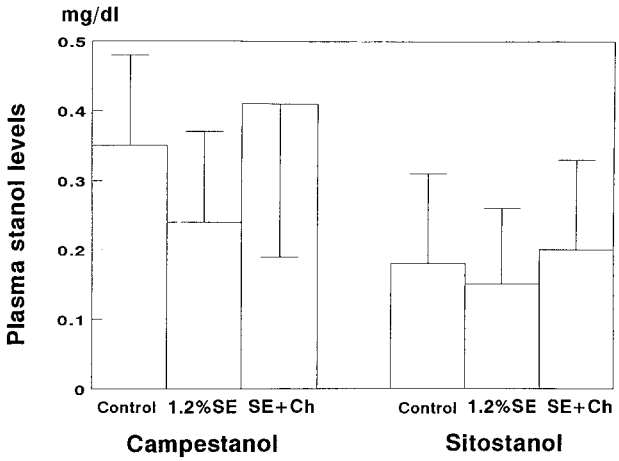

Fig 4. Effect of 1.2% SE feeding on plasma stanol concentrations in rabbits. Data are presented as means ± SD. SE + Ch: 1.2% SE + 0.2% Ch. There is no significant change in stanol concentrations in plasma.

Table 1. Effect of SE Feeding on Biliary Lipid Concentrations and Output

	Cholesterol	Campestanol	Campesterol	Sitostanol	Sitosterol
Control					
Concentration (μg/mL)	9.2 ± 1.0	0.11 ± 0.06	1.1 ± 0.4	0.2 ± 0.1	1.8 ± 0.3
Output (μg/h)	209 ± 48	2.1 ± 0.5	25.8 ± 8.0	5.2 ± 2.4	40.5 ± 10.0
1.2% SE					
Concentration (μg/mL)	15.8 ± 8.6	0.48 ± 0.10*	0.6 ± 0.2	0.6 ± 0.1†	0.9 ± 0.2†
Output (μg/h)	338 ± 150	10.1 ± 2.6†	11.8 ± 3.3‡	12.8 ± 3.8‡	18.0 ± 4.5‡

NOTE. Data are presented as means ± SD; 1.2% SE, feeding of 1.2% stanol fatty acid esters for 4 weeks.
*$P < .001$ compared with controls.
†$P < .01$ compared with controls.
‡$P < .05$ compared with controls.

1.2% SE for 4 weeks. Biliary campestanol concentration increased more than 4 times ($P < .001$) and sitostanol increased 3 times ($P < .01$) while campesterol and sitosterol concentrations decreased 45% (P = NS) and 50% ($P < .01$), respectively, in rabbits fed 1.2% SE alone. Biliary outputs (μg/h) of campestanol and sitostanol increased 5 times ($P < .01$) and 2.4 times ($P < .05$) while campesterol and sitosterol decreased 54% ($P < .05$) and 56% ($P < .05$), respectively, after feeding of 1.2% SE. Biliary cholesterol outputs increased 62% in rabbits fed 1.2% SE alone; this change was not statistically significant.

Hepatic cholesterol concentrations increased 5.7-fold in the Ch-fed group (13.4 ± 1.9 mg/g; $P < .0001$) compared with baseline control values (2.3 ± 0.2 mg/g; Fig 5). After addition of 1.2% SE to the Ch diet, hepatic cholesterol levels decreased 64% (4.9 ± 2.2 mg/g; $P < .0001$). However, after feeding of 1.2% SE alone, hepatic cholesterol levels (2.5 ± 0.3mg/g) did not differ from baseline.

The bile acid pool size was 212 ± 11 mg in controls and 188 ± 28 mg in rabbits fed 1.2% SE alone, but increased 89% ($P < .01$) to 401 ± 66 mg in the Ch group (Fig 6). After addiation of 1.2% SE to the Ch diet, the bile acid pool size (264 ± 41mg) did not increase significantly.

Percent cholesterol absorption in rabbits fed the Ch diet (0.3 g/d) was 73.0% ± 8.1% of intake and was not significantly different from that in the untreated control group (63.8% ± 15.3%). However, after addition of 1.2% SE to the Ch diet, percent cholesterol absorption decreased 43.8% ($P < .001$) to 41.0% ± 11.5%, which was similar to that in rabbits fed 1.2% SE alone (40.0% ± 7.5%). The percent absorption in both 1.2% SE and 1.2% SE + 0.2% Ch groups were significantly lower than untreated baseline control values ($P < .001$ and $P < .05$, respectively).

Cholesterol 7α-hydroxylase activity reflecting classic bile acid synthesis was inhibited only in the Ch-fed group (13.5 ± 9.8 pmol/mg/min; $P < .01$), in which the bile acid pool had expanded 89% (Fig 6) compared with baseline but was not significantly decreased in the 1.2% SE or the Ch + 1.2% SE group (Table 2).

Cholesterol 27-hydroxylase activity (alternative bile acid synthesis) was not changed significantly in any of the treated groups compared with controls (Table 2).

As expected, HMG-CoA reductase activity reflecting hepatic cholesterol synthesis significantly decreased in the groups fed Ch (from 28.1 ± 7.9 to 7.0 ± 3.9 pmol/mg/min; $P < .0001$)

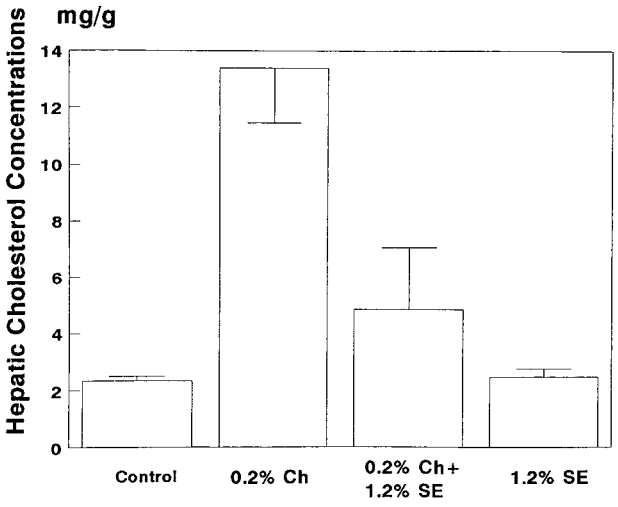

Fig 5. Effect of 1.2% SE feeding on hepatic cholesterol concentrations in rabbits. Data are presented as means ± SD. Hepatic cholesterol levels in rabbits fed 0.2% Ch alone are significantly higher than the controls but decrease significantly after addition of 1.2% SE to the 0.2% Ch diet. There is no significant difference between controls and rabbits fed 1.2% SE alone.

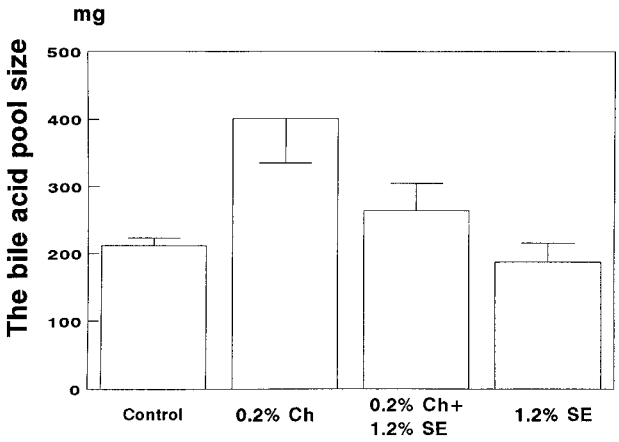

Fig 6. Effect of 1.2% SE feeding on bile acid pool size in rabbits. Data are presented as means ± SD. The bile acid pool size expands significantly only in rabbits fed 0.2% Ch alone.

Table 2. Hepatic Enzyme Activities in Rabbits

	Control	Ch	1.2% SE + Ch	1.2% SE
Cyp7a	30.8 ± 8.7 (n = 7)	13.5 ± 9.8† (n = 5)	19.8 ± 10.7 (n = 5)	25.8 ± 6.1 (n = 3)
Cyp27	27.8 ± 8.0 (n = 5)	32.1 ± 10.8 (n = 4)	29.3 ± 9.0 (n = 4)	26.5 ± 5.5 (n = 3)
HMG-CoA	28.1 ± 7.9 (n = 7)	7.0 ± 3.9* (n = 9)	6.6 ± 2.9* (n = 5)	10.3 ± 5.2† (n = 3)

NOTE. Data are presented as means ± SEM (pmol/mg/min). Cyp7a, cholesterol 7α-hydroxylase; Cyp27, cholesterol 27-hydroxylase; Ch, 0.2% cholesterol; SE, stanol fatty acid esters.

*$P < .001$ compared with controls.

†$P < .01$ compared with controls.

and Ch + 1.2% SE (6.6 ± 2.9 pmol/mg/min; $P < .001$), in which both plasma and hepatic cholesterol concentrations increased. However, in rabbits fed 1.2% SE alone, in which plasma and hepatic cholesterol levels did not increase, hepatic HMG-CoA reductase activity unexpectedly decreased 63% (10.3 ± 5.2 pmol/mg/min; $P < .01$).

The results of LDL receptor-mediated binding are summarized in Table 3. Hepatic LDL receptor-mediated binding decreased 42% ($P < .05$) in rabbits fed Ch, 57.7% ($P < .05$) in rabbits fed Ch + 1.2% SE, and 57% ($P < .05$) in rabbits fed 1.2% SE alone. These reductions were consistent with the decrease in HMG-CoA reductase activity in the respective rabbit groups.

DISCUSSION

This study showed that SE incorporated into the diet with cholesterol inhibited intestinal cholesterol absorption to reduce plasma and hepatic cholesterol concentrations in cholesterol-fed rabbits. The dose of stanols required to reduce cholesterol absorption was approximately 3 times (1.07 g stanols *v* 0.3 g cholesterol) as much as the cholesterol intake.

We have previously reported[24] that feeding of 0.2% sitosterol alone (0.3 g/d) to NZW rabbits for 10 days reduced baseline plasma cholesterol levels. We believe that in the present study, the significant reduction of plasma cholesterol levels in rabbits fed 1.2% SE + Ch was caused by the inhibitory effect of SE on cholesterol absorption. Addition of 1.2% SE to the Ch diets reduced percent cholesterol absorption to 41%, which was similar to the percent absorption (40%) in rabbits fed 1.2% SE alone. In addition, when 0.33% or 0.66% SE was added to the Ch diet, there was neither a decrease in percent cholesterol absorption nor a reduction in plasma cholesterol levels. This fact suggests that to decrease the intestinal cholesterol absorption, a critical mass of SE must be present compared with the amount of cholesterol. As long as the proportion of dietary plant stanols has not reached this critical level to displace cholesterol from micelles, cholesterol absorption remains relatively intact. However, because the micelles do not differentiate cholesterol from stanols, the further increase of intestinal stanols with similar structures saturates the micelles and displaces cholesterol from the micellar mixture. Because cholesterol must be solublized in micelles to reach the enterocyte for absorption, the significant reduction of micellar cholesterol below the critical level decreases the opportunity for cholesterol to be picked up. These results suggest that the stanols compete with cholesterol for space in the micelles while the enterocyte can differentiate cholesterol from the stanols, although there is only a very small difference in the side chain with 5α-dihydro reduction. This study showed that doubling of SE to 2.4% of intake did not further reduce cholesterol absorption or lower plasma cholesterol levels in the cholesterol-fed rabbits. This finding indicated that 1.2% SE had saturated the micelle, and greater dietary intake could not further displace cholesterol from micelles. In addition to our speculation of micellar competition mentioned above, it might also be possible that stanols inhibit intestinal cholesterol absorption by competing with cholesterol for a surface receptor on the membrane of enterocytes, which is assumed to transport cholesterol into enterocytes. Recently, it has been reported that adenosine triphosphate–binding cassette transporter ABC-1 is a key gene involved in the active pathway for the efflux of cholesterol from cells[25] and is the target gene of orphan nuclear receptor LXRα.[26] Repa et al[27] suggested that dietary cholesterol, which is transformed into oxysterols, the ligand for LXRα, activates LXRα, which up-regulates ABC-1 in the intestine to decrease cholesterol absorption by pumping cholesterol back into the lumen. However, in our study, reduction of cholesterol absorption in rabbits fed SE + Ch compared with those fed Ch alone was not attributable to the effect of dietary cholesterol on ABC-1 mentioned above because both rabbit groups are fed similar amounts of cholesterol. In addition, there is no report indicating that plant stanols are ligands for LXRα.

Table 3. LDL Receptor-Mediated Binding in Rabbits Fed Cholesterol and Stanol Fatty Acid Esters

	Control	0.2% Ch	0.2% Ch + 1.2% SE	1.2% SE
LDL binding (ng/mg protein)	120.5 ± 41.0 (n = 8)	69.9 ± 37.7* (n = 6)	51.0 ± 37.7* (n = 5)	51.8 ± 14.5* (n = 3)

NOTE. Data are presented as means ± SD.

*$P < .05$ compared with controls.

As expected, hepatic HMG-CoA reductase activity was significantly suppressed by dietary cholesterol, even when SE was added to the diets, because hepatic cholesterol concentrations increased. However, it was unexpected to find that feeding of 1.2% SE (sitostanol + campestanol + fatty acids) alone, which did not increase hepatic cholesterol levels but decreased plasma levels, would also inhibit hepatic HMG-CoA reductase. In this study, feeding of 1.2% SE reduced intestinal cholesterol absorption, which might be expected to enhance hepatic cholesterol synthesis (reflected by increased HMG-CoA reductase activity). The enzyme assays were carefully performed in duplicate, and the results were repeated. Therefore, the inhibitory effects of the stanol fatty acid esters on hepatic HMG-CoA reductase are different from those of free sterols (for example, sitosterol) and do not appear to be related to the inhibitory effect on intestinal absorption of cholesterol. In addition, results from LDL receptor-mediated binding measurements were consistent with the changes in HMG-CoA reductase activity in rabbits fed 1.2% SE. This result may be an important additional advantage of this treatment.

As expected, cholesterol feeding was associated with inhibited cholesterol 7α-hydroxylase because increased dietary cholesterol induced expansion of the bile acid pool size.[28] In comparison, cholesterol 7α-hydroxylase activity was not inhibited in rabbits fed Ch + 1.2% SE because the bile acid pool size did not increase significantly. However, it is interesting to consider how SE feeding could prevent expansion of the bile acid pool. We hypothesize that SE might also interfere with ileal bile acid transporter function, which plays an important role in controlling the size of bile acid pool in rabbits.[29] Blocking of cholesterol absorption with SE may offset the up-regulatory effect of dietary cholesterol on stimulating ileal bile acid transporter function. As a result, ileal bile acid reabsorption is not increased by dietary cholesterol, the bile acid pool size does not enlarge, and cholesterol 7α-hydroxylase is not inhibited. Prevention of the expansion of the bile acid pool by SE feeding, which prevented inhibition of cholesterol 7α-hydroxylase, may also play a considerable role in reduction of plasma cholesterol levels because we previously reported that inhibition of cholesterol 7α-hydroxylase (bile acid synthesis) was associated with increased plasma cholesterol levels,[24] but up-regulation of cholesterol 7α-hydroxylase resulted in decreased plasma cholesterol levels.[30]

This study also showed that although abundant SEs were fed to the animals, plasma stanol concentrations did not increase. This result supports the idea that the SEs are poorly absorbed and therefore are safe agents for lowering plasma cholesterol levels.

In conclusion, our results suggest that addition of a critical mass of SE to the diet inhibited intestinal cholesterol absorption by displacing cholesterol from micelles to reduce plasma cholesterol levels.

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