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Dietary Plant Sterol Esters Must Be Hydrolyzed to Reduce Intestinal Cholesterol Absorption in Hamsters^{1–3}

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Abstract

Background: Elevated concentrations of LDL cholesterol are associated with the development of atherosclerosis and therefore are considered an important target for intervention to prevent cardiovascular diseases. The inhibition of cholesterol absorption in the small intestine is an attractive approach to lowering plasma cholesterol, one that is addressed by drug therapy as well as dietary supplementation with plant sterols and plant sterol esters (PSEs).

Objective: This study was conducted to test the hypothesis that the cholesterol-lowering effects of PSE require hydrolysis to free sterols (FSs).

Methods: Male Syrian hamsters were fed atherogenic diets (AIN-93M purified diet containing 0.12% cholesterol and 8% coconut oil) to which one of the following was added: no PSEs or ethers (control), 5% sterol stearate esters, 5% sterol palmitate esters (PEs), 5% sterol oleate esters (OEs), 5% sterol stearate ethers (STs; to mimic nonhydrolyzable PSE), or 3% FSs plus 2% sunflower oil. The treatments effectively created a spectrum of PSE hydrolysis across which cholesterol metabolism could be compared. Metabolic measurements included cholesterol absorption, plasma and liver lipid concentration, and fecal neutral sterol and bile acid excretion.

Results: The STs and the PEs and SEs were poorly hydrolyzed (1.69–4.12%). In contrast, OEs were 88.3% hydrolyzed. The percent hydrolysis was negatively correlated with cholesterol absorption ($r = -0.85$; $P < 0.0001$) and positively correlated with fecal cholesterol excretion ($r = 0.92$; $P < 0.0001$), suggesting that PSE hydrolysis plays a central role in the cholesterol-lowering properties of PSE.

Conclusions: Our data on hamsters suggest that PSE hydrolysis and the presence of FSs is necessary to induce an optimum cholesterol-lowering effect and that poorly hydrolyzed PSEs may lower cholesterol through an alternative mechanism than that of competition with cholesterol for micelle incorporation. *J Nutr* 2015;145:1402–7.

Keywords: cholesterol absorption, micelles, hamsters, plant sterol esters, phytosterol esters, intestinal hydrolysis

Introduction

Circulating LDL cholesterol is associated with the development of atherosclerosis and is therefore considered an important therapeutic target for prevention of cardiovascular diseases. The inhibition of cholesterol absorption in the small intestine is an attractive approach to lowering LDL cholesterol, and dietary supplementation with plant sterols or their FA esters is an effective, nondrug means to accomplish this. A number of mechanisms have been proposed to explain how

plant sterols (or stanols) lower plasma cholesterol levels. Evidence for intracellular mechanisms is emerging whereby plant sterols alter expression of enterocyte sterol transporters, thus shifting the balance between basolateral secretion and apical excretion (1, 2). Historically, however, the primary focus has been on the physiochemical interactions of plant sterols with cholesterol and other biomolecules in the gut lumen. The following mechanisms have been proposed by which these interactions reduce cholesterol uptake: 1) cocrystallization of plant sterols and cholesterol forming insoluble crystals, 2) interaction of plant sterols with digestive enzymes, and 3) competition between cholesterol and plant sterols for solubilization into dietary mixed micelles (3). The latter mechanism is currently the most widely accepted; however, direct evidence for its validity is limited.

Many food products are now available containing plant sterols or their FA esters. Because esterification significantly increases sterol solubility, plant sterol esters (PSEs)⁷ are more

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³ Supplemental Tables 1 and 2 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at <http://jn.nutrition.org>.

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⁷ Abbreviations used: FS, free sterol; OE, oleate ester; PE, palmitate ester; PSE, plant sterol ester; ST, stearate ether.

easily incorporated in food products. However, the effectiveness of PSEs vs. unesterified plant sterols for reducing uptake of cholesterol has not been thoroughly investigated; the same is true for the degree to which inhibition of cholesterol absorption by PSEs results from hydrolysis to free sterols (FSs). It has been assumed that the molecular component of PSE that competes with cholesterol is the FS and that this moiety must be freed from the FA through hydrolysis of the ester bond in order to be effective. However, definitive evidence confirming this assumption remains lacking. In addition, an examination of the effect of sterol hydrophobicity on micelle incorporation suggests that low sterol polarity hinders micelle solubility (4). Considering the highly nonpolar nature of esterified sterols, we predict that PSE would be excluded from micelle incorporation. In fact, studies in our laboratory have demonstrated the ability of both unesterified sitosterol and stigmasterol, but not intact PSE, to displace cholesterol from micelles (5, 6). This suggests that the FA moiety prevents PSE from competing with cholesterol for micelle solubilization. Furthermore, we observed in hamsters that dietary plant sterol stearates were unable to reduce cholesterol absorption compared with a control diet, and that the stearate esters were <5% hydrolyzed (7), thus supporting the notion that the competitive mechanism necessitates hydrolysis. To test the hypothesis that dietary PSEs must be hydrolyzed in order to impart their cholesterol-lowering effect, we prepared PSEs and a sterol ether possessing a range of susceptibilities to hydrolysis and investigated their relative hydrolysis in an animal model.

Methods

Animals and diets. Sixty male Syrian hamsters (BioBreeders) weighing 73–87 g were divided into 6 experimental groups of 10. Our previous work (8) in hamsters fed 5% PSEs in a high protein demonstrated a 30–50% change in parameters of interest, including cholesterol absorption and non-HDL-cholesterol concentration. Power calculations suggested 100% power to detect such changes with a sample size of 10. All hamsters were housed in polycarbonate cages with sawdust bedding and were given free access to food for 29 d. They were maintained in a humidity- and temperature-controlled (25°C) room with a 12-h light-dark cycle. During the first 4 d, all animals were given a control diet for acclimation, after which treatment diets were administered for the duration of the study. The control diet was an AIN-93M purified diet (9) containing 0.12% (wt:wt) cholesterol and 8% coconut oil. Each treatment diet consisted of the control diet with replacement of 5% cornstarch with an equivalent mass of sterol stearate ethers (STs), sterol stearate esters, sterol palmitate esters (PEs), sterol oleate esters (OEs), or 3% FSs plus 2% high oleic acid sunflower oil to mimic fully hydrolyzed PSE (Supplemental Table 1). The AIN-93 mineral and vitamin mixes, casein, dextrinized cornstarch, fibers, and coconut oil were purchased from Dyets, Inc. Choline bitartrate, L-cystine, and cholesterol were purchased from Sigma Chemicals. Cornstarch, sucrose, and soybean oil were purchased from a local grocery store. All procedures were approved by the Institutional Animal Care and Use Committee of the University of Nebraska-Lincoln.

Food consumption was recorded twice per week and body weight was recorded weekly. Feces were collected on day 21 for neutral sterol and bile acid analysis. On day 29, hamsters were asphyxiated by carbon dioxide gas and the thoracic cavity was opened to allow for removal of blood by cardiac puncture using 10-mL syringes. Blood was then placed into 10-mg EDTA-coated tubes and centrifuged to separate the plasma and RBCs. Plasma was then placed into cryotubes and frozen at –80°C until analyzed for lipoprotein concentration. Bile was removed from gallbladders by aspiration with preweighed 1-mL syringes, diluted with saline, and transferred to microcentrifuge tubes for immediate freezing in dry ice. Livers were excised, weighed, and quickly frozen in dry ice and stored at –80°C until analyzed for lipid concentration.

Sterol ester and ether synthesis. Soybean sterols (Archer Daniels Midland Co.) were esterified with palmitic, stearic, or oleic acid as previously described (10). The sterol ST was prepared by alkylation of the sodium alkoxide. Sodium hydride (60% suspension in oil) was washed with a small volume of hexane under an atmosphere of nitrogen. The resulting pyrophoric powder was maintained at all times under an atmosphere of nitrogen. A solution of sterol in tetrahydrofuran was cautiously added to a suspension of the washed sodium hydride in tetrahydrofuran, followed by iodooctadecane. The reaction presented a mixture of the desired octadecyl (stearate) ethers, octadecene as a by-product of elimination, and recovered sterol. The octadecyl ether was purified by multiple recrystallizations from ethyl acetate and analyzed for purity by proton and carbon NMR (¹H and ¹³C NMR).

Analytic procedures. Previously published methods were used to measure liver lipid concentration (11), plasma lipoprotein cholesterol concentration (12), and fecal bile acids and neutral sterols (13). The extent of PSE hydrolysis was calculated as the ratio of fecal unesterified plant sterols relative to total (unesterified + esterified) plant sterols. Cholesterol absorption efficiency was measured by the dual isotope ratio method using [³H]sitostanol (nonabsorbed reference molecule) and [¹⁴C] cholesterol (14, 15).

Statistical analysis. One-factor ANOVA analysis was used to compare study endpoints of the treatment groups. Mean values were analyzed for treatment differences using the Tukey's multiple comparison procedure, and mean values were considered significantly different at *P* < 0.05. Scheffé correction was used where unequal variance was suspected (e.g., fecal sterol excretion), which gave the same results as Tukey's procedure. Association between experimental endpoints was analyzed by Pearson's product-moment correlation analysis. Statistical procedures were conducted using JMP 8 (SAS Institute, Inc.).

Results

Body weight and food intake. Modest yet statistically significant differences existed between some treatment groups throughout the study period (Supplemental Table 2). Because of random assignment of hamsters to treatments at the beginning of the study, the mean body weight of the ST group was significantly and consistently lower than the control at each week of measurement. However, body weight gain was not significantly different among any of the groups throughout the study (data not shown), indicating no effect of diet on hamster growth.

TABLE 1 Cholesterol absorption and plasma cholesterol concentration in male hamsters fed diets containing various PSEs¹

	Control diet	ST diet	Stearate ester diet	PE diet	OE diet	FS diet
Cholesterol absorption, %	64.9 ± 2.1 ^a	36.9 ± 0.8 ^c	56.3 ± 1.8 ^{a,b}	53.5 ± 3.7 ^b	14.9 ± 3.8 ^d	12.8 ± 1.7 ^d
Non-HDL cholesterol, mmol/L	6.73 ± 0.35 ^a	6.15 ± 0.32 ^a	6.22 ± 0.33 ^a	5.89 ± 0.26 ^a	2.81 ± 0.18 ^b	2.84 ± 0.15 ^b
HDL cholesterol, mmol/L	2.42 ± 0.09 ^a	1.68 ± 0.09 ^b	2.24 ± 0.08 ^a	2.18 ± 0.06 ^a	1.67 ± 0.13 ^b	1.57 ± 0.07 ^b

¹ Values are means ± SEMs (*n* = 10). Labeled means in a row without a common letter differ, *P* < 0.05. FS, free sterol; OE, oleate ester; PE, palmitate ester; PSE, plant sterol ester; ST, stearate ether.

TABLE 2 Liver weight and liver lipid concentrations in male hamsters fed diets containing various PSEs¹

	Control diet	ST diet	Stearate ester diet	PE diet	OE diet	FS diet
Total cholesterol, $\mu\text{mol/g}$	37.5 \pm 2.21 ^a	11.1 \pm 0.60 ^c	24.8 \pm 2.11 ^b	28.4 \pm 2.02 ^b	6.46 \pm 0.32 ^c	6.09 \pm 0.27 ^c
Esterified cholesterol, $\mu\text{mol/g}$	30.9 \pm 2.23 ^a	5.37 \pm 0.55 ^c	18.9 \pm 2.02 ^b	21.9 \pm 1.98 ^b	2.14 \pm 0.34 ^c	1.79 \pm 0.34 ^c
Free cholesterol, $\mu\text{mol/g}$	6.61 \pm 0.14 ^a	5.69 \pm 0.13 ^b	5.91 \pm 0.10 ^b	6.50 \pm 0.11 ^a	4.32 \pm 0.04 ^c	4.30 \pm 0.10 ^c
TG, $\mu\text{mol/g}$	1.91 \pm 0.10 ^a	1.51 \pm 0.10 ^b	1.61 \pm 0.10 ^b	1.67 \pm 0.10 ^b	2.33 \pm 0.19 ^{a,b}	1.98 \pm 0.07 ^{a,b}
Phospholipid, $\mu\text{mol/g}$	17.0 \pm 0.28 ^b	16.9 \pm 0.19 ^b	17.1 \pm 0.18 ^b	17.8 \pm 0.28 ^{a,b}	17.9 \pm 0.16 ^{a,b}	18.3 \pm 0.41 ^a
Liver weight, g	5.61 \pm 0.10 ^a	5.61 \pm 0.13 ^a	5.38 \pm 0.15 ^{a,b}	4.94 \pm 0.10 ^b	3.90 \pm 0.12 ^c	3.62 \pm 0.18 ^c

¹ Values are means \pm SEMs ($n = 10$). Labeled means in a row without a common letter differ, $P < 0.05$. FS, free sterol; OE, oleate ester; PE, palmitate ester; PSE, plant sterol ester; ST, stearate ether.

No significant differences in food intake were observed among the groups throughout the study. Overall mean food intake during week 4 for all hamsters combined was 6.6 ± 0.2 g/d.

Intestinal cholesterol absorption and plasma cholesterol concentration. Cholesterol absorption was significantly decreased in all groups compared with the control group except for stearate ester; cholesterol absorption in the OE and FS groups was significantly lower than in all other groups (Table 1). Non-HDL-cholesterol concentration was also significantly lower in the OE and FS groups than in the control, ST, stearate ester, and PE groups (Table 1). HDL-cholesterol concentration in hamsters fed ST, OE, and FS was similar and significantly lower than in the control, stearate ester, and PE groups (Table 1).

Liver weight and lipids. Liver weights (Table 2) for the stearate ester and ST groups did not differ from control ($P > 0.05$). PE group liver weight was significantly lower than that of the control and ST groups but not different from the stearate ester group. OE and FS were equivalently and significantly lower than all other groups ($P < 0.0001$). All treatments lowered total cholesterol significantly compared with the control group (Table 2), and ST, OE, and FS lowered total cholesterol significantly more than stearate ester or PE. The same trend between groups was seen in esterified cholesterol levels (Table 2). With the exception of PE, all treatments significantly lowered free cholesterol compared with the control. OE and FS lowered free cholesterol significantly relative to all other groups. Liver TGs (Table 2) of ST, stearate ester, and PE group were significantly lower than control but did not differ from the OE or FS group, and the OE and FS groups were no different than control. Phospholipid concentrations (Table 2) did not differ between control, ST, stearate ester, PE, and OE groups. The FS group, however, was significantly greater than the control, ST, and stearate ester groups, but no different than the PE or OE group.

Fecal sterols. Fecal bile acids (Table 3) were significantly lowered relative to control only in hamsters fed OE and FS. The

OE group had significantly lower fecal bile acids than did the ST, stearate ester, and PE groups, but not more than the FS group. Fecal neutral sterol content (Table 3) was significantly higher in the ST group than in the control group and in the stearate ester and PE groups than in the control and ST groups. The OE and FS fecal neutral sterol excretion was significantly greater than all other groups. Fecal plant sterol excretion was significantly elevated in all treatments relative to the control group. The percent of PSE hydrolysis was similar between ST and stearate ester, and PE was hydrolyzed significantly more than ST but not more than stearate ester. OE was hydrolyzed more than ST, stearate ester, and PE. FS hydrolysis was greater than all other groups.

Correlations. Percent hydrolysis was correlated negatively with percent cholesterol absorption ($r = -0.85$, $P < 0.0001$) and plasma non-HDL cholesterol ($r = -0.91$, $P < 0.0001$) and positively with fecal cholesterol excretion ($r = 0.92$, $P < 0.0001$; Figure 1A–C). Fecal cholesterol excretion was also significantly correlated with non-HDL cholesterol ($r = -0.84$, $P < 0.0001$; Figure 1D).

Discussion

The purpose of this study was to determine the relation between (or the degree to which) the susceptibility of PSEs to hydrolysis in the intestinal lumen and their ability to lower cholesterol. Studies conducted as early as the 1950s (16, 17) and later (18, 19) suggested that in order for dietary plant sterols to inhibit cholesterol absorption, they must be in an unesterified (free) state. However, few studies stating this hypothesis endeavored to quantify the degree to which dietary PSEs were hydrolyzed. Fewer still have studied hydrolysis in the hamster, a model with many similarities to human metabolism, which make it a uniquely important tool for such mechanistic work. These similarities include a low endogenous cholesterol synthesis rate, expression of cholesterol ester transfer protein, and uptake of up to $\sim 80\%$ of LDL cholesterol via the LDL receptor pathway.

TABLE 3 Fecal neutral sterol and bile acid excretion in male hamsters fed diets containing various PSEs¹

	Control diet	ST diet	Stearate ester diet	PE diet	OE diet	FS diet
Bile acids, $\mu\text{mol} \cdot \text{d}^{-1} \cdot 100 \text{ g BW}^{-1}$	0.58 \pm 0.04 ^{a,b}	0.57 \pm 0.04 ^{a,b}	0.75 \pm 0.05 ^a	0.71 \pm 0.04 ^a	0.37 \pm 0.04 ^c	0.43 \pm 0.05 ^{b,c}
Neutral sterol, $\mu\text{mol} \cdot \text{d}^{-1} \cdot 100 \text{ g BW}^{-1}$	2.84 \pm 0.00 ^d	11.9 \pm 0.30 ^b	6.72 \pm 0.47 ^c	6.72 \pm 0.46 ^c	21.1 \pm 0.79 ^a	21.7 \pm 0.71 ^a
Total plant sterol, $\mu\text{mol} \cdot \text{d}^{-1} \cdot 100 \text{ g BW}^{-1}$	1.31 \pm 0.10 ^a	345 \pm 11 ^b	358 \pm 16 ^b	362 \pm 16 ^b	359 \pm 16 ^b	387 \pm 16 ^b
Esterified plant sterol, $\mu\text{mol} \cdot \text{d}^{-1} \cdot 100 \text{ g BW}^{-1}$	0.0 \pm 0.0 ^c	339 \pm 11 ^a	346 \pm 16 ^a	348 \pm 15 ^a	42.5 \pm 4.2 ^b	0.0 \pm 0.0 ^c
Free plant sterol, $\mu\text{mol} \cdot \text{d}^{-1} \cdot 100 \text{ g BW}^{-1}$	1.31 \pm 0.10 ^c	5.81 \pm 0.12 ^c	11.2 \pm 0.82 ^c	14.9 \pm 0.83 ^c	317 \pm 13 ^b	387 \pm 16 ^a
PSE hydrolysis, %		1.69 \pm 0.03 ^d	3.13 \pm 0.19 ^{c,d}	4.12 \pm 0.15 ^c	88.3 \pm 0.81 ^b	100 \pm 0.0 ^a

¹ Values are means \pm SEMs ($n = 10$). Labeled means in a row without a common letter differ, $P < 0.05$. BW, body weight; FS, free sterol; OE, oleate ester; PE, palmitate ester; PSE, plant sterol ester; ST, stearate ether.

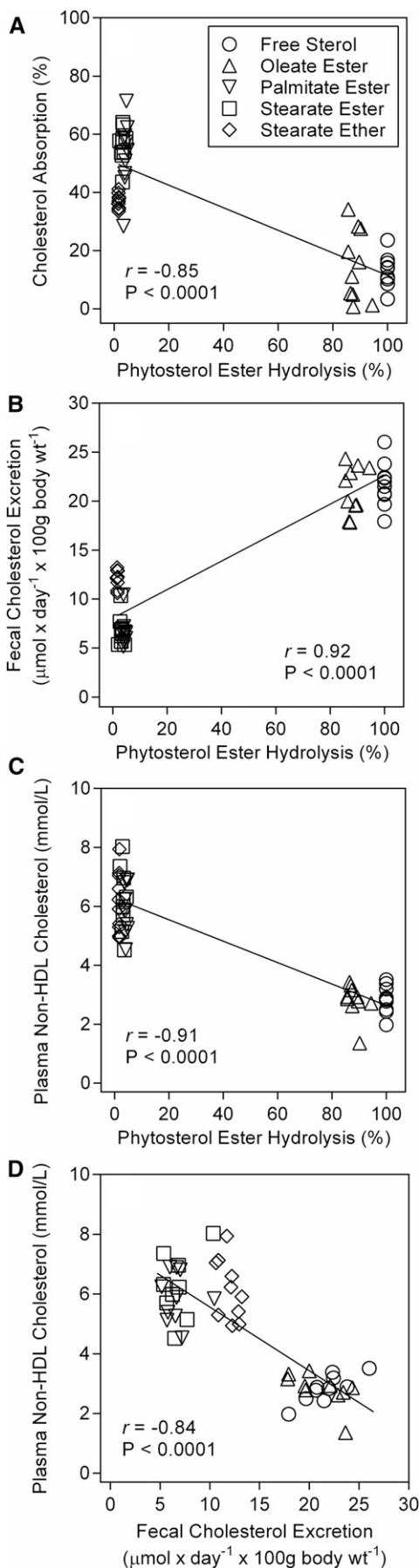


FIGURE 1 Pearson correlations between percent of PSE hydrolysis and cholesterol absorption (A), fecal cholesterol excretion (B), and

Research in our laboratory suggested that hydrolysis and hypocholesterolemic effects are linked; plant sterol stearates undergo minimal hydrolysis (0.88–4.69) in hamsters and produce no hypocholesterolemic effects (7). Therefore, the current study tested the hypothesis that hydrolysis of PSEs is required to elicit a cholesterol-lowering effect.

In this study, we synthesized 1 sterol ether and 4 sterol ester molecules that would be hydrolyzed to varying degrees when ingested. These relative levels of hydrolysis aligned very well with the predictions based on *in vitro* work previously conducted in this laboratory (10). In the current study, however, the range of hydrolysis was much less evenly distributed across the entire spectrum, with ST, stearate ester, and PE clustered at the lower end of the spectrum (~2–4% hydrolyzed), and OE and FS clustered at the upper end (88–100% hydrolyzed). Without coverage of the middle of the spectrum, we were left to speculate about the cholesterol-lowering efficiency of PSE that are only partially hydrolyzed. Consequently, the minimally effective degree of hydrolysis for maximally inhibiting cholesterol absorption could not be determined in this study. Such a dose could possibly be determined in subsequent studies using a mixture of PSEs designed to create precise degrees of hydrolysis.

Despite the clustering of data points at each end of the hydrolytic spectrum, changes in cholesterol metabolism among treatment groups support our hypothesis that hydrolysis is vital to the cholesterol-lowering action of PSEs. With the exception of ST, percent cholesterol absorption and degree of hydrolysis were inversely associated. Percent hydrolysis was also positively associated with fecal cholesterol excretion. These data are in agreement with early studies exploring the effects of plant sterols esterified to FAs of similar and dissimilar structure to our treatments. Experiments in a rat model showed that free sitosterol and sitosteryl oleate were nearly identical in their abilities to lower liver cholesterol compared with control (17). Sitosteryl palmitate, on the other hand, was significantly less capable of lowering liver cholesterol, agreeing with our conclusions that palmitate and OEs undergo differential hydrolysis, which accounts for the divergent cholesterol-lowering capabilities.

In support of our findings regarding the correlation between sterol ester hydrolysis and cholesterol-lowering effects, oleate and SEs of plant sterols fed to rats resulted in equivalently lowered liver cholesterol and increased fecal cholesterol excretion compared with a control group, with no change observed in the SE group (20). OEs were then found to be 99.5% hydrolyzed whereas SEs were only 19.2% hydrolyzed. Furthermore, in 2-wk-old chicks, a diet containing 1% cholesteryl caprate, an ester of a ten-carbon SFA, resulted in lower plasma and liver cholesterol compared with a diet containing 1% free cholesterol only (16). Also, a diet containing 1% free cholesterol and 1.38% soy sterol caprate resulted in elevated plasma and liver cholesterol compared with a diet containing 1% free cholesterol and 1.38% free soy sterols. These data align with ours in demonstrating that sterol esters of SFAs are incompletely hydrolyzed and thus impede the absorption or intestinal activity of their sterol moieties. Conversely, in a rat study (18), decanoate (caprate) and OEs of the same plant sterols possessed equivalent cholesterol-lowering abilities, contrasting other work performed in similar and different animal models (16, 20).

plasma non-HDL cholesterol (C) and between fecal cholesterol excretion and plasma non-HDL cholesterol (D) in male hamsters fed diets containing various PSEs ($n = 60$). PSE, plant sterol ester.

Previous work from our laboratory demonstrated that plant sterol stearates containing different plant sterol moieties failed to lower cholesterol when consumed at 2.5% of the diet in a hamster model of hypercholesterolemia (7). This was hypothesized to be the result of poor stearate ester hydrolysis. It was further hypothesized that nonhydrolyzed PSE may, at high doses, impart a modest cholesterol-lowering effect by forming an "oil phase" in the intestinal lumen that solubilizes cholesterol and prevents its micelle incorporation, thus lowering cholesterol absorption. Considering in this study that stearate esters were poorly hydrolyzed, yet still managed to lower liver esterified cholesterol and increase fecal neutral sterol excretion, the possibility exists that stearate esters at 5% of the diet succeeded in creating an oil phase capable of partitioning cholesterol away from micelles but 2.5% was insufficient. In opposition to this theory are our data regarding cholesterol absorption in which stearate esters, regardless of changes in liver cholesterol and fecal neutral sterol excretion, were unable to lower cholesterol absorption in relation to the control group. Furthermore, we were unable to observe a difference in cholesterol partitioning between oil and aqueous phases (data not shown).

A unique strength of our study design, in addition to the ability to compare treatment groups across the extent of PSE hydrolysis, was the ability to compare cholesterol metabolism between PSE varying in the nature of the esterified FA. In the case of stearate ester and PE, no differences were observed other than a slight lowering of liver free cholesterol in PE compared with stearate ester and a slightly lower liver weight in PE than in the control, which was not observed in stearate ester. Given that stearate ester and PE were hydrolyzed to the same extent, with only the few differential metabolic effects noted previously, it is likely that the FA moiety of the PSE does not play a primary role in the molecule's metabolism. This supports the hypothesis that the primary mechanism of PSE takes place through micellar cholesterol displacement and is dependent on hydrolysis above any other physical property of the PSE.

The comparison of stearate ester and ST groups was also informative in that there was no evidence of a difference in percent hydrolysis between the 2, but their metabolic effects regarding cholesterol metabolism varied greatly. Notably, although stearate ester did not alter cholesterol absorption compared with the control, ST lowered it by 43%. Also, ST lowered liver esterified cholesterol by 83%, whereas stearate ester only lowered it by 39%. The ST treatment group was included to represent a PSE that was fully intact with no hydrolysis. Given our hypothesis that hydrolysis of the PSE molecule is required for cholesterol lowering, the ST group represents an unexpected anomaly. Because hydrolysis of stearate ester and ST was similar with differential metabolic effects, it may be assumed that any differences in metabolism are caused by a structural difference between the 2 molecules. In this case, the only structural difference is that of a carbonyl group present on the first carbon of the stearic acid molecule in stearate ester that is not present on the ST molecule. As mentioned previously, poorly hydrolyzed PSE such as stearate ester and PE may work through an oil-phase mechanism. It is possible that the ST works through this mechanism as well and may act as a more potent oil-phase generator as a result of its unique chemistry. Remodeling of luminal nonpolar lipids, whereby TGs are hydrolyzed to FFAs, monoglycerides, and diglycerides, is necessary for efficient cholesterol absorption (21, 22). The ST molecule may interfere with remodeling lipases and prevent hydrolysis of lipids, resulting in a suboptimal luminal environment for cholesterol absorption.

Biliary bile acid composition has been shown to be diet labile. As a method of quantifying the biologic importance of compositional changes in bile acid mixtures, the relative hydrophobicity of each bile acid in a sample of bile, as calculated by its migration on an HPLC column in a reverse-phase system, may be used to arrive at a hydrophobicity index (23, 24). Work in our laboratory has shown PSEs (25) and free stearic acid (26) to be capable of inducing bile acid composition changes significant enough to alter the hydrophobicity index. Although the current study used the same 3% sterol equivalent of plant sterols as did Carr et al. (25), the changes in bile acid metabolism were not great enough to alter the hydrophobicity index (data not shown). This may be because of the use of a greater number of bile acids in the current analysis that may have balanced out any changes seen in the other bile acids analyzed previously. Also, differences existed between the methods used in the 2 studies. Direct UV detection of bile acids with a different HPLC system was used in the current study compared with fluorometric detection of NAD as an enzymatic by-product used previously. In addition, there seemed to be a high degree of variability within treatments of the current study that made detecting statistical differences between treatments difficult.

In summary, our findings indicate that hydrolysis of PSEs is necessary to induce an optimum cholesterol-lowering effect, suggesting that the FS molecule is active in lowering cholesterol and that competition with cholesterol for micellar incorporation is the primary mechanism through which plant sterols act. Additionally, poorly hydrolyzed PSEs may contribute a secondary cholesterol-lowering effect by producing an oil phase either by self-aggregation or by reducing the efficiency of other lipases. Finally, altering bile acid metabolism enough to affect the thermodynamic properties of micelle formation may not be an important mechanism by which PSEs affect cholesterol lowering.

Acknowledgments

TJC and TPC designed the study; JH and PHD synthesized sterol esters and ethers; TJC conducted the research; TJC and TPC analyzed the data; TJC, PHD, and TPC wrote the paper; TPC had primary responsibility for final content. All authors read and approved the final manuscript.

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ONLINE SUPPORTING MATERIAL

Supplemental Table 2. Hamster body weight

	Control diet	Stearate ether diet	Stearate ester diet	Palmitate ester diet	Oleate ester diet	Free sterol diet
				g		
Week 0	83.6 ± 0.8 ^a	79.6 ± 0.6 ^b	82.3 ± 1.2 ^{ab}	79.3 ± 0.8 ^b	81.2 ± 1.0 ^{ab}	81.3 ± 0.8 ^{ab}
Week 1	89.6 ± 0.9 ^a	84.2 ± 0.7 ^b	87.7 ± 1.1 ^{ab}	85.5 ± 1.0 ^{ab}	87.7 ± 0.9 ^{ab}	83.7 ± 1.8 ^b
Week 2	95.1 ± 1.2 ^a	89.1 ± 1.0 ^b	94.7 ± 1.2 ^a	91.2 ± 1.3 ^{ab}	94.9 ± 1.0 ^a	90.2 ± 1.5 ^{ab}
Week 3	99.9 ± 1.3 ^a	93.7 ± 0.9 ^b	99.1 ± 1.0 ^a	95.2 ± 1.3 ^{ab}	97.7 ± 1.3 ^{ab}	93.4 ± 1.4 ^b
Week 4	103.8 ± 1.5 ^a	97.1 ± 0.9 ^{bc}	102.2 ± 1.0 ^{ab}	98.8 ± 1.4 ^{abc}	101.5 ± 1.5 ^{abc}	96.7 ± 1.1 ^c

Values are means ± SEM (n = 10). Means within a row having different superscripts are significantly different (P < 0.05).