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Efficacy of plant sterols is not influenced by dietary cholesterol intake in hypercholesterolemic individuals

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Abstract

Plant sterols (PSs) reduce plasma total and low-density lipoprotein cholesterol (LDL-C) levels by reducing cholesterol absorption; however, it is not known whether the level of dietary cholesterol intake has an impact on the efficacy of PSs on blood lipids. The purpose of this study was to determine the effect of high vs low dietary cholesterol levels on the lipid-lowering efficacy of free PSs. The study was a semirandomized, double-blind, crossover trial consisting of four 28-day feeding phases each separated by a 4-week washout period. Otherwise healthy hypercholesterolemic subjects (n = 22) consumed each of (*a*) low-cholesterol control (C^-S^-), (*b*) high-cholesterol control (C^+S^-), (*c*) 22 mg PSs per kilogram of body weight with a low-cholesterol diet (C^-S^+), and (*d*) 22 mg PSs per kilogram of body weight with a high-cholesterol diet (C^+S^+). Blood was drawn on the first and last 2 days of each phase to measure plasma total cholesterol, LDL-C, high-density lipoprotein cholesterol, and triacylglycerols as well as plasma campesterol and β-sitosterol concentrations. Dietary cholesterol had no effect on PS efficacy as a cholesterol-lowering agent because no interaction was found between the 2 factors. However, dietary cholesterol and PS intake had significant independent effects on plasma total cholesterol, LDL-C, and high-density lipoprotein cholesterol levels. β-Sitosterol levels in plasma increased ($P < .0001$) as a result of PS supplementation. Data from the present study indicate that, although PSs and dietary cholesterol exert independent effects on plasma cholesterol, PS efficacy is not affected by varying levels of cholesterol intake.
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1. Introduction

Plant sterols (PSs) exist at the forefront of nutraceutical research for the development of food products that lower plasma cholesterol concentrations. Plant sterols are naturally occurring plant fats similar in structure to cholesterol except for alterations in their side chains and/or double bonds [1,2]. Whereas 50% to 60% of dietary cholesterol typically undergoes absorption [3], the absorption of PSs is less than 5% [4]. Discrimination in the absorption of different sterols depends on their structure and occurs during their efflux to the intestinal lumen [5]. Plant sterols act at the intestinal level, reducing cholesterol absorption by 40% to 60% [6-8] through competition with cholesterol molecules for uptake into mixed micelles [9,10]. In addition, PSs may affect the expression of adenosine triphosphate–binding cassette proteins after being converted to liver X receptor agonists [2]. However, recent animal studies have shown that PSs decrease blood cholesterol levels independently of intestinal adenosine triphosphate–binding cassette transporters gene expression [11-14]. Other mechanisms are thought to be involved in the cholesterol-lowering action of PSs. During lipolysis in the intestine, PSs were shown to co-crystallize with cholesterol, resulting in a poorly absorbable mixture. However, in vivo experiments are needed to confirm this mechanism [15]. Another potential mechanism involves the binding of PS esters to cholesterol esterase, decreasing its activity, and therefore limiting the rate of absorption of free cholesterol. Alternatively, PS esters could attract endogenous and exogenous cholesterol to a lower part of the intestine where absorption is limited [15].

Factors that might influence the cholesterol-lowering action of PSs include their form, frequency of intake, and the diet background. Sterols and stanols, the hydrogenated form of sterols, have produced similar reductions in plasma cholesterol [16-18]. In addition, one single daily dose of PSs

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given at lunch appears to be as effective as a dose provided at each of 3 meals [19]. However, other factors can influence the activity of PSs. The level of dietary cholesterol intake accompanying PS treatment may alter the inhibitory effect of PSs on cholesterol absorption [20-22]. In fact, administration of PSs to gerbils fed different levels of cholesterol showed an interaction between dietary cholesterol and PS activity [22]. In humans, the cholesterol-lowering efficacy of PSs has been independently studied at either low or high levels of cholesterol intakes, with similar reductions in low-density lipoprotein cholesterol (LDL-C) and total cholesterol (TC) being observed in both contexts [6,18,23,24]. Although it has also been suggested that higher intakes of dietary cholesterol may improve the efficacy of PSs [21,25], no human study to date has directly compared high- and low-cholesterol diets in terms of their effect on PS activity. Therefore, the purpose of the present study was to compare the effect of PSs given at low- and high-cholesterol intakes on the lipid profile and to examine the effect of dietary cholesterol on plasma PSs during PS supplementation. The present null hypothesis was that no interaction exists between dietary cholesterol intake and the effect of dietary PSs on plasma lipids and PSs.

2. Subjects and methods

2.1. Subjects

Otherwise healthy hypercholesterolemic men and postmenopausal women (n = 22) between the ages of 45 and 85 years were recruited into the study. Subjects were required to complete a medical history questionnaire, provide a blood sample for determination of plasma lipid concentrations, and undergo a thorough medical examination before they were accepted in the trial. Inclusion criteria included a plasma LDL-C concentration between 3.5 and 8.0 mmol/L and triacylglycerol level lower than 4 mmol/L. Exclusion criteria included history of recent or long-term use of oral hypolipidemic therapy and long-term use of insulin, systemic antibodies, corticosteroids, androgens, or phenytoin. Subjects were also excluded if they had a myocardial infarction, coronary artery bypass, or other major surgical procedures within the last 6 months and a recent onset of angina, congestive heart failure, inflammatory bowel disease, pancreatitis, or hypothyroidism. Significant preexisting diseases including cancer, long-term use of laxatives, and smoking or consumption of more than 2 drinks per day were also considered to be part of the exclusion criteria. The study was conducted at the Mary Emily Clinical Nutrition Research Unit at McGill University. On their first visit to the unit, 10 mL of blood was drawn from subjects for lipid screening purposes. A second screening blood draw was performed for a complete blood analysis before they were accepted into the study. Subjects were provided a clear explanation of the study protocol and signed a consent form. The study was approved by the Human Ethical Review Committee of the Faculty of Medicine at McGill University.

2.2. Experimental design and diets

The study was a semirandomized, 4-phase, crossover, double-blind feeding trial where subjects were fed 4 different controlled diets each over 28 days, separated by 4-week washout periods during which they resumed their habitual diets. During the feeding phases, subjects were provided 3 meals daily from a 3-day rotating menu designed to provide the same amount of energy and nutrients, except cholesterol, during all phases of the clinical trial. Meals were prepared in the metabolic kitchen of the research unit at McGill University, and their composition was similar to the typical Western diet. The nutrient composition of the study diet is illustrated in Table 1. Subjects received 30% of energy from fat (10% saturated fat), 15% from proteins, and 55% from carbohydrates. Energy required by each subject was calculated using the Mifflin equation [26] and further adjusted to meet individual requirements as we monitored their weight to prevent fluctuations of more than 1 kg. The nutrient content of the basal diet was calculated using the FOOD PROCESSOR (Food Processor, Salem, OR), a computerized dietary analysis system with a Canadian database. Diets differed in their cholesterol and PS content: diet C^-S^- (low-cholesterol control) contained 80 mg cholesterol per 4186 kJ with no added PSs; diet C^-S^+ contained 80 mg cholesterol per 4186 kJ and 22 mg free PSs per kilogram of body weight; diet C^+S^+ contained 200 mg cholesterol per 4186 kJ and 22 mg free PSs per kilogram of body weight; and diet C^+S^- (high-cholesterol control) contained 200 mg cholesterol per 4186 kJ and no added PSs. The free PS treatment (Forbes Medi-Tech, Vancouver, BC, Canada) derived from soybeans had a purity of 96% and contained 78.7% β-sitosterol, 10.6% campesterol, and 10.7% others. Diet C^-S^- was given to all subjects in their first phase, used as an adaptation period for volunteers to assess caloric needs and satiety levels. This was followed by diet C^-S^+ where PS treatment was introduced. For the 2 subsequent phases, subjects were randomly assigned to one of diets C^+S^- and C^+S^+ to reduce the error term associated with diet sequencing. The free sterol treatment was provided as a white, odorless, tasteless powder mixed thoroughly with 15 g of margarine and served as a single dose with the breakfast meal. During the feeding phases, subjects were

Table 1
Nutrient composition of the study diet for a daily intake of 12 558 kJ

	Weight (g)	% of energy intake
Carbohydrates	419.0	55.0
Protein	118.0	15.0
Monounsaturated fats	39.0	11.4
Polyunsaturated fats	26.6	7.8
Saturated fats	27.4	8.0
Dietary fiber	39.1	–

asked to have their daily breakfast at the unit under the staff's supervision to ensure compliance. Subjects were permitted to take their lunch and dinner packs home for consumption, but were not allowed to eat foods not provided to them by the metabolic kitchen. Coffee and alcohol were also prohibited, but low-calorie decaffeinated beverages were offered with meals. Subjects were asked to weigh themselves each morning at the clinic before breakfast.

On days 1, 2, 28, and 29 of each phase, fasted blood was collected from volunteers; and plasma was separated and stored at −80°C for further analyses. On day 29, an additional blood sample was drawn for complete blood count and biochemistry analyses to monitor subjects' health status during the study.

2.3. Analyses

2.3.1. Plasma lipid concentrations

Total cholesterol, high-density lipoprotein cholesterol (HDL-C), and triacylglycerol concentrations were measured using an autoanalyzer and commercial enzymatic kits (Roche Diagnostics, Indianapolis, IN). Briefly, TC was determined enzymatically using cholesterol esterase and cholesterol oxidase. High-density lipoprotein cholesterol was measured after the precipitation of apolipoprotein B–containing lipoproteins with dextran sulfate and magnesium sulfate by polyethylene glycol–modified enzymes [22]. Low-density lipoprotein cholesterol was calculated using the Friedewald equation [27].

2.3.2. Plasma PS levels

Plasma campesterol and β-sitosterol concentrations were measured by gas-liquid chromatography. Internal standard 5-α-cholestane was added to plasma samples that were then saponified with 4 mL of methanolic potassium hydroxide. Sterols were extracted twice from the mixture with 4 mL of petroleum ether and were injected into a gas-liquid chromatograph equipped with a flame ionization detector (HP 5890 Series II; Hewlett Packard, Palo Alto, CA) and a 30-m column. Campesterol and β-sitosterol plasma concentrations were determined in duplicate by identifying the peak sizes and expressing them relative to 5-α-cholestane internal standard.

2.3.3. Statistical analysis

Data were expressed as mean ± SEM. End points and percentages of change for lipoprotein cholesterol and PSs data were compared using a crossover, repeated-measures, 2-way analysis of variance model for mixed designs for determination of the effect of PSs, dietary cholesterol, and their interaction. Initial values for lipid data were taken as averages of days 1 and 2, whereas end points were taken as averages of days 28 and 29. As for PS data, start and end point values were averages of duplicate measurements from days 1 and 29, respectively. The accepted level of significance was $P < .05$. The statistical software used was SAS version 8.0 (SAS Institute, Cary, NC).

3. Results

3.1. Subject baseline characteristics

The ratio of men to women enrolled in the study was 1.2, and the average age was 60.0 ± 2.5 years. At baseline, the average body mass index of subjects was 27.7 ± 1.1 kg/m^2; and the average TC, LDL-C, HDL-C, and triacylglycerol concentrations were 5.75 ± 1.13, 3.63 ± 0.13, 1.45 ± 0.09, and 1.87 ± 0.34 mmol/L, respectively.

3.2. Subject compliance and dropout rate

Twenty-two subjects started and completed the study; there were no dropouts from the clinical trial. The meals and portions were adapted to subjects' energy requirements. All individuals tolerated the diet well and reported no adverse effects; subjects were unable to distinguish between dietary treatments because they reported no differences in smell, taste, or color across diets.

3.3. Blood biochemistry and weight changes during the feeding trial

Blood biochemical analyses remained within the reference ranges throughout the study period. Diets were designed for weight maintenance; consequently, there were no significant group mean weight changes across the 4 phases as illustrated in Table 2. Similarly, mean initial and final weights were not significantly different across groups.

3.4. Plasma lipid profile in response to diet

Initial lipid levels of subjects did not show any significant difference across treatments. Given the semirandomized design of the study, it was expected that treatment sequence would have a statistically significant effect on blood lipids. Therefore, an appropriate washout period separating the treatment phases was included aiming at eliminating any carryover effect and minimizing the sequence effect.

3.4.1. Total cholesterol

Mean plasma cholesterol values for each of the treatments are presented in Table 3. Dietary cholesterol had a significant effect ($P = .0001$) on TC end point values, whereas the PS effect was not significant. In addition, there was no interaction between dietary cholesterol level and PS treatment, indicating that dietary cholesterol level did not affect

Table 2
Weight changes across different PS treatments

Treatment	Initial average weight (kg)	Final average weight (kg)	% Change
C^-S^-	78.2 ± 3.5	77.5 ± 3.5	−0.91 ± 0.26
C^-S^+	77.0 ± 3.46	76.4 ± 3.46	−0.74 ± 0.26
C^+S^-	76.0 ± 3.3	75.8 ± 3.3	−0.28 ± 0.22
C^+S^+	76.8 ± 3.2	76.4 ± 3.2	−0.37 ± 0.24

No statistically significant difference in weight changes between treatment groups.

Table 3
Plasma lipid levels in response to PS treatment with low (80 mg/4186 kJ) and high (200 mg/4186 kJ) dietary cholesterol

	Concentrations		$P^{2\,a}$		% Change	P^2	
	Start (mmol/L)	End (mmol/L)	Main effect of PSs on end points	Main effect of dietary cholesterol on end points		Main effect of PSs on % change	Main effect of dietary cholesterol on % change
TC			.16	.0001		.27	.0004
C^-S^-	5.99 ± 0.11	5.44 ± 0.09			−8.87 ± 1.91		
C^-S^+	6.18 ± 0.11	5.34 ± 0.09			−12.8 ± 1.79		
C^+S^-	6.10 ± 0.11	5.88 ± 0.09			−3.95 ± 1.85		
C^+S^+	5.97 ± 0.11	5.73 ± 0.09			−4.05 ± 1.79		
LDL-C			.03	.005		.30	.001
C^-S^-	4.20 ± 0.10	3.80 ± 0.08			−8.80 ± 2.75		
C^-S^+	4.20 ± 0.09	3.63 ± 0.08			−11.9 ± 2.58		
C^+S^-	4.05 ± 0.09	4.04 ± 0.08			−0.40 ± 2.67		
C^+S^+	3.95 ± 0.09	3.86 ± 0.08			−2.50 ± 2.59		
HDL-C			.0005	.0001		.83	.36
C^-S^-	1.05 ± 0.03	1.02 ± 0.01			−0.87 ± 2.10		
C^-S^+	1.16 ± 0.03	1.10 ± 0.01			−3.13 ± 1.98		
C^+S^-	1.25 ± 0.03	1.19 ± 0.01			−4.58 ± 2.04		
C^+S^+	1.28 ± 0.03	1.22 ± 0.01			3.20 ± 1.98		
Triacylglycerol			.82	.11		.88	.05
C^-S^-	1.64 ± 0.11	1.35 ± 0.05			−13.80 ± 4.37		
C^-S^+	1.80 ± 0.11	1.35 ± 0.05			−17.16 ± 4.10		
C^+S^-	1.77 ± 0.11	1.46 ± 0.05			−7.95 ± 4.23		
C^+S^+	1.62 ± 0.11	1.44 ± 0.05			−5.84 ± 4.11		

80 mg cholesterol per 4186 kJ, no PSs; C^-S^+: 80 mg cholesterol per 4186 kJ, with PSs; C^+S^-: 200 mg cholesterol per 4186 kJ, no PSs; C^+S^+: 200 mg cholesterol per 4186 kJ, with PSs. n = 22, $P < .C^-S^-$: 05.

[a] P value for 2-way analysis of variance.

PS cholesterol-lowering activity. At the end of the dietary phases, TC plasma concentrations were 5.44 ± 0.09, 5.34 ± 0.09, 5.88 ± 0.09, and 5.73 ± 0.09 mmol/L for treatments C^-S, C^-S^+, C^+S^-, and C^+S^+, respectively. The main effect of dietary cholesterol demonstrated TC end point values 7.2% lower ($P < .0001$) after the low-cholesterol treatments (C^-S^- and C^-S^+) relative to the high-cholesterol treatments (C^+S^- and C^+S^+). On the other hand, the main effect of PSs shows no statistically significant difference in plasma TC when comparing low- and high-PS treatments. Overall, plasma TC was decreased by 4.0% ± 1.3% in the high-cholesterol treatments (C^+S^- and C^+S^+) and by 10.8% ± 1.3% in the low-cholesterol treatments (C^-S^- and C^-S^+) relative to baseline. The difference between the 2 groups was significant ($P = .0004$).

3.4.2. LDL-cholesterol

Dietary cholesterol and PSs exerted significant effects ($P < .05$) on LDL-C end point values; however, there was no interaction between the 2 variables. The LDL-C end point values are summarized in Table 3, and individual responses of subjects to treatments and controls are presented in Fig. 1. The LDL-C concentrations were 3.80 ± 0.08, 3.63 ± 0.08, 4.04 ± 0.08, and 3.86 ± 0.08 mmol/L after treatments C^-S^-, C^-S^+, C^+S^-, and C^+S^+, respectively. Main effects of dietary cholesterol and PSs show that LDL-C end point values were 5.9% lower ($P < .01$) after the low-cholesterol treatments (C^-S^- and C^-S^+) relative to the high-cholesterol treatments (C^+S^- and C^+S^+) and that PS treatments (C^-S^+ and C^+S^+) resulted in 4.4% lower ($P < .05$) LDL-C end points compared with control treatments (C^-S^- and C^+S^-). Percentages of change in LDL-C are summarized in Table 3. Dietary cholesterol, but not PS intake, elicited a significant effect on changes in plasma LDL-C. The mean LDL-C reduction from baseline in subjects on the high-cholesterol diet was 1.4% ± 1.9%, whereas a low-cholesterol intake was associated with

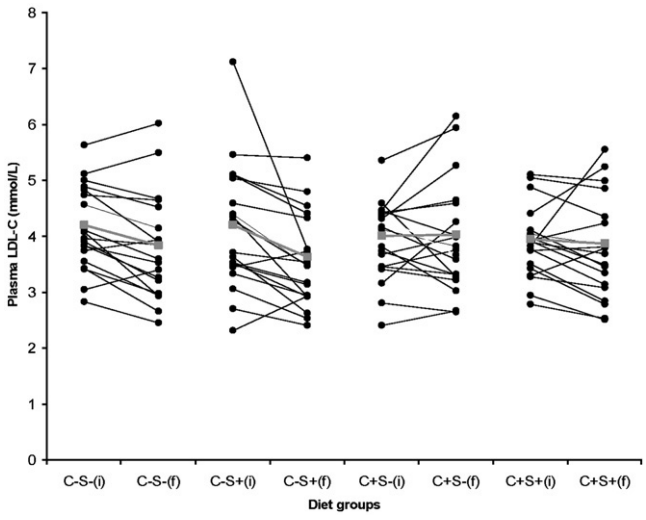

Fig. 1. Initial and final LDL-C values in response to PS treatments and controls. —■—: group mean. C^-S^-: 80 mg cholesterol per 4186 kJ, no PSs; C^-S^+: 80 mg cholesterol per 4186 kJ, with PSs; C^+S^-: 200 mg cholesterol per 4186 kJ, no PSs; C^+S^+: 200 mg cholesterol per 4186 kJ, with PSs. n = 19, $P < .05$. I indicates initial; F, final.

a reduction of 10.4% ± 1.9% (P = .001). Fig. 1 shows that one subject had a baseline LDL-C of 7.1 mmol/L and an end point of 3.7 mmol/L. Our results were verified after removing lipid values corresponding to the subject of interest. The TC and LDL-C end points and percentages of change were not affected as a result of the correction.

3.4.3. High-density lipoprotein cholesterol

Dietary cholesterol and PSs showed significant effects on HDL-C end point values; however, as reported for other cholesterol data, no interaction was found. The HDL-C end points are summarized in Table 3. At the end of the dietary periods, plasma concentrations were 1.02 ± 0.01, 1.10 ± 0.01, 1.19 ± 0.01, and 1.22 ± 0.01 mmol/L in treatments C^-S^-, C^-S^+, C^+S^-, and C^+S^+, respectively (P < .001). The main effects of dietary cholesterol and PSs show that HDL-C end point values were 11.6% lower (P = .0001) in the low-cholesterol treatments (C^-S^- and C^-S^+) compared with the high-cholesterol treatments (C^+S^- and C^+S^+) and that controls (C^-S^- and C^+S^-) resulted in lower (P = .0005) HDL-C end points than PS treatments (C^-S^+ and C^+S^+) by 4.5%. Percentages of change in HDL-C are summarized in Table 3. No significant difference in percentages of change was observed across treatments.

3.4.4. Triacylglycerol

Triacylglycerol end points and percentages of change are summarized in Table 3. There were no significant effects of dietary cholesterol and PSs on plasma triacylglycerol end point concentrations and percentages of change.

3.5. Heterogeneity of subjects' responses to plant sterol therapy

Only 8 subjects had increased LDL-C levels relative to baseline after PS administration. In 7 of those subjects, the increase was seen after the high-cholesterol PS treatment. When values were matched with the corresponding low-cholesterol PS (C^-S^+) levels, no relationship was found, as there was a decrease in LDL-C during this phase in the same subjects. Furthermore, baseline LDL-C levels throughout treatments were correlated with end point levels (r = 0.72, P < .0001); however, no correlation was found between baseline and LDL-C changes across treatments.

Table 4
Changes in plasma PSs in response to diet treatments

	Treatment			
	C^-S^-	C^-S^+	C^+S^-	C^+S^+
β-Sitosterol (μmol/L)				
Start	5.18 ± 0.57[a]	6.08 ± 0.57[a]	5.95 ± 0.58[a]	5.64 ± 0.58[a]
End	5.06 ± 0.60[a]	6.45 ± 0.60[b]	5.66 ± 0.60[a]	6.78 ± 0.60[b]
Change	0.05 ± 0.08[a]	0.15 ± 0.08[a]	−0.01 ± 0.08[a b]	0.28 ± 0.08[a c]
Campesterol (μmol/L)				
Start	13.21 ± 0.72[a]	14.62 ± 0.72[a]	12.70 ± 0.72[a]	12.70 ± 0.72[a]
End	12.83 ± 0.56[a]	13.17 ± 0.56[a]	12.92 ± 0.56[a]	12.95 ± 0.56[a]
Change	0.04 ± 0.06[a]	−0.05 ± 0.06[a]	0.04 ± 0.06[a]	0.07 ± 0.06[a]

Values with different superscript letters are significantly different. Significance set at P < .05.

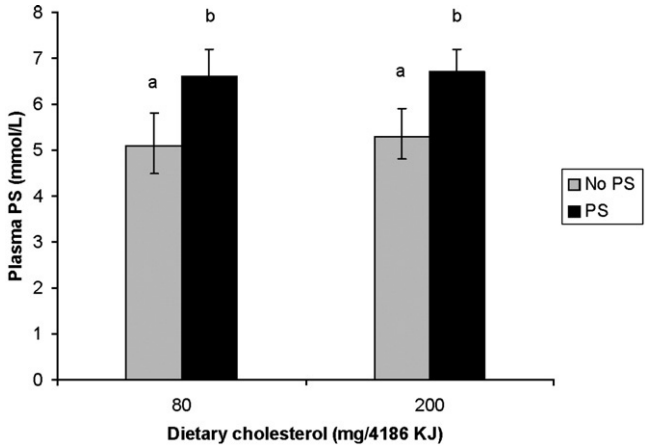

Fig. 2. End point plasma β-sitosterol levels in response to PS treatment with low (80 mg/4186 kJ) and high (200 mg/4186 kJ) cholesterol intake. Explanation of graph: C^-S^-: 80 mg cholesterol per 4186 kJ, no PSs; C^-S^+: 80 mg cholesterol per 4186 kJ, with PSs; C^+S^-: 200 mg cholesterol per 4186 kJ, no PSs; C^+S^+: 200 mg cholesterol per 4186 kJ, with PSs. Percentage of change: C^-S^-, 5%; C^-S^+, 15%; C^+S^-, −1%; C^+S^+, 28%. n = 22, P < .05.

3.6. Plasma PS levels in response to diet

Dietary cholesterol did not affect the levels of plasma PSs across different treatments. However, intake of PSs increased (P < .0001) plasma β-sitosterol, but not campesterol, levels. Plasma β-sitosterol levels before and after the different diet treatments are presented in Table 4. At the end of the dietary periods, plasma concentrations of β-sitosterol were 5.06 ± 0.60, 6.45 ± 0.60, 5.66 ± 0.60, and 6.78 ± 0.60 mmol/L in treatments C^-S^-, C^-S^+, C^+S^+, and C^+S^- (Fig. 2), respectively. Plasma β-sitosterol values were 21.6% lower (P < .0001) in the controls (C^-S^- and C^+S^-) than in the PS treatments (C^-S^+ and C^+S^+). Dietary cholesterol failed to change plasma levels of campesterol and β-sitosterol. However, β-sitosterol plasma levels were elevated (P < .01) by PS intake across treatments. The high-PS diets (C^-S^+ and C^+S^+) increased plasma β-sitosterol concentration by 21.4% compared with 1.1% for the control diets (C^-S^- and C^+S^-).

4. Discussion

Results of the present study show for the first time that dietary cholesterol level does not impact PS efficacy as a cholesterol-lowering agent in humans, despite independent actions of PS and cholesterol intake on lipid levels. It has not been possible from human studies on PSs to elucidate the effect of varying dietary PS concentrations because of differences in study design, PS dose, and matrices used in different trials. Moreover, this is the first human clinical trial to provide a direct comparison of the effect of PSs administered at 2 different levels of cholesterol intake using a crossover design and a precisely controlled diet. Our

results agree with the pattern seen in previous human studies on PS cholesterol-lowering effect. Reductions of 4% to 9% in TC and 8% to 16% in LDL-C were observed upon administration of varying doses of PSs in the context of a low-cholesterol diet (135-300 mg/d) [6,23-25,28-30]. Similarly, reductions of up to 9% and 15% in TC and LDL-C, respectively, were seen in studies using PSs in the context of a high-cholesterol diet (500 mg/d) [18,31]. The only study having established a direct comparison of PS effect with low and high dietary cholesterol was conducted on gerbils [22]. Our results do not align with the conclusion of that study, which suggests that a higher cholesterol intake enhances the lipid-lowering activity of PSs. Hayes et al [22] showed that the greatest reduction in plasma cholesterol (42%) corresponded to the lowest ratio (1:1) of PSs to cholesterol in the diet, suggesting that with low dietary cholesterol, extra PSs may not lower plasma cholesterol further, whereas with high dietary cholesterol, PSs induce a dose-dependent reduction in plasma cholesterol. However, it is important to note that the results of the present study cannot be directly compared with the outcomes of the animal trial of Hayes et al, especially because the doses of both the PSs and the dietary cholesterol were lower in the present trial than the ones used in the latter study. The PS dose administered to gerbils was equivalent to 2.5 g/d for humans, whereas the levels of dietary cholesterol studied were 250, 500, and 2500 mg/d of cholesterol. In the present study, the average daily PS intake was 1700 mg, whereas dietary cholesterol varied from 160 to 640 mg, respectively. Both PS and cholesterol intakes could account for the discrepancy between the results of the 2 studies. Because PSs are suggested to have more than one mechanism of action through which they reduce cholesterol, results from the animal trial of Hayes et al could be explained by the fact that above a certain threshold of cholesterol intake and/or PSs, competition with cholesterol for micelle incorporation is replaced or complemented by other mechanisms involved in cholesterol absorption such as the inhibition of cholesterol esterase or co-crystallization with cholesterol [15], making reductions in cholesterol absorption more efficient at the different sites of action. However, the intake of cholesterol administered in this study and representing the range seen in a North American diet did not show any relationship with the efficacy of PSs.

Comparing adjusted end points and percentages of change for plasma TC, LDL-C, HDL-C, and triacylglycerol across treatment phases, dietary cholesterol was shown to have a significant effect on lipoprotein cholesterol concentrations. Although saturated fat has been shown to be the primary plasma cholesterol modulator [32-35], the present study emphasizes the impact of dietary cholesterol on lipoprotein cholesterol, as we were able to observe a limited yet significant effect of cholesterol intake on plasma cholesterol in the context of a diet low in saturated fats. Our results agree with the study conducted by Jones et al [36] showing an increase of 7% in TC as a result of an increase in dietary cholesterol in subjects fed a low–saturated fat diet.

The choice of the study population was based on the fact that hypercholesterolemic individuals are the major target for lipid-lowering agents. Although hypercholesterolemia is often associated with changes in cholesterol absorption such as the case of sitosterolemia [37] and autosomal dominant hypercholesterolemia [38], other studies have shown similar PS efficacy in hypercholesterolemic and normocholesterolemic subjects [2]. In addition, cholesterol absorption was seen to be similar in these 2 populations [39]; therefore, results of the present study are likely to be applicable to normocholesterolemic individuals.

The heterogeneity of subject response was tested by examining subset responsiveness observed among individuals with increased LDL-C. Only one subject had increased plasma LDL-C after the $C^{-}S^{+}$ treatment, whereas the corresponding levels in the $C^{+}S^{+}$ intervention decreased. Similarly, 7 subjects in the $C^{+}S^{+}$ intervention showed an increase from baseline in their LDL-C plasma levels; however, the same subjects experienced a reduction in their LDL-C levels after the $C^{-}S^{+}$ treatment, suggesting that these subjects are hyperresponders to dietary cholesterol intake. We can conclude that there was no consistency across treatments in the response to PS and dietary cholesterol in "nonresponders" for a specific intervention. When the relationship of LDL-C levels at baseline with end points and changes was assessed, a strong correlation with end points but not with changes was recorded. Therefore, the effect of PSs and dietary cholesterol on LDL-C levels in the blood did not vary significantly as a function of individual baseline characteristics. These results reinforce previous research showing similar cholesterol absorption and response to PS therapy in normocholesterolemic and hypercholesterolemic populations [39]; however, further information should be available about the origins of high baseline LDL-C to draw conclusions on the effect of individual genetic variations on the response to PSs.

The independent action of PSs in the low– and high–dietary cholesterol contexts, although significant, did not reach the values seen previously in the literature where plasma LDL-C levels were reported to be 10% to 15% lower than those observed on control diets [6,16-18,23,30,40]. Contrary to previous research using a fixed daily amount of PSs [18,41-43], subjects in the present trial were administered 22 mg/kg body weight. The average daily dose of PSs was 1.7 g, with subjects consuming as little as 1.1 g or as much as 2.5 g. Knowing that the National Cholesterol Education Program recommendation to lower LDL-C significantly is 2 g of PSs per day [44], variations in the dose of PSs administered between subjects might have affected the results. Moreover, the time of intake of PSs may have affected their cholesterol-lowering action. In the present study, PSs were consumed as a single daily dose in the morning, in contrast with a single afternoon dose given in the study from Plat et al [19]. Nevertheless, even if the efficacy of a single morning dose of PSs is less than the efficacy of the same dose consumed throughout the day,

the results from the present study emphasize that the level of dietary cholesterol intake does not influence the efficacy of PSs consumed as a single dose.

End point plasma PS concentrations were not different across high- and low-cholesterol groups. In addition, the fixed effect of cholesterol was not significant, demonstrating that dietary cholesterol had no significant effect on the absorption of PSs. Plant sterols are absorbed via the same pathway as cholesterol [15]; therefore, it would be expected that an increased competition due to a higher cholesterol intake would reduce the amount of PSs entering the circulation. However, it has been shown that exogenous cholesterol has a very little contribution to the level of cholesterol in the blood as compared with endogenously synthesized cholesterol [45,46]. Moreover, the absorption of cholesterol and PSs may be different along the intestine as a result of a complex sequence between influx and efflux of intraluminal cholesterol and PSs crossing the apical brush border membrane of the enterocyte [47]; therefore, a change in the dietary cholesterol is less likely to induce a change in PS bioavailability.

In conclusion, the present study shows for the first time in humans that although dietary cholesterol significantly and directly affects plasma cholesterol, it does not impact PS lipid-lowering efficacy in hypercholesterolemic individuals. The lack of interaction between PS and dietary cholesterol levels supports using PS as a cholesterol-lowering agent in the context of a low-cholesterol and low–saturated fat diet.

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