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ORIGINAL ARTICLE

Phytosterol Intake and Dietary Fat Reduction are Independent and Additive in their Ability to Reduce Plasma LDL Cholesterol

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Abstract We studied the interrelationship of diet and plant sterols (PS) on plasma lipids, lipoproteins and carotenoids. Mildly hypercholesterolemic men ($n = 13$) and postmenopausal women ($n = 9$) underwent four randomized, crossover, double-blind, controlled feeding periods of 23 days each. The design consisted of two levels of PS (0 and 3.3 g/day) and two background diets having fat content either typical of the American diet (total and saturated fat at 33.5 and 13.2% of energy, respectively), or a Step 1 type of diet (total and saturated fat at 26.4 and 7.7% of energy, respectively). Plasma total cholesterol (TC), high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, Apo A1 and Apo B were 4.3, 5.3, 4.5, 2.8 and 2.5% lower, respectively ($P \leq 0.0001$; <0.0001, 0.0016, 0.0006, and 0.0069), with the Step 1 diet than with the typical American diet. Diet had no effect on TC/HDL cholesterol ($P = 0.1062$). Plant sterol intake lowered TC, LDL cholesterol, and Apo B by 9.0, 12.4 and 6.1% and TC/HDLC by 9.6% ($P \leq 0.0001$ for all), respectively, without affecting HDL cholesterol and Apo A1 ($P = 0.2831$ and 0.732). The PS effect in lowering plasma TC and LDL cholesterol was independent of and additive to the effect due to dietary fat reduction. Responses of plasma carotenoids to PS intake were consistent with the literature.

Keywords Plant sterols · LDL cholesterol · Typical American diet · Step 1 diet

Abbreviations

Apo A1	Apolipoprotein A1
Apo B	Apolipoprotein B
BHNRC	Beltsville Human Nutrition Research Center
HDL	High density lipoprotein
HPLC	High-performance liquid chromatography
HRT	Hormone-replacement therapy
LDL	Low density lipoprotein
NCEP	National Cholesterol Education Program
PS	Plant sterols
SEM	Standard error of the mean
SFA	Saturated fatty acid
TAD	Typical American Diet
TC	Total cholesterol
TAG	Triacylglycerides
USDA	US Department of Agriculture

Introduction

The landmark study by Miettinen et al. [1] reported the year-long efficacy of a margarine enriched with plant stanol-esters in reducing the low density lipoprotein (LDL) cholesterol concentrations of mildly hypercholesterolemic adults. Since then, numerous human studies on the efficacy

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and safety of plant sterols (PS) and plant stanols, either in free forms or as esters, and alone or in combination with each other, have been published. The LDL cholesterol-lowering effects of PS and plant stanols are equivalent and additive to those of diet or lipid-lowering medications and the optimal daily intake is 2–2.5 g/day [2–5].

Effects of PS incorporated into different food forms [6] have been studied but not the impact of the fat level and composition of the background diet. Earlier studies have suggested that increasing saturated fat and cholesterol intakes may enhance the PS induced LDL cholesterol-lowering effect [7, 8]. A review of the literature found that the background diets in previous studies were either uncontrolled, such as free-living subjects consuming their habitual diets [1, 9, 10]; semi-controlled, such as freeing-living subjects receiving dietary counseling [9, 11]; or controlled but unvaried [12–14]. The only study in which fat intakes were varied by design did not quantify the effect of dietary fat [15]. A direct comparison of diets with varying fat level and composition has not been reported to date.

In the United States, the recommendation for individuals with elevated serum LDL cholesterol concentrations is to replace their customary diets with a "therapeutic lifestyle change (TLC) diet" consisting of saturated fat intake at <7% of calories, and cholesterol intake at <200 mg/day and including the use of viscous fiber and plant stanols/sterols as an initial step towards achieving the LDL cholesterol goal [16]. We designed the present study to determine the effect of three daily servings of PS in the presence of two practical background diets differing in fat level and composition on responses in lipids, lipoproteins, retinol, tocopherols, and carotenoids. These diets represent a typical American diet (TAD, relatively high in fat and saturated fat) and a TLC like diet (Step 1 diet).

Experimental Procedure

Study Design

The four dietary treatments consisted of two background diets (TAD and Step 1) in combination with two levels of PS (0 and 3.3 g/day) in a double-blind, randomized cross-over design, with each participant consuming each of the diets for 28 days. The fundamental differences between the TAD and Step 1 diets were their fat levels (33.5 vs. 26.4% of the calories for TAD and Step 1 diet, respectively) and fat composition (saturated/*cis*-monounsaturated/*cis*-polyunsaturated/*trans* fat at 13.2/11.5/6.5/2.4 for the TAD and 7.7/8.9/7.1/2.6 for the Step 1 diet). Blood samples were collected during the fourth week, after 22 days of feeding. As it has been established that new steady states for plasma total cholesterol (TC), high density lipoprotein (HDL) cholesterol, and TAG are reached in 3–4 weeks [17], study subjects were then switched to the next diet with no washout between periods. The study protocol was approved by the Johns Hopkins University, Bloomberg School of Public Health, Committee on Human Research (Baltimore, MD). A detailed description of the subject recruitment procedures and selection criteria has been presented elsewhere [13]. Subjects were blinded to the four experimental diets. The salad dressings and margarines, with and without added PS, were prepared specifically for this study and supplied color-coded and blinded to the investigators by Unilever Foods, NA (Englewood Cliffs, NJ). Biological samples were coded and blinded to the analysts.

Diets

The four experimental diets were planned using data from the United States Department of Agriculture (USDA) National Nutrient Database for Standard Reference, Release 19 [18]. Typical American diet and Step 1 diets were composed of foods commonly eaten in the United States. Ranch salad dressing (8 g fat/serving) and a 60% fat spread (8.4 g fat/serving) were fed as part of the TAD diets, and Italian salad dressing (3.8 g fat/serving) and a 32% fat spread (4.5 g fat/serving) were fed a part of the Step 1 diets. Salad dressings and spreads were either fortified with 1.1 g PS per serving (equivalent to 1.8 g/serving of PS as esters from vegetable oil sources) and used in +PS diets, or having no added PS and used in the –PS diets. One serving of margarine was included in the breakfast, and one serving each of margarine and salad dressing were served at dinner for a total of zero (−PS) or 3.3 g/day of add PS (+PS).

A 7-day menu rotation was used. From Monday through Friday all subjects consumed breakfast and dinner at the Human Study Facility of Beltsville Human Nutrition Research Center (BHNRC) under the supervision of a dietitian. At breakfast, each subject was provided with a carry-out lunch to be consumed off-site that day. Snacks were included in the daily menu, which the subjects were allowed to eat either at dinner or later in the evening. Meals for the weekend were packaged for home consumption and were provided to the subjects, with written instructions, after dinner on Friday. Unlimited amounts of coffee, tea, and diet sodas were allowed, but all additives (sugar and milk) for coffee and tea were provided with the meals. Only foods provided by the Human Study Facility were allowed during the study.

Each weekday morning the subjects were weighed before breakfast when they arrived at the Facility. Energy intake was adjusted in 200- or 400-kcal increments to

maintain initial body weight. The subjects were fed the same items and the same proportions of each item relative to total dietary energy intake. Therefore, the relative amounts of all nutrients, other than those provided by the margarine and salad dressing, were constant for all subjects. Each day, the subjects completed a questionnaire detailing beverage intake, factors related to dietary compliance, exercise, medications, and illnesses. The questionnaires were reviewed routinely by a study investigator and all problems identified were discussed with the subject during the next meal.

During each feeding period, two composites of the 7-day menu cycle were made at two energy levels for each of the four diets. The food items were collected and prepared as though they were to be consumed. They were then homogenized in a blender with ice added to prevent heat build-up. The blended samples were freeze-dried in preweighed containers and weighed again after drying. The samples were then pulverized, and a weekly composite for each energy level was prepared by mixing together 15% by weight of each day's freeze-dried sample. The eight diet composites were analyzed for dry matter, crude protein, crude fat, total dietary fiber, ash, and fatty acid composition (Covance Laboratories Inc., Madison, WI) and sterol content and composition [14]. The fatty acid composition of the food composites was analyzed by using method AOCS 996.06. Carotenoid concentrations were estimated by using the US Department of Agriculture–Nutrition Coordinating Center carotenoid database for US foods [19].

Blood Collection and Analysis

Baseline (i.e., pretreatment) blood samples were collected on two separate days during the week immediately before the start of the first controlled feeding period. Blood samples were also collected on days 22 and 24 of the fourth week of each controlled feeding period. Procedures for blood sampling and processing were those described in the protocol for the Lipid Research Clinics Program [20]. Plasma concentrations of TC, HDL cholesterol, and TAG were measured enzymatically with commercial kits (Sigma Chemical Company, St Louis, MO) at the Lipid Research Clinic Laboratory at the George Washington University Medical Center, which maintains standardization with the Centers for Disease Control and Prevention, US Department of Health and Human Services. Low density lipoprotein cholesterol was calculated using the Friedewald equation [21]. Plasma concentrations of apolipoproteins A1 (Apo A1) and B (Apo B) were measured by rate nephelometry (Beckman ICS Immunochemical Analyzer; Beckman Instruments, Fullerton, CA). For each analysis, samples from an individual subject were processed in the same analytical run. Plasma concentrations of lutein, α- and β-cryptoxanthin, lycopene, α- and β-carotene, total carotenoids, retinol, and α-, γ-, and δ-tocopherol were measured by HPLC as previously described [22, 23]. The precision and accuracy of the HDL chromatograph (HPLC) system was verified by using standard reference material 968b (National Institute of Standards and Technology, Gaithersburg, MD).

Statistical Analyses

All analyses were performed using SAS (SAS, Cary, NC) for Windows (v.9.1 2002–2003). The analytic plan was designed a priori and described a mixed-effects model for analysis of the data for repeated measurements [24]. For each variable the mean of two sample measurements taken during week 4 of each feeding period was analyzed. Individual differences were accounted for using the baseline value as a covariate, and by including a random subject effect in the model. The remaining time series (residual) correlation was modeled as one parameter autoregressive. Contrasts between diets, PS, gender and diet and/or gender and/or PS were tested by a *F*-test for differences among groups. Significant differences between baseline adjusted means (LS mean) were determined by Tukey–Kramer *t*-tests [25]. Plasma carotenoids were analyzed statistically with and without lipid standardization. A SAS Proc TTest [25] was used to compare the mean baseline values of men and women, and lipoprotein cholesterol values under baseline and TAD–PS intakes.

Results

Subjects

The characteristics of the subjects prior to the start of the intervention are shown in Table 1. Fourteen men and nine postmenopausal women (who were not using hormone replacement therapy) completed the four dietary intervention periods. Data from one male subject were dropped from the analyses because of suspicion of noncompliance. The male participants were younger on average than the female participants. At baseline, mean plasma lipid and lipoprotein cholesterol concentrations for men and women were not different. Plasma concentrations of TC and LDL cholesterol were higher at baseline than during the TAD–PS controlled dietary periods ($P = 0.0189$ and 0.018, respectively), whereas TAG and HDL cholesterol were comparable ($P > 0.05$ for both).

Diets

The compositions of the TAD and the Step 1 diet, which were formulated by adding control or test spreads and salad

Table 1 Age, body mass index, energy intake, and baseline plasma lipids, lipoproteins, retinol, tocopherols, and carotenoids

	Men ($n = 13$)	Women ($n = 9$)[a]	All ($n = 22$)
Age (years)	47.0 ± 3.0	61.0 ± 2.4	51.7 ± 2.4
Body mass index (kg/m^2)	28.2 ± 0.8	27.8 ± 1.0	28.0 ± 0.6
Energy intake[b] (MJ/day)	12.10 ± 0.51	9.39 ± 0.39	10.99 ± 0.44
Baseline blood lipids and lipoproteins			
TAG (mmol/L)	1.46 ± 0.19	1.84 ± 0.39	1.62 ± 0.20
TC (mmol/L)	5.80 ± 0.20	5.97 ± 0.22	5.87 ± 0.14
HDL cholesterol (mmol/L)	1.34 ± 0.12	1.43 ± 0.10	1.38 ± 0.08
LDL cholesterol (mmol/L)	3.80 ± 0.16	3.71 ± 0.13	3.76 ± 0.10
TC/HDLC	4.66 ± 0.34	4.30 ± 0.26	4.51 ± 0.23
Apolipoprotein A1 (g/L)	1.49 ± 0.05	1.59 ± 0.09	1.53 ± 0.06
Apolipoprotein B (g/L)	0.88 ± 0.05	0.91 ± 0.05	0.89 ± 0.04
Apo B/Apo A1	0.62 ± 0.05	0.59 ± 0.05	0.61 ± 0.04
Baseline blood carotenoids, retinol, and tocopherols			
Retinol (μmol/mmol TC)	0.45 ± 0.03	0.41 ± 0.07	0.43 ± 0.03
δ-Tocopherol (μmol/mmol TC)	0.04 ± 0.01	0.03 ± 0.01	0.04 ± 0.00
γ-Tocopherol (μmol/mmol TC)	1.17 ± 0.11	1.08 ± 0.14	1.13 ± 0.08
α-Tocopherol (μmol/mmol TC)	7.40 ± 0.41	6.82 ± 0.79	7.16 ± 0.40
Lutein (μmol/mmol TC)	0.07 ± 0.00	0.05 ± 0.01	0.06 ± 0.00
Zeaxanthin (μmol/mmol TC)	0.03 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
α-Cryptoxanthin (μmol/mmol TC)	0.02 ± 0.00	0.01 ± 0.00	0.02 ± 0.00
β-Cryptoxanthin (μmol/mmol TC)	0.06 ± 0.00	0.04 ± 0.00	0.05 ± 0.00
Lycopene (μmol/mmol TC)	0.08 ± 0.01	0.07 ± 0.01	0.07 ± 0.00
α-Carotene (μmol/mmol TC)	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
β-Carotene (μmol/mmol TC)	0.11 ± 0.01	0.11 ± 0.02	0.11 ± 0.01
Total carotenoids (μmol/mmol TC)	0.39 ± 0.02	0.34 ± 0.02	0.37 ± 0.01

Values are mean ± SEM

[a] Women were postmenopausal and were not receiving hormone replacement therapy

[b] Average daily energy intake from all four diets

dressings to a common experimental background diet, are listed in Table 2. The TAD was designed to contain 34% of energy from fat with a ratio of saturated to total monounsaturated to total polyunsaturated fatty acids (S:M:P) of 1:1:0.5. The analytical results showed that the fat energy contribution was slightly lower than planned at 33.5%, but the S:M:P ratio was achieved. The ratio of saturated to *cis*-monounsaturated to *cis*-polyunsaturated fatty acids was 1.0:0.9:0.5. The Step 1 diet was planned to have less than 30% of energy from fat, to have less than 7% of energy from saturated fat, and to have an S:M:P ratio of 1:1.5:1. The analytical results showed that fat contributed 26.4% of energy and that saturated fatty acids contributed 7.7% of energy. The target S:M:P ratio was achieved, and the ratio of saturated to *cis*-monounsaturated to *cis*-polyunsaturated fatty acids was 1.0:1.2:0.9. The *trans* fat content of both diets was close to the mean US population intake of 2.6% of energy [26]. Both diets were designed to provide less than 250 mg cholesterol/day for the average subject, and the Step 1 diet was designed to have approximately 30% less cholesterol than the TAD. The analysis of the dietary composites showed that the TAD had a total fat content of close to those reported in the 2005–2006 NHANES [27], and a slightly higher saturated fat content. The TAD and Step 1 diets had approximately equal dietary fiber content and provided about 26 g dietary fiber per day. This amount of fiber is considerably higher than the average intake reported for the US diet of 18.0 g/day and 14.3 g/day for men and women aged 19 years and over, respectively [27], but was constant across all treatments.

Excluding the PS-enriched foods, the TAD and the Step 1 diets contained comparable amounts of dietary noncholesterol sterols, with both diets providing less than 400 mg PS/day for intake of the average subject. Sitosterol and campesterol accounted for at least 70% of the PS. The profile of noncholesterol sterols in the two +PS controlled diets reflected that of the soybean PS ester extract incorporated in the spread and dressings. From the 3.3 g/day

Table 2 Diet composition of the typical American diet (TAD) and Step 1 type diet

	TAD	Step 1
Protein (% of energy)	16.2	17.4
Carbohydrate (% of energy)	50.3	56.2
Fat (% of energy)	33.5	26.4
Saturated	13.19	7.67
12:0	2.17	0.17
14:0	1.27	0.43
16:0	4.74	3.89
18:0	3.64	2.51
cis-Monounsaturated	11.49	8.94
18:1	11.08	8.59
cis-Polyunsaturated	6.49	7.10
18:2	5.80	6.60
18:3	0.62	0.53
trans	2.35	2.57
18:1	1.96	2.13
18:2	0.33	0.44
Dietary fiber (g/1,000 kcal)	11.6	12.0
Cholesterol (mg/1,000 kcal)	96.3	68.7
Plant sterols[a] (mg/1,000 kcal)	145.7	143.0

[a] The percentage distribution of PS in the +PS diets is brassicasterol 2.5, campesterol 24.6, campestanol 0.8, stigmasterol 18.2, β-sitosterol 47.7, β-sitostanol 1.3, D5-avenasterol 1.2; the percentage distribution of PS in the −PS diets is brassicasterol 2.2 campesterol 19.2, campestanol 1.5, stigmasterol 9.7, β-sitosterol 53.3, β-sitostanol 2.7, D5-avenasterol 3.8

intake of PS in the +PS diets, 1.56 g was sitosterol and 0.81 g was campesterol.

The carotenoid-containing foods in the four experimental diets were the same, were fed in equal amounts in proportion to energy intake, and provided α- and β-carotene intakes that exceeded those reported in the 2005–2006 NHANES [27]. The calculated carotenoid content of the common background diet contained 4.82 mg/1,000 kcal total carotenoids, 1.69 mg/1,000 kcal β-carotene, 0.39 mg/1,000 kcal α-carotene, 2.05 mg/1,000 kcal lycopene, and 0.64 mg/1,000 kcal lutein + zeaxanthin.

Responses of Plasma Lipids and Lipoproteins to PS Intake and Diet

Body mass index had no significant effect on any of the dependent variables studied. There was also no effect of sex on lipids or lipoproteins; therefore, data from both sexes were combined for analyses and reporting.

Main Effects

Independent of PS, plasma concentrations of TC, HDLC, LDLC, Apo A1 and Apo B were lower by 4.4, 5.3, 4.5, 2.8, and 2.5%, respectively, with the Step 1 diet substituting for the TAD. Diet had no significant effect on plasma concentrations of TAG or the ratio of TC to HDLC (TC/HDLC). Independent of the type of diet, the consumption of 3.3 g PS/day reduced plasma concentrations of TC, LDLC, and TC/HDLC by 9.0, 12.4, and 9.6%, respectively. Plasma TAG, HDLC, and Apo A1 concentrations were not significantly affected by PS intake. In response to PS intake, plasma Apo B concentrations were reduced from 0.82 to 0.77 g/L, and the ratio of Apo B to Apo A1 was reduced from 0.60 to 0.55 (Table 3).

Interaction Effects

Diet × PS interactions existed for the plasma concentration of Apo B ($P = 0.0035$) and the Apo B/Apo A1 ratio ($P = 0.0011$). When PS were not added to the diets, the plasma Apo B concentration after consumption of the TAD (0.85 g/L) was higher than that after consumption of the Step 1 diet (0.80 g/L). With PS intake, Apo B concentrations after consumption of both diets (to 0.77 g/L) were not significantly different. Without PS intake, there was no effect of diet on the ratio of Apo B–Apo A1. Subsequent to PS intake, however, the reduction in this ratio after consumption of the TAD was greater than after consumption of the Step 1 diet (Table 3).

Responses of Plasma Retinol, Tocopherols, and Carotenoids to PS Intake and Diet

The effects of diet and PS intake on the absolute and TC standardized plasma concentrations of retinol; α-, γ-, and δ-tocopherols; lutein; zeaxanthin; α- and β-cryptoxanthins; lycopene; and α- and β-carotene are presented in Table 4 with responses from each sex combined (the sex effect and the diet × PS interaction were not significant, $P > 0.05$). Absolute plasma concentrations of all compounds except retinol and δ-tocopherol were reduced by the intake of 3.3 g PS/day. After standardization for TC, the effects of PS were limited to cryptoxanthins, lycopene, and carotenes; the reductions were 5.9% for α-cryptoxanthin, 7.8% for β-cryptoxanthin, 24.7% for lycopene, 14.3% for α-carotene, and 21.0% for β-carotene.

Diet had no effect on the absolute concentrations of retinol, α-tocopherol, β-cryptoxanthin, lycopene, and α-carotene. Concentrations of γ-tocopherol, lutein, zeaxanthin, and α-cryptoxanthin were higher, and the concentration of β-carotene was lower with a TAD background diet. Subsequent to standardization for TC, plasma concentrations of γ- and δ-tocopherols and zeaxanthin remained higher after the consumption of the TAD than after the Step 1 diet. There was no diet effect on concentrations of retinol, α-tocopherol, lutein, cryptoxanthins, and lycopene.

Table 3 Effects of diet and intake of plant sterols (PS) on plasma lipids and lipoproteins

	Treatments[1]					Diet effect		PS effect			*P* value (*F* test)		
	Step 1		TAD										
	−PS	+PS	−PS	+PS	SEM	Step 1	TAD	−PS	+PS	SEM	Diet	PS	Diet × PS
TAG (mmol/L)	1.53[2]	1.41	1.52	1.41	0.08	1.47	1.46	1.52	1.41	0.07	0.8739	0.0530	0.6933
TC (mmol/L)	5.31^{c}	4.83^{a}	5.55^{d}	5.06^{b}	0.10	5.07	5.30	5.43	4.94	0.10	<0.0001	<0.0001	0.9147
HDLC (mmol/L)	1.24^{a}	1.25^{a}	1.31^{b}	1.32^{b}	0.03	1.25	1.32	1.29	1.29	0.03	<0.0001	0.2831	0.5247
LDLC (mmol/L[4])	3.38^{b}	2.95^{a}	3.55^{b}	3.10^{a}	0.08	3.17	3.32	3.46	3.03	0.07	0.0016	<0.0001	0.7873
TC/HDLC	4.53^{b}	4.12^{a}	4.47^{b}	4.03^{a}	0.09	4.33	4.25	4.50	4.07	0.08	0.1062	<0.0001	0.7496
Apo A1 (g/L)	1.40^{a}	1.40^{a}	1.44^{ab}	1.45^{b}	0.02	1.40	1.44	1.42	1.42	0.02	0.0006	0.7324	0.7476
Apo B (g/L)	0.80^{b}	0.77^{a}	0.85^{c}	0.77^{a}	0.02	0.79	0.81	0.82	0.77	0.02	0.0069	<0.0001	0.0035[3]
Apo B/Apo A1	0.59^{b}	0.56^{a}	0.61^{b}	0.54^{a}	0.01	0.58	0.58	0.60	0.55	0.01	0.9814	<0.0001	0.0011[4]

[1] Among four treatments, values in a row with different superscripts differ, $P < 0.05$ (Tukey–Kramer)

[2] LS mean

[3] In the presence of PS, no diet effect was detected for plasma Apo B level ($P = 0.9982$). In the absence of PS Apo B after TAD feeding was higher than that after Step 1 feeding ($P = 0.0006$)

[4] The PS induced lowering of Apo B/Apo A1 was greater after TAD feeding (a 11.5% reduction, $P < 0.0001$) than after Step 1 feeding (a 5.1% reduction, $P = 0.0277$)

Concentrations of α- and β-carotene were lower after consumption of the TAD background diet. The higher TC-corrected plasma retinol concentration after PS intake in the present study was mainly due to the significant reduction in TC related to PS, because there was no effect of PS on the uncorrected plasma retinol concentration.

Discussion

The main objective of the study was to investigate, whether the magnitude of reduction in blood concentration of LDL cholesterol in response to PS intake is different between a diet representing what average Americans eat and a diet representing what health professionals recommend for blood cholesterol management. Within the context of the present study design, a diet effect and a PS effect were observed. Because there was no diet × PS interaction, our data demonstrate experimentally, for the first time, that the effects of PS and diet on plasma lipoprotein cholesterol concentrations are additive, at least for diets ranging in composition from the Step 1 to the TAD.

The magnitude of PS induced reduction in plasma TC and LDL cholesterol concentrations found in this study is comparable with the changes reported in the literature in which 2–3 g PS/day was consumed in conjunction with various background diets. Under Step 1 feeding alone, three servings of PS produced a 12.7% reduction in LDL cholesterol which is consistent with our prior study in which two servings of PS were fed and a 9.7% reduction in LDL cholesterol was observed [13]. Most importantly, these data show that a 16.9% reduction in plasma LDL cholesterol concentration and a 7.8% increase in the TC/HDL cholesterol ratio were accomplished by switching from a TAD to a Step 1 +PS diet. For the reduction in LDL cholesterol, 72.7% was attributed to the PS and 27.3% to the switch from the TAD to the Step 1 diet. For the reduction in the TC/HDL cholesterol ratio, 100% was attributed to PS.

Because of the power gained from using a crossover design of this study, we were able to report for the first time a diet × PS interaction for Apo B and Apo B/Apo A1. The diet effect on Apo B was detectable only in the absence of PS which may be explained by the greater effect of PS on Apo B masking the weaker diet effect. Apo B/Apo A1 ratio has been suggested to be a better index than TC/HDL cholesterol ratio, non-HDL cholesterol/HDL cholesterol ratio, or LDL cholesterol/HDL cholesterol ratio to predict coronary risk [28]. As such, our finding of a greater PS effect on Apo B/Apo A1 ratio with TAD feeding than Step 1 feeding is of practical significance as the majority of the Americans are consuming diets closer to the TAD than the Step 1 diet [27].

Consistent with the literature, the present study found no effect of PS on plasma TAG and Apo A1 concentrations, whereas LDL cholesterol and Apo B concentrations, and thus the Apo B/Apo A1 ratio, were lowered by PS intake. The diet effects on TC, HDL cholesterol, LDL cholesterol, Apo A1, and Apo B were as expected. A diet effect on TAG was not observed possibly because the number of subjects was insufficient for such a purpose [29]. The subject sample size in this study was determined based on the detection of a significant difference in LDL cholesterol and not TAG.

Table 4 Effects of diet and intake of plant sterols (PS) on plasma retinol, tocopherols and carotenoids

Plasma concentration	Diet effect		PS effect			*P* value (*F* test)	
	Step 1	TAD	−PS	+PS	SEM	Diet	PS
Retinol							
μmol/L	2.25[a]	2.28	2.28	2.24	0.09	0.6153	0.4965
μmol/mmol TC	0.44	0.43	0.42	0.45	0.02	0.3130	0.0062
δ-Tocopherol							
μmol/L	0.20	0.23	0.22	0.21	0.02	0.0019	0.5149
μmol/mmol TC	0.04	0.04	0.04	0.04	<0.01	0.0142	0.5034
γ-Tocopherol							
μmol/L	5.80	6.49	6.40	5.89	0.32	0.0006	0.0108
μmol/mmol TC	1.14	1.22	1.18	1.18	0.06	0.0191	0.8451
α-Tocopherol							
μmol/L	36.12	35.85	38.36	33.60	1.68	0.7908	<0.0001
μmol/mmol TC	6.95	6.72	6.95	6.72	0.25	0.1155	0.1160
Lutein							
μmol/L	0.30	0.33	0.34	0.30	0.02	0.0073	0.0011
μmol/mmol TC	0.06	0.06	0.06	0.06	<0.01	0.0592	0.2663
Zeaxanthin							
μmol/L	0.11	0.13	0.13	0.11	0.01	<0.0001	0.0013
μmol/mmol TC	0.02	0.03	0.02	0.02	<0.01	<0.0001	0.1784
α-Cryptoxanthin							
μmol/L	0.083	0.091	0.095	0.080	0.004	0.0119	<0.0001
μmol/mmol TC	0.016	0.017	0.017	0.016	0.001	0.1837	0.0104
β-Crytpoxanthin							
μmol/L	0.253	0.260	0.279	0.233	0.011	0.3198	<0.0001
μmol/mmol TC	0.049	0.048	0.051	0.047	0.002	0.5325	0.0008
Lycopene							
μmol/L	0.340	0.335	0.399	0.275	0.02	0.6653	<0.0001
μmol/mmol TC	0.066	0.063	0.073	0.055	<0.01	0.0925	<0.0001
α-Carotene							
μmol/L	0.142	0.132	0.152	0.122	0.01	0.0969	<0.0001
μmol/mmol TC	0.027	0.024	0.028	0.024	<0.01	0.0059	0.0033
β-Carotene							
μmol/L	0.501	0.438	0.545	0.394	0.02	<0.0001	<0.0001
μmol/mmol TC	0.097	0.081	0.100	0.079	0.01	<0.0001	<0.0001

[a] LS mean

Qualitatively, the responses of plasma retinol, tocopherols, and carotenoids to PS intake observed in the present study were as expected. Quantitatively, the responses are consistent with those reported by us previously but greater than those reported by others in studies in which comparable amounts of PS were consumed, particularly for α- and β-carotene and lycopene. Richelle et al. [30] recently identified reductions in absorption of β-carotene and α-tocopherol as a mechanism for the PS intake-associated reductions in the circulation. We found higher plasma TC standardized concentrations of γ-tocopherols and zeaxanthin after consumption of the TAD than the Step 1 diet. This finding is consistent with the knowledge that an increase in fat intake promotes the absorption of tocopherols and carotenoids [31]. The observation that the TAD, as compared with the Step 1 diet, produced significant reductions in TC-corrected plasma α- and β- carotene concentrations was unexpected and unexplainable, but is probably not physiologically important because the differences are insignificant in the context of their typical ranges in plasma.

In conclusion, our study is the first example of a direct comparison of the influence of diets versus the influence of PS intake on blood lipoprotein concentrations. Our findings

confirm that the LDL cholesterol-reducing effects resulting from PS intake and from switching from the TAD to the Step 1 diet are independent of each other, and that both are important but the effect of PS intake is quantitatively far greater. Our findings should not be taken as a suggestion that diet is of no consequence, because in fact the diet effect and the PS effect are additive. A limitation of the present study, as in most other published studies of PS and plant stanols, is the possible lack of generalizability because study participation was restricted to mildly hypercholesterolemic adults and, in our case, middle-aged male and non-HRT using postmenopausal female subjects. It is important to extend this study to larger and more varied groups.

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Conflict of interest statement J.T. Judd, M. Kramer, B.A. Clevidence, and D.J. Baer, no conflicts of interest. S.C. Chen was an employee of Unilever Foods, NA at the time the dietary portion of the study was conducted. G.W. Meijer is an employee of Unilever.

References

1. Miettinen TA, Puska P, Gylling H, Vanhanen H, Vartiainen E (1995) Reduction of serum cholesterol with sitostanol-ester margarine in a mildly hypercholesterolemic population. N Engl J Med 333:1308–1312
2. Katan MB, Grundy SM, Jones P, Law M, Miettinen T, Paoletti R (2003) Efficacy and safety of plant stanols and sterols in the management of blood cholesterol levels. Mayo Clin Proc 78:965–978
3. Ostlund RE Jr (2002) Phytosterols in human nutrition. Annu Rev Nutr 22:533–549
4. Lichtenstein AH (2002) Plant sterols and blood lipid levels. Curr Opin Clin Nutr Metab Care 5:147–152
5. Plat J, Mensink RP (2005) Plant stanol and sterol esters in the control of blood cholesterol levels: mechanism and safety aspects. Am J Cardiol 96:15D–22D
6. Nestel P, Cehun M, Pomeroy S, Abbey M, Weldon G (2001) Cholesterol-lowering effects of plant sterol esters and non-esterified stanols in margarine, butter and low-fat foods. Eur J Clin Nutr 55:1084–1090
7. Miettinen TA, Vanhanen H (1994) Dietary sitostanol related to absorption, synthesis and serum level of cholesterol in different apolipoprotein E phenotypes. Atherosclerosis 105:217–226
8. Pelletier X, Belbraouet S, Mirabel D, Mordret F, Perrin JL, Pages X, Debry G (1995) A diet moderately enriched in phytosterols lowers plasma cholesterol concentrations in normocholesterolemic humans. Ann Nutr Metab 39:291–295
9. Hallikainen MA, Sarkkinen ES, Gylling H, Erkkila AT, Uusitupa MI (2000) Comparison of the effects of plant sterol ester and plant stanol ester-enriched margarines in lowering serum cholesterol concentrations in hypercholesterolaemic subjects on a low-fat diet. Eur J Clin Nutr 54:715–725
10. Weststrate JA, Meijer GW (1998) Plant sterol-enriched margarines and reduction of plasma total- and LDL-cholesterol concentrations in normocholesterolaemic and mildly hypercholesterolaemic subjects. Eur J Clin Nutr 52:334–343
11. Maki KC, Davidson MH, Umporowicz DM, Schaefer EJ, Dicklin MR, Ingram KA, Chen S, McNamara JR, Gebhart BW, Ribaya-Mercado JD, Perrone G, Robins SJ, Franke WC (2001) Lipid responses to plant-sterol-enriched reduced-fat spreads incorporated into a National Cholesterol Education Program Step I diet. Am J Clin Nutr 74:33–43
12. Jones PJ, Ntanios FY, Raeini-Sarjaz M, Vanstone CA (1999) Cholesterol-lowering efficacy of a sitostanol-containing phytosterol mixture with a prudent diet in hyperlipidemic men. Am J Clin Nutr 69:1144–1150
13. Judd JT, Baer DJ, Chen SC, Clevidence BA, Muesing RA, Kramer M, Meijer GW (2002) Plant sterol esters lower plasma lipids and most carotenoids in mildly hypercholesterolemic adults. Lipids 37:33–42
14. Vanstone CA, Raeini-Sarjaz M, Parsons WE, Jones PJ (2002) Unesterified plant sterols and stanols lower LDL-cholesterol concentrations equivalently in hypercholesterolemic persons. Am J Clin Nutr 76:1272–1278
15. Gylling H, Miettinen TA (1999) Cholesterol reduction by different plant stanol mixtures and with variable fat intake. Metabolism 48:575–580
16. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report (2002) Circulation 106:3143–3421
17. Kris-Etherton PM, Dietschy J (1997) Design criteria for studies examining individual fatty acid effects on cardiovascular disease risk factors: human and animal studies. Am J Clin Nutr 65:1590S–1596S
18. Composition of foods, agriculture handbook no. 8, sections 1–22. In: US Department of Agriculture HNIS (ed), US Government Printing Office, 1976–1990
19. Holden JM, Eldridge AL, Beecher GR, Buzzard IM, Bhagwat SA, Davis CS, Douglass LW, Gebhardt SE, Haytowitz DB, Schakel S (1999) Carotenoid content of US foods: an update of the database. J Food Comp Anal 12:169–196
20. Hainline A, Karon J, Lippel K (1982) Manual of laboratory operations: lipid and lipoprotein analysis. In: USGPO HEW (ed) Lipid research clinics program pub no. (NIH) 75–628 (rev.). National Heart Lung and Blood Institute, Bethesda
21. Friedewald WT, Levy RI, Fredrickson DS (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 18:499–502
22. Khachik F, Beecher GR, Goli MB, Lusby WR, Smith JC Jr (1992) Separation and identification of carotenoids and their oxidation products in the extracts of human plasma. Anal Chem 64:2111–2122
23. Bieri JG, Brown ED, Smith JC Jr (1985) Determination of individual carotenoids in human plasma by high performance liquid chromatography. J Liq Chromatogr 8:473–484
24. Littell RC, Milliken GA, Stroup WW, Wolfinger RD (1996) SAS system for mixed models. SAS, Cary
25. SAS/STAT (2003) User's guide. Version 9.1.3. Cary, NC: SAS Institute Inc.
26. Allison DB, Egan SK, Barraj LM, Caughman C, Infante M, Heimbach JT (1999) Estimated intakes of *trans* fatty and other fatty acids in the US population. J Am Diet Assoc 99:166–174 (quiz 175–176)

27. What we eat in America, NHANES 2005–2006: documentation and data files (2008). In: US Department of Agriculture ARS (ed)
28. Walldius G, Jungner I, Aastveit AH, Holme I, Furberg CD, Sniderman AD (2004) The apoB/apoA–I ratio is better than the cholesterol ratios to estimate the balance between plasma pro-atherogenic and antiatherogenic lipoproteins and to predict coronary risk. Clin Chem Lab Med 42:1355–1363
29. Ginsberg HN, Kris-Etherton P, Dennis B, Elmer PJ, Ershow A, Lefevre M, Pearson T, Roheim P, Ramakrishnan R, Reed R, Stewart K, Stewart P, Phillips K, Anderson N (1998) Effects of reducing dietary saturated fatty acids on plasma lipids and lipoproteins in healthy subjects: the DELTA study, protocol 1. Arterioscler Thromb Vasc Biol 18:441–449
30. Richelle M, Enslen M, Hager C, Groux M, Tavazzi I, Godin JP, Berger A, Metairon S, Quaile S, Piguet-Welsch C, Sagalowicz L, Green H, Fay LB (2004) Both free and esterified plant sterols reduce cholesterol absorption and the bioavailability of beta-carotene and alpha-tocopherol in normocholesterolemic humans. Am J Clin Nutr 80:171–177
31. Parker RS, Swanson JE, You CS, Edwards AJ, Huang T (1999) Bioavailability of carotenoids in human subjects. Proc Nutr Soc 58:155–162