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Oral absorption of phytosterols and emulsified phytosterols by Sprague-Dawley rats

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Abstract

Clinical studies have demonstrated that consumption of phytosterol esters in lipid-based foods decreases serum concentrations of total and LDL cholesterol. These substances represent minimal potential for adverse effects when consumed orally because of their low bioavailability. However, some studies have reported estrogenic and other effects in laboratory animals treated parenterally with phytosterols, demonstrating that these substances may have the potential to cause adverse effects if absorbed. Water-soluble phytosterols have been prepared by formulation with emulsifiers to expand delivery options to include non–lipid-based foods. However, emulsifiers are used as excipients in the formulation of lipophilic pharmaceuticals to increase solubility, thereby increasing their absorption. Therefore, oral consumption of emulsified water-soluble phytosterols could potentially increase their absorption. In the current study, absorption of phytosterols prepared as water-soluble emulsified micelles with two different food-grade emulsifiers was evaluated in Sprague-Dawley rats and compared with absorption of non-micellar free phytosterols and esterified phytosterol mixtures dissolved in a lipophilic vehicle (soybean oil). Rats were dosed via gavage with 42 mg/kg of formulated phytosterol preparations. Blood was collected at 8, 16, 24, and 32 hours, extracted with hexane, derivatized with benzoyl chloride, and analyzed by high-performance liquid chromatography to determine concentrations of β-sitosterol, and campesterol. Plasma concentrations and $AUC_{0-32hours}$ [μg/mL/h] of β-sitosterol and campesterol were lower in plasma obtained from rats treated with emulsified phytosterol preparations than in animals treated with free phytosterols dissolved in soybean oil. Because the pharmacokinetic profile of water-soluble phytosterols is similar to that of phytosterols administered in a lipid vehicle, the safety profile is likely to be the same as that of phytosterols and phytosterol esters in currently used applications.

Keywords: Phytosterols; β-Sitosterol; Campesterol; Stigmasterol; Cholesterol

1. Introduction

Phytosterols are naturally occurring substances found in plants and that are structurally similar to cholesterol [1]. Although numerous unique phytosterols have been identified, β-sitosterol, campesterol, and stigmasterol account for the largest proportion in most sources [2]. While similar in structure to cholesterol, these phytosterols possess substitutions at position C-24 that are responsible for their poor absorption [3–5].

Phytosterols inhibit cholesterol absorption from the gastrointestinal system. Many clinical studies have demonstrated significant decreases in serum cholesterol concentrations after consumption of foods into which phytosterols were incorporated [6–8]. In those studies, phytosterols were administered as fatty acid esters to increase solubility and to facilitate incorporation into lipid-based foods. However, the cholesterol-lowering activity is attributable to the free phytosterols as they are hydrolyzed to free sterols and fatty acids in the gut [9,10].

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Laboratory animals administered phytosterols parenterally have demonstrated adverse effects including evidence of estrogenicity [11–13]. In contrast, oral exposure to phytosterols does not appear to cause adverse effects. Laboratory rats consuming dietary mixtures of phytosterol or pure β-sitosterol demonstrated no evidence of estrogenic activity even in assays sensitive enough to respond to weakly estrogenic phytochemicals such as coumesterol [14,15]. From these studies, it appears that the adverse effects of phytosterols are dependent on bioavailability.

Water-soluble phytostanols have been formulated as lecithin emulsified micelles with potential applications in non–lipid-based foods [16]. However, micellar preparations of lipophilic pharmaceuticals are formulated specifically to increase their solubility and hence also their absorption from the gut [17–19]. Phytosterols formulated with emulsifiers into water-soluble micelles may be more soluble within the gut and have the potential for increased absorption when consumed orally. The present studies were conducted to compare the absorption of emulsified phytosterol micelles with absorption of phytosterols and phytosterol esters in laboratory rats.

2. Methods and materials

2.1. Materials

Reference compounds campesterol ($C_{28}H_{48}O$; MW 400.7), stigmasterol ($C_{24}H_{48}O$; MW 412.7), β-sitosterol ($C_{29}H_{50}O$; MW 414.7), and other chemicals including KOH, ethanol, benzoyl chloride, 1,2-dichloroethane, and pyridine were obtained from Sigma Chemical (St. Louis, MO).

2.2. Phytosterols and phytosterol esters

The free phytosterols (lot #4763-36-6) used in this work were obtained from soybean oil distillates through a multistep purification process (Cargill NutriProducts, Eddyville, IA). The product used was a mixture of phytosterols. The relative composition of the sterols was 53% β-sitosterol, 20% campesterol, 20% stigmasterol, and 7% other phytosterols. Phytosteryl esters were produced by trans-esterification of free phytosterols with fatty acid methyl esters derived from canola oil (Cargill NutriProducts, Eddyville, IA). Phytosterols and phytosterol esters were dissolved in commercial soybean oil (SBO) at 8.2 wt%. The sterol content of soybean oil was not measured in the current study. However, in typical commercial oil it is present at very low concentrations (0.1–0.2%). The concentration used in the current study was high enough that the background concentration was considered to be negligible. These lipid-based delivery vehicles were used where indicated in the animal exposure studies.

2.3. Spray-dried phytosterol-lecithin micelles (powder and aqueous mixture)

Hydroxylated, deoiled soybean lecithin (Precept 8120, Central Soya, Fort Wayne, IN) was selected for preparation of emulsified phytosterols to endow the product with a higher hydrophile-lipophile balance value and therefore also a product with greater water dispersibility. Phytosterols and soybean lecithin were dissolved in hexane at a ratio of 1 part phytosterol to 2 parts hydroxylated lecithin. The solution was spray dried to a fine powder and then dried in a vacuum oven at 60°C and 1 Hg of absolute pressure to produce a water-soluble powder. Where indicated in the animal exposure studies, dry phytosterol powder was diluted and mixed with hot water at a concentration of 0.3% sterols (0.9% of the powder). This blend was mixed first with a hand-held homogenizer and then homogenized at 7500 psi using an APV Gaulin (Derby, England, UK), model 15 homogenizer. The resulting liquid dispersion was used in the animal trial.

2.4. Dry mixture of phytosterols and lecithin

Powdered phytosterols were melted and spray-microprilled to an approximate average particle size of ~10 μm. The powder was dry mixed with hydroxylated lecithin (Precept 8120, Central Soya) at a ratio of 1 part phytosterol to 2 parts lecithin. The resulting powder was used in the animal trial. To administer this mixture, the requisite amount of dry powder was suspended in water and dosed by oral gavage.

2.5. Spray-dried phytosterol-monoglyceride micelles (powder and aqueous mixture)

Mixed phytosterols were melted with saturated monoglycerides made from fully saturated soybean oil consisting of palmitic (6.7%) and stearic (91.4%) acids (Dimodan PVK, Danisco Cultor, New Century, KS), and Tween 60 (Quest International) in a ratio of 1 part sterols to 1.25 parts monoglycerides with a total Tween 60 concentration of 3.6%. The melt mixture was spray-prilled to a powder. Where indicated in the animal exposure studies, dry phytosterol powder was slurried with hot water, heated to 85°C, and passed through a two-stage homogenizer at 3000 and 500 psi on the first and second stages, respectively. The equipment used were a Microthermics HTST/UHT pasteurizer (Raleigh, NC) and a NIRO two-stage homogenizer (Hudson, WI). The ratio of water to powder was such that the resulting dispersion contained 0.20% free sterols. The resulting liquid dispersion was used in the animal trial.

2.6. Animal exposure

Male Sprague-Dawley rats were obtained from Charles River Laboratories (Portage, MI). On arrival, the animals weighed approximately 250 g. Animals were acclimated for

7 days before initiating studies. After an overnight fast, animals ($n = 16$) were dosed with the phytosterol formulation via oral gavage and at 8, 16, 24, and 32 hours, four animals per time point were sacrificed and blood was withdrawn by cardiac puncture. Dosages for the animals were determined based on an anticipated human total phytosterol consumption amount of 3 g/day and an estimated body weight of 70 kg. The total dose per animal was 10.7 mg per 250-g rat.

2.7. Analytical sterol standards and test sample preparation

For this assay, stigmasterol was used as an internal standard as it was not present in blank rat plasma and showed no absorption in oral dosing studies (data not shown). Stock solutions of 1 mg/mL of campesterol, β-sitosterol and the internal standard stigmasterol were prepared in chloroform. Standards were prepared by dilution of the stock solutions of campesterol and β-sitosterol into chloroform with a constant amount of the internal standard. The solvent was evaporated in a vortex evaporator for 30 minutes and then taken up in 250 μL of blank rat plasma obtained from untreated rats. For the plasma samples, 100 μL of the internal standard stock solution was added to the assay vessel, the solvent was evaporated, and 250 μL of unknown samples was added.

2.8. Analytical determination of phytosterol concentrations in rat plasma

Phytosterol concentrations in rat plasma were assayed by high-performance liquid chromatography (HPLC) after derivatization with benzoyl chloride after the procedure of Kasama et al. [20] with slight modifications. Standard and test plasma samples were spiked with the internal standard, stigmasterol, then saponified by addition of 1 mL of 1 mol/L ethanolic potassium hydroxide (containing 5.61 g of KOH in 100 mL of ethanol), the solution was mixed vigorously, then incubated at 80°C in a water bath for 1 hour. After the incubation, 0.5 mL of distilled water was added to each tube. Phytosterols were then extracted by addition of 5 mL of hexane to the reaction mixture followed by vigorous mixing. Test tubes were centrifuged for 5 minutes at 1500 rpm to separate the aqueous (bottom) and organic (top) phases. To facilitate removal of the organic phase, the aqueous layer was frozen in an ethanol-dry ice bath. The organic layer containing the phytosterols was transferred to a new test tube and the organic solvent evaporated in a vortex evaporator. The phytosterol samples were derivatized by solubilization in 1.0 mL of freshly prepared benzoyl chloride reagent (containing 1.0 mL benzoyl chloride, 20 mL 1,2-dichloroethane, and 2 mL pyridine) and incubation for 30 minutes at room temperature. The samples were mixed with a 2.0-mL aliquot of 1,2-dichloroethane and acidified with 2.0 mL of 0.1 mol/L hydrochloric acid. Samples were mixed vigorously and centrifuged 5 minutes at 1500 rpm. The aqueous layer was discarded and the organic layer was washed twice with 2 mL of distilled water, the aqueous layer being discarded after each washing step. The resultant organic layer, which contained the derivatized phytosterols, was evaporated in a vortex evaporator for 2 hours, then dissolved in 250 μL of acetonitrile. The samples were transferred to microinserts and analyzed by HPLC (Waters, Alliance 2690), using a Vydac C8 (25 cm × 4.6 mm) column with detection at 228 nm and a column temperature of 50°C. The injection volume was 20 μL and the column was developed with a mobile phase of acetonitrile: 0.5% acetic acid in water [92.5:7.5 v/v] with a 20-minute run time. Under these conditions, the accuracy and precision for campesterol was 1.4% error and 5.6% coefficient of variation and for β-sitosterol was 2.8% error and 4.2% coefficient of variation.

2.9. Statistical analysis

From the plasma samples, the time course of absorption was determined for the phytosterol compounds. Pharmacokinetic data were determined using the suite of programs from the Kinetica software package (Inna Phase Corp., Philadelphia, PA). For mean plasma concentrations at the indicated time points and area-under-the-curve (AUC) values for specified formulations, a statistical comparison (using the Student *t* test) was performed to compare groups treated with free sterols in SBO to experimental groups.

3. Results

3.1. Analytical validation

To validate the ability to detect phytosterols in rat plasma, pure campesterol, stigmasterol, and β-sitosterol were added to plasma from untreated rats. After extraction and derivatization, the samples were analyzed via HPLC as described above (see “Methods and materials” section). Significant concentrations of cholesterol were detected owing to the similarity in structure between cholesterol and the phytosterols. Retention times for cholesterol, campesterol, stigmasterol, and β-sitosterol were approximately 9.97, 11.2, 12.1, and 13.1 minutes, respectively (Fig. 1). Although the chromatogram absorption peaks the for all phytosterols were relatively small, clear separation was observed between the different phytosterols and other substances were not detected, indicating that this was an acceptable method for analysis of phytosterols in plasma. Baseline levels of campesterol and β-sitosterol were identified at a concentration of approximately 1.6 μg/mL and 10 μg/mL, respectively, in blank rat plasma, but stigmasterol was not present (Fig. 2).

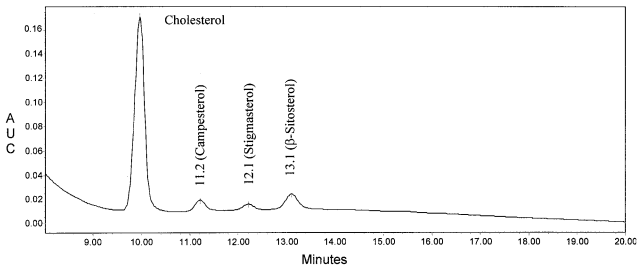

Fig. 1. Chromatogram of pure phytosterols prepared in rat blank plasma. The sterol peaks shown at retention times of 11.2, 12.1, and 13.1 minutes are campesterol, stigmasterol, and β-sitosterol, respectively. An aliquot of the free phytosterol mixture that was used in the formulations was spiked into blank rat plasma, then derivatized as described in the "Methods and materials" section. The large peak with a retention time of 10 minutes is cholesterol.

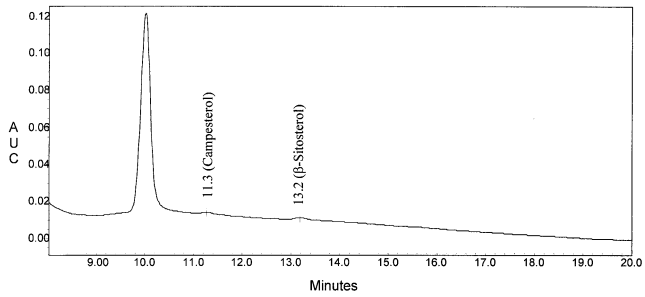

Fig. 3. Plasma phytosterols profile at 16 hours after administration of spray-dried phytosterol-lecithin micelles by oral gavage. Plasma samples were taken at 16 hours and prepared for HPLC analysis as described in the "Methods and materials" section. For illustrative purposes, the internal standard was not spiked to the samples.

3.2. Plasma phytosterol concentrations

Initially, phytosterol concentrations were evaluated in samples of rat plasma obtained at numerous time points within the first 8 hours after oral exposure but none of the phytosterols were identified at concentrations greater than that observed in plasma from untreated animals (data not shown). Therefore, the time points at which blood was collected were extended up to 32 hours after oral exposure to the indicated phytosterol mixture. Campesterol and β-sitosterol were detected in the plasma at concentrations greater than that observed in plasma from untreated rats. However, stigmasterol was not detected in any of the samples (Fig. 3).

The highest plasma concentrations of β-sitosterol were observed after administration of free sterols in SBO (Table 1). Similarly, β-sitosterol absorption, as indicated by the AUC (741.56 μg/mL/h) was highest in animals treated with free sterols in SBO (Table 2). In contrast, very low concentrations of β-sitosterol were detected at all time points after administration of sterol esters in SBO. At some time points, the concentration was actually even below that detected in the plasma from untreated animals (24 hours; Table 1). Correspondingly, β-sitosterol absorption was significantly lower than that observed after administration of unesterified phytosterols in SBO (AUC = 238.82 μg/mL/h). The plasma concentrations of β-sitosterol observed after administration of the lecithin emulsified phytosterols either in a liquid or as a dry powder were consistently lower than the concentrations observed after exposure to free sterols in SBO. However, they were higher than the values observed after exposure to phytosterol esters in SBO (Table 1). Compared with free sterols, the absorption was significantly lower after administration of the emulsified phytosterol in liquid (AUC = 318.11 μg/mL/h) or dry (AUC = 565.99 μg/mL/h) format or by administration of phytosterols mixed with hydroxylated soy lecithin (AUC = 214.34 μg/mL/h). Two alternatively formulated phytosterol micelles were prepared by mixing phytosterols with monoglycerides and polysorbate 60. As observed with lecithin-emulsified phytosterols, only small concentrations of β-sitosterol were detected at any time point after administration (Table 1). Furthermore, the absorption was approximately the same for both of these formulations as indicated by the AUC (Table 2). These results indicate that emulsification of phytosterols decreases the absorption of β-sitosterol compared with the absorption observed with free sterols administered in a lipophilic vehicle. Bioavailability was slightly greater than that observed with sterol esters in SBO.

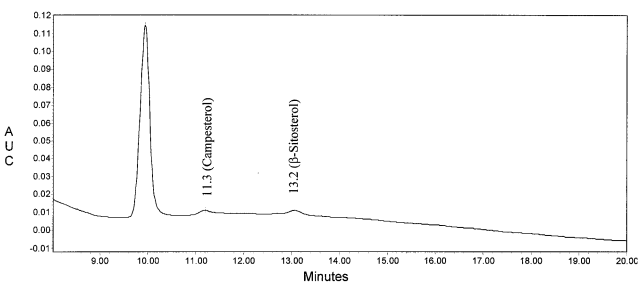

Fig. 2. Chromatogram of rat blank plasma. Plasma from untreated rats was processed as described in the "Methods and materials" section. Campesterol (11.3 minutes) and β-sitosterol (13.2 minutes) were consistently present in blank plasma. The large peak with a retention time of 10 minutes is cholesterol.

Plasma concentrations of campesterol were also monitored after administration of the phytosterol preparations described above. The concentrations of campesterol were consistently lower than β-sitosterol concentrations regardless of formulation, possibly because of the greater proportion of β-sitosterol in the original phytosterol mixture. As observed with β-sitosterol, plasma concentrations of campesterol were highest in animals treated with free sterols dissolved in SBO (Table 3). However, unlike β-sitosterol, the plasma concentrations of campesterol were nearly identical regardless of whether the animals were treated with free phytosterols or esterified phytosterols (Tables 2 and 3). Compared with free phytosterols dissolved in SBO, the plasma concentrations of campesterol were lower after administration of emulsified phytosterols. Compared with phytosterols dissolved in SBO (AUC = 319.86 μg/mL/h),

Table 1
Mean plasma concentrations of β-sitosterol (μg/mL) after oral administration of different formulations*

Formulation	8 h	16 h	24 h	32 h
Blank plasma	8.87 ± 0.04	12.63 ± 1.00†	12.67 ± 0.91‡	9.92 (*n* = 1)
Free sterols in SBO	27.23 ± 5.35	25.46 ± 4.04	27.24 ± 4.05	25.44 ± 4.61
Sterol esters in SBO	8.06 ± 9.32†	11.61 ± 7.24†	3.44 ± 4.63‡	13.48 ± 9.48†
Sterol-lecithin (liquid)	13.71 ± 8.69†	9.34 ± 6.06‡	10.67 ± 8.41	12.09 ± 10.65†
Sterol-lecithin (dry)	18.75 ± 4.85	22.75 ± 3.74	19.55 ± 6.92	19.74 ± 13.45
Sterol-lecithin (mixture)	7.26 ± 3.90‡	16.71 ± 3.42†	1.20 ± 1.82‡	3.24 ± 2.67‡
Sterol-monoglyceride (liquid)	19.21 ± 9.24	15.06 ± 3.65†	14.99 ± 6.53†	18.32 ± 5.21†
Sterol-monoglyceride (dry)	15.55 ± 5.95†	19.01 ± 6.10	20.42 ± 10.83	12.98 ± 3.35‡

* Animals were dosed and samples were analyzed as described in the Methods Section. Results for the formulation dosing are indicated as the mean plasma β-sitosterol concentrations ± standard deviation with a sample size of 4. Blank plasma determinations have a sample size of 2, except where noted. Within each column, statistical significance (Student *t* test) from the reference formulation (Free sterols in SBO) is represented as (†) for *P* values < 0.05 and (‡) for *P* values < 0.01.

the absorption of campesterol was significantly lower in animals treated with lecithin emulsified phytosterols either in liquid (AUC = 66.95 μg/mL/h) or dry (AUC = 142.65 μg/mL/h) format. Similarly, campesterol absorption was also low in animals treated with a soybean lecithin phytosterol (non-emulsified) mixture (AUC = 119.11 μg/mL/h). Campesterol absorption after administration of phytosterols emulsified with monoglycerides and polysorbate 60 was approximately the same as that of free sterols in SBO when administered as a reconstituted liquid (AUC = 309.54 μg/mL/h). However, it was significantly reduced when administered as a liquid (AUC = 193.3μg/mL/h).

4. Discussion

Phytosterols are structurally similar to cholesterol [1] except that they possess substitutions at C-24. This substitution appears to be responsible for inhibiting their absorption from the gastrointestinal system [3–5]. Although Mellanen et al. demonstrated estrogenic activity of phytosterols when evaluated in vitro [21], these compounds exhibit relatively little potential for toxicity because they are poorly absorbed.. Long-term studies on the oral consumption of phytosterols in humans at doses > 25 g/day showed no evidence of estrogenic activity or other adverse effects [22,23]. Laboratory rats consuming dietary phytosterol mixtures or pure β-sitosterol had little evidence of adverse effects even in assays sensitive enough to detect effects of weakly estrogenic substances such as coumesterol [14,15]. However, studies on parenteral exposure of laboratory animals to phytosterols have demonstrated adverse effects including estrogenicity [11–13]. From these studies it can be concluded that the potential for phytosterols to cause adverse effects is directly dependent on their bioavailability.

Addition of esterified phytosterols to processed foods decreases serum cholesterol concentrations in humans [6–8]. In many clinical studies, phytosterols were administered as fatty acid esters to increase solubility and facilitate incorporation into lipid-based foods. Water-soluble phytosterols have been produced by formulation with emulsifiers for applications in non–lipid-based foods [16]. With this increased emphasis on formulation of phytosterols, it is prudent to evaluate the effect of emulsification on phytosterol absorption, particularly in light of the enhancement of oral bioavailability of emulsified pharmaceutical preparations [17–19]. Furthermore, the evidence for estrogenic effects (e.g., aromatase inhibition) observed in rats after consumption of diets supplemented with phytosterols was greater in magnitude when the diet also contained cholic

Table 2
AUC_{0-32h} (μg/mL/h) for β-sitosterol and campesterol after exposure to phytosterol formulations*

Formulation	β-Sitosterol	Ratio	Campesterol	Ratio
Blank plasma	293.20 ± 43.7	0.40‡	138.00 ± 16.0	0.43‡
Free sterols in SBO	741.56 ± 50.0	1.00	319.86 ± 36.2	1.00
Sterol esters in SBO	238.82 ± 170.1	0.32‡	309.36 ± 84.8	0.97
Sterol-lecithin (liquid)	318.11 ± 143.1	0.43‡	66.95 ± 66.6	0.21‡
Sterol-lecithin (dry)	565.99 ± 87.2	0.76†	142.65 ± 34.9	0.45‡
Sterol-lecithin (mixture)	214.34 ± 39.0	0.29†	119.11 ± 45.8	0.37‡
Sterol-monoglyceride (liquid)	437.20 ± 187.0	0.59†	193.30 ± 95.4	0.60†
Sterol-monoglyceride (dry)	491.68 ± 104.6	0.66‡	309.54 ± 51.0	0.97

* AUC was calculated for β-sitosterol and campesterol using standard pharmacokinetic analysis. The ratio of AUC_{test}/AUC_{ref} was calculated using Free sterols in SBO as a reference. Within each column, statistical significance (Student's *t*-test) from the reference formulation is represented as (†) for *P* values < 0.05 and (‡) for *P* values < 0.01.

Table 3
Mean plasma concentrations of campesterol (μg/mL) after oral administration of different formulations*

Formulation	8 h	16 h	24 h	32 h
Blank plasma	4.69 ± 0.49	6.65 ± 1.04	4.61 ± 0.39	5.20 (n = 1)
Free sterols in SBO	11.24 ± 4.86	12.11 ± 5.20	11.21 ± 3.55	10.85 ± 1.73
Sterol esters in SBO	10.26 ± 8.57	14.15 ± 3.67	7.19 ± 5.52	14.13 ± 8.53
Sterol-lecithin (liquid)	1.64 ± 2.90†	2.98 ± 2.66†	2.06 ± 3.26†	3.37 ± 5.68†
Sterol-lecithin (dry)	3.35 ± 4.50	5.51 ± 5.36	5.72 ± 6.91	6.52 ± 10.66
Sterol-lecithin (mixture)	6.70 ± 4.19	5.72 ± 3.33	1.77 ± 2.17‡	1.42 ± 1.04‡
Sterol-monoglyceride (liquid)	11.37 ± 6.25	4.93 ± 3.92	5.11 ± 2.63	8.06 ± 3.15
Sterol-monoglyceride (dry)	9.16 ± 2.52	11.04 ± 2.60	13.83 ± 6.85	9.31 ± 1.34

* Animals were dosed and samples were analyzed as described in the "Methods and materials" section. Results for the formulation dosing are indicated as the mean plasma campesterol concentrations ± SD with a sample size of 4. Blank plasma determinations have a sample size of 2 except where noted. Within each column, statistical significance (Student t test) from the reference formulation (Free sterols in SBO) is represented as (†) for P values < 0.05 and (‡) for P values < 0.01.

acid [24]. This effect may be attributable to an ability of cholic acid to increase the absorption of phytosterols, as it does with supplemental dietary cholesterol in rats [25,26].

Previous studies evaluated the bioavailability of phytosterols in rats by administration of radiolabeled substances [5,27]. In the present studies, phytosterol bioavailability was evaluated by development of an analytical method to directly quantitate their concentration in rat plasma. At time points up to 32 hours after oral exposure to mixed phytosterol preparations, blood samples were extracted, derivatizated with benzoyl chloride, and analyzed via HPLC to determine plasma concentrations of β-sitosterol, campesterol, and stigmasterol. Stigmasterol was not detected in any of the samples. Small concentrations of β-sitosterol and campesterol were detected in the plasma at all time points in untreated rats, suggesting that the animals were exposed to low concentrations of these phytosterols in the feed. Mean plasma concentrations for β-sitosterol (Table 1) and campesterol (Table 3) were relatively flat, whereas stigmasterol was not detected in any sample at any time point. Plasma concentrations of β-sitosterol were generally higher than concentrations of campesterol at most time points regardless of the formulation in which they were delivered. Although β-sitosterol may be absorbed more efficiently than campesterol in rats [28] bioavailability is dependent on both the absorption efficiency and starting concentration. The observation in the current study that plasma concentrations of β-sitosterol were higher that those of campesterol could be attributable, at least in part, to the fact that the starting material contained 2.65 times more β-sitosterol than campesterol.

Plasma concentrations of β-sitosterol and campesterol were greatest in rats administered free sterols in SBO. In all formulations evaluated, phytosterols were poorly absorbed, showing maximal plasma concentrations approximately 1.5 to 2.5 times the baseline level. There was also a substantial amount of variability in the determinations. The poor absorption of the phytosterol marker compounds coupled with the variability make it difficult to compare the impact of the formulation on the absorption of the compounds. However, the results demonstrated that, compared with mean plateau campesterol and β-sitosterol concentrations from sterols administered in soybean oil, none of the formulations produced with lecithin either emulsified or simply in combination with emulsifier used to formulate water-soluble phytosterols showed increased uptake of phytosterols.

The results from the current study indicate that plasma concentrations of water-soluble phytosterol preparations are similar to or lower than those of free phytosterols and phytosterol esters dissolved in a soybean oil in Sprague-Dawley rats. Because of the similarity in pharmacokinetic profiles, plasma concentrations and bioavailability of emulsified phytosterols are not likely to differ from phytosterols or phytosterol esters delivered in a lipid vehicle. Therefore, the safety profile of emulsified phytosterols is likely to be similar to that of current commercially marketed phytosterol formulations.

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