No Changes in Serum Fat-Soluble Vitamin and Carotenoid Concentrations With the Intake of Plant Sterol/Stanol Esters in the Context of a Controlled Diet

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Spreads enriched with plant sterol and stanol esters have been shown to possess similar cholesterol-lowering properties; however, their comparative capacity to alter circulating levels of other fat-soluble compounds has not been fully assessed. To compare actions of sterol and stanol ester consumption on serum fat-soluble vitamin and carotenoid concentrations, 15 hypercholesterolemic subjects were fed each of 3 fixed foods treatment diets over 21 days using a randomized crossover controlled design. Diets contained either (1) margarine (M), (2) margarine with sterol esters (MSE; 1.92 g/d), or (3) margarine with stanol esters (MSA; 1.76 g/d). No significant differences were found in initial or final serum fat-soluble vitamin and carotenoid concentrations among the 3 phases. Serum retinol and α- and γ-tocopherol concentrations at baseline and endpoint and percentage changes relative to baseline for MSE and MSA were not significantly different from those of the M diet. After adjusting for total cholesterol reduction, no changes for α- and γ-tocopherol were found. Serum vitamins D and K, lycopene, and lutein concentrations and percentage changes did not differ across diets. Serum concentrations at baseline and endpoint and percentage changes for α- and β-cryptoxanthin and α- and γ-carotene were not different among the diets, nor did serum α- and γ-carotene concentrations to total cholesterol ratios differ. Serum lutein, β-cryptoxanthin, and α-carotene concentrations increased over time. In conclusion, our results show no effect of consumption of esterified plant sterols or stanols on serum fat-soluble vitamin or carotenoid concentrations compared with a control diet.

ELEVATED PLASMA cholesterol levels are a major risk factor in the development of coronary heart disease (CHD). Although hypercholesterolemic patients with CHD risk can benefit from drug therapies,[1] enriching food products with plant sterols and stanols also favorably reduces circulatory cholesterol concentrations.[2-6] Consumption of plant sterols and stanols provides no obvious adverse effects,[5-9] but is not recommended for individuals with phytosterolemia.[10] Retinol, vitamins D and K, tocopherols, and carotenoids are fat-soluble, and are absorbed similarly to other lipids in the gut. Since the lipid-lowering effects of phytosterols are mainly due to the inhibition of intestinal cholesterol absorption, it is possible that the absorption of other fat-soluble compounds, such as vitamins, might also be compromised by plant sterol or stanol ester consumption.[11]

Data are equivocal, however, in defining such an action. Gylling and Miettinen[12] found that serum vitamin D and retinol concentrations, as well as the α-tocopherol to cholesterol ratio remained unaffected by consumption of stanol esters, while those of α-tocopherol and α- and β-carotene were reduced over a 6-week period in moderately hypercholesterolemic postmenopausal women. When stanol esters were compared with a control diet, serum vitamin D increased, but the α-tocopherol to cholesterol ratio and retinol concentration remained unchanged. Stanol ester feeding reduced α-carotene in comparison to a control diet.[12] Similarly, Gylling et al[13] also found that consumption of 2.5 g/d of sitostanol ester–enriched spread decreased serum α-tocopherol and carotene concentrations. In contrast, Hallikainen and Uusitupa[14] reported no changes in serum retinol concentration after 8 weeks of consumption of 2.3 g/d stanol esters, while serum β-carotene and α-tocopherol concentrations were reduced. These investigators also reported no effect of diet when the ratios of serum β-carotene and α-tocopherol to cholesterol were compared. Weststrate and Meijer[15] found reductions in α- and β-carotene and lycopene concentrations with the consumption of 3 g/d of sterol ester in normocholesterolemic and mildly hypercholesterolemic subjects. Hendriks et al[11] found similar results with consumption of 3 different levels of plant sterol esters (0.85 g/d, 1.61 g/d, or 3.24 g/d). However, after correcting for the reduction in plasma lipid levels, only plasma α- and β-carotene concentrations were found to be reduced. These investigators concluded that although there was no significant difference between groups, the 1.61-g/d dose of plant sterol esters appeared to provide the best combination of cholesterol-lowering with minimal reductions in other plasma fat-soluble components.

Despite these findings, effects of plant sterol and stanol esters on serum fat-soluble vitamin and carotenoid concentrations have not been cross-compared under precisely controlled dietary conditions. Therefore, the aim of this study was to investigate whether diets enriched with sterol and stanol esters reduce serum fat-soluble vitamin and carotenoid concentrations in hypercholesterolemic male subjects using a fixed foods feeding trial. The null hypothesis tested was that over 3 weeks, feeding margarine alone, versus margarine enriched with nonhydrogenated or hydrogenated plant sterols, would not influence serum fat-soluble vitamin and carotenoid concentrations.

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MATERIALS AND METHODS

Human Subjects

Hypercholesterolemic male subjects between the ages of 37 and 61 years participated in the trial. Subjects were screened for total circulating cholesterol and triglyceride concentrations, and those within the range of 6.0 to 10.0 mmol/L total circulating cholesterol and less than 3.0 mmol/L triglyceride concentrations were chosen for the study. Subjects were asked to answer a family history questionnaire to screen for familial hyperlipidemia, diabetes, hypothyroidism, or heart disease. Individuals who had been on drug therapy for hypercholesterolemia during the previous 2 months were excluded. The experimental protocol was approved by the Ethical Review Committee of McGill University. The study protocol was explained to the subjects prior to their providing informed consent.

Protocol and Diet

A randomized, double-blind, placebo-controlled crossover feeding design was used. Three coded margarine mixtures were provided containing (1) margarine alone (M), (2) margarine with 8% (wt/wt) esterified plant sterols (MSE), or (3) 8% (wt/wt) esterified plant stanols (MSA). Sterol composition of the spreads is given in Table 1. In order to reduce the error term associated with diet pattern, subjects were randomly assigned to one of six predetermined Latin squares, where each square possessed 3 sequenced phases and 3 subjects. In this manner, balance of the crossover design was ensured.

All subjects were provided with a North American solid foods diet during each of the dietary phases. Each dietary phase consisted of a 21-day feeding period followed by a 5-week wash-out. The diet was formulated to meet Canadian Recommended Nutrient Intakes and provide fat, fiber, and carbohydrate subcomponents consistent with Health Canada Recommendations. Dietary protein, carbohydrate, and fat made up 15%, 50%, and 35% of ingested energy, respectively. The dietary fat was comprised of 15%, 10%, and 10% of energy as monounsaturated, saturated, and polyunsaturated fats, using a blend of olive oil–, butter-, and a corn oil–based margarine, respectively. The diet was designed using a 3-day rotating meal cycle to provide variety over the 21-day feeding period. Meals were prepared in the metabolic kitchen at the Mary Emily Clinical Nutrition Research Unit. Subjects were required to consume at least 2 meals per day at the Nutritional Research Unit under supervision of the unit's staff, although meals other than breakfast were available for take-out. Subjects were prohibited from consuming any food other than those provided by the Nutrition Research Unit throughout the study.

A predictive equation based on each subject's weight, height, age, and level of activity was used to formulate the amount of food consumed by each subject for maintaining individual weight balance. Margarine mixtures, either as such or enriched with 8% sterol or stanol esters, were incorporated into the diet at a mean level of 23 g margarine/d, which corresponded to 1.92 g esterified plant sterols or 1.76 esterified plant stanols/d/70 kg body weight. Daily margarine doses were divided into 3 equal portions and added to each meal. Fat-soluble vitamin and carotenoid concentrations of the 3 rotating meal cycles are given in Table 2.

Table 1. Plant Sterol and Stanol Composition of Spreads Used in the Feeding Experiment

	Stanol Ester		Sterol Ester	
Total Sterols	mg · kg^{-1}	% (wt:wt)	mg · kg^{-1}	% (wt:wt)
Cholesterol	202	0.26	470	0.56
Brassicasterol	173	0.23	1,251	1.49
Campesterol	1,646	2.14	21,611	25.8
Campestanol	21,304	27.83	770	0.92
Stigmasterol	743	0.98	16,106	19.23
β-sitosterol	3,868	5.05	38,402	45.85
Sitostanol	46,770	61.11	1,610	1.92
Δ5-avenasterol	133	0.17	838	1.01
Others	1,700	2.22	2,700	3.22
Total	76,539	100	83,758	100

Table 2. Fat-Soluble Vitamin and Carotenoid Concentrations in Three Rotating Dietary Foods

	μmol/kg (wet weight)		
Food Components	Day 1	Day 2	Day 3
Retinol	0.15	0.39	0.19
λ-tocopherol	0.79	1.21	0.16
α-tocopherol	6.15	8.28	3.47
Lutein & zeaxanthin	3,031.78	2,870.26	1,654.49
α-cryptoxanthin	48.95	60.56	27.96
β-cryptoxanthin	27.56	87.07	120.59
Lycopene	259.00	944.73	1,176.27
α-carotene	163.62	6.80	141.54
β-carotene	399.83	269.8	270.3

Blood samples (20 mL) were collected from subjects before breakfast on days 0, 8, 15, and 21 of the trial for lipid analyses. Serum taken on days 0 and 21 was obtained after a 15-minute centrifugation at 1,500 rpm and immediately labeled and stored at -80°C for later use in fat-soluble vitamin analyses. On days 0 and 21 of each phase, an additional 15 mL of blood was drawn to perform complete blood analyses to monitor the health of the subjects. Subjects also underwent complete physical examinations and urine analysis at the beginning and end of each dietary phase to evaluate the clinical safety of the margarine products.

Determination of Serum Fat-Soluble Vitamin and Carotenoid Levels

The extraction procedure was as described by Barua et al.[16,17] In brief, a solution of retinol acetate (100 μL of 5 μmol/L) or a solution of β-apo-8'-carotene (50 μL of 5 μmol/L) in methanol was added to 200 μL serum as an internal standard to quantify vitamin and carotenoid levels, respectively. The serum was treated with 200 μL ethanol and 500 μL ethyl acetate. The mixture was vortexed and centrifuged at 2,000 rpm each time for 1 minute. The supernatant was collected in a test tube and pellet was broken and extracted twice with 500 μL ethyl acetate and then with 500 μL hexane. The mixture was vortexed and centrifuged at each step. Organic phases were pooled and 500 μL double-distilled water was added, vortexed, and centrifuged as before. The supernatant phase was carefully transferred to a test tube and evaporated under a gentle stream of nitrogen. The residue was then dissolved in ice-cold dichloromethane (40 μL) and methanol (60 μL) and immediately transferred to a glass low-volume insert, placed inside a 1-mL vial and was capped. Duplicates of each sample were prepared. Aliquots of 30 μL were injected into a high-performance liquid chromatograph (HPLC; Shimadzu Corp Analytical Instruments Plant, Kyoto, Japan). Peaks were determined by injecting a series of standards (La Roche, Toronto, Canada).

The HPLC system included Shimadzu model 6A pumps, auto injector (SIL-6A), system controller (SCL-6A), and UV-VIS spectrophotometer detector (SPD-6A). A Waters C-18 μm "Resolve" column (30 cm x 3.9 mm id) was preceded by a C-130 guard column (Upchurch Scientific, Oak Harbor, WA). The detector was connected to a dual-channel Shimadzu C-R4 integrator. The chromatography mobile phase was a mixture of acetonitrile/dichloromethane/methanol/1-octanol (90:15:10:0.1, vol:vol:vol:vol). The flow rate was set at 1.0 mL/min.

Samples for each subject were run continuously, first for vitamins with the detector wavelength set at 300 nm, then with the detector set at 450 nm consecutively for carotenoid analyses.

Analysis of Data

All data were expressed as the mean ± SD. To standardize fat-soluble vitamins and carotenoids, serum concentrations of each compound were divided by plasma total cholesterol concentration. The effect of diet, time, and time by diet on vitamins, carotenoids, and their ratios to total cholesterol were determined using a crossover repeated-measures analysis of variance model. Data at commencement and end of each dietary period were used for this analysis. Tests for associations between variables were also performed using Pearson correlation coefficient analyses. A level of statistical significance at $P < .05$ was used in all analyses. The data were analyzed using the Procedure-General Linear Model of SAS (version 6.12) software.[18]

RESULTS

Subject Compliance and Drop-out Rate

Eighteen subjects were recruited into the study protocol, with 16 completing all 3 treatments. However, data from only 15 subjects were included in final analyses because 2 subjects left the study at the end of the first phase due to personal reasons and a third was terminated due to reported failure to fast before blood withdrawal. Weight changes were monitored daily and food amounts adjusted accordingly where necessary. There was no significant weight change detected in subjects while consuming any of the 3 margarine mixtures.[6]

Serum Vitamin Profile in Response to Treatment

Plasma lipid levels for this study have been reported elsewhere.[6] Significant reductions in serum total and low-density lipoprotein (LDL)-cholesterol were observed in the sterol and stanol ester groups compared to control.[6] Serum vitamin concentrations are presented in Table 3. In general no time, diet, and time by diet effects were found among the 3 phases for fat-soluble vitamins. There was no difference in the mean baseline retinol concentrations across the various diets. After 21 days of treatment, mean retinol concentrations and percentage changes relative to baseline were not significantly different among the diets (Table 3).

Serum vitamin D concentrations at baseline were not different among the groups. After 21 days of treatment, serum vitamin D concentrations and percentage changes were not significantly different in the 3 diets (Table 3).

The concentrations of α-tocopherol and γ-tocopherol (vitamin E) at baseline and endpoint were unchanged across diets. After percentage changes relative to baseline were compared, no significant differences were observed for these 2 vitamins among the diets (Table 3). After α- and γ-tocopherol concentrations were corrected for total cholesterol, no significant differences were found across diets (Table 3).

Vitamin K concentrations at the beginning of the trial were not significantly different among the 3 groups. Likewise, after 21 days, diet had no effect on serum vitamin K concentration or percentage changes relative to baseline (Table 3). There was no association between vitamin and cholesterol concentrations.

Serum Carotenoid Profile in Response to Treatment

Serum carotenoid levels are presented in Table 4. In general, no diet or time by diet effects for carotenoids were found across diets. Serum lutein concentrations at baseline and endpoint and percentage changes relative to baseline were not significantly different between the phases. When the effect of time was considered, serum lutein concentrations increased ($P < .04$) over the feeding period (Table 4).

Serum α- and β-cryptoxanthin concentrations at baseline were not significantly different across diets. The effect of diet on these 2 types of cryptoxanthin concentrations for day 21 and on percentage changes relative to baseline were not significantly different in the stanol and sterol ester diets compared with the control diet. Serum β-cryptoxanthin concentration increased ($P < .02$) over the feeding period (Table 4).

Effect of diets on serum lycopene concentrations were not significant on baseline data, and also no differences were found on endpoint and percentage changes relative to baseline data (Table 4).

Serum α-carotene and β-carotene concentrations at commencement of each dietary phase were not significantly different among the diets. After 21 days of treatment, no significant differences were observed across the diets for both types of carotenes and their percentage changes relative to baseline. Serum α-carotene concentration increased ($P < .01$) over time (Table 4). After serum α- and β-carotene concentrations were standardized by plasma total cholesterol concentration, no sig-

Table 3. Serum Retinol, α- and γ-Tocopherol, Vitamins D and K, and Their Ratios to Total Cholesterol in Sterol and Stanol Ester–Enriched and Control Diets (N = 15)

Vitamins	Sterol Ester	Stanol Ester	Control
Retinol (μmol/L)			
Day 0	3.40 ± 1.01	2.88 ± 0.73	3.21 ± 0.77
Day 21	2.79 ± 0.77	3.11 ± 0.81	2.88 ± 0.70
% change*	−17.9	7.9	−10.3
γ-tocopherol (μmol/L)			
Day 0	3.83 ± 1.93	3.50 ± 2.21	3.82 ± 1.97
Day 21	2.87 ± 1.78	3.15 ± 1.74	3.58 ± 2.44
% change	−24.9	−9.8	−6.3
γ-tocopherol:TC†			
Day 21	0.52 ± 0.31	0.57 ± 0.35	0.61 ± 0.42
% change	−6.7	7.8	2.7
α-tocopherol (μmol/L)			
Day 0	35.01 ± 12.12	28.90 ± 18.86	33.90 ± 11.46
Day 21	29.12 ± 8.29	33.17 ± 9.14	32.05 ± 10.42
% change	−16.8	11.8	−3.4
α-tocopherol:TC†			
Day 21	5.28 ± 1.43	5.68 ± 1.51	5.34 ± 1.55
% change	0	36.1	5.3
Vitamin D (nmol/L)			
Day 0	31.40 ± 8.71	29.78 ± 10.69	32.61 ± 11.46
Day 21	30.63 ± 9.22	32.89 ± 12.59	35.30 ± 12.19
% change	−2.5	10.4	8.2
Vitamin K (nmol/L)			
Day 0	1.61 ± 0.92	1.54 ± 1.08	1.62 ± 0.97
Day 21	1.83 ± 1.01	1.21 ± 0.50	1.63 ± 0.66
% change	13.6	−21.1	1

Abbreviation: TC, total cholesterol.
*Percent change relative to day 0.
†μmol/mmol.

Table 4. Serum Lutein, α- and β-Cryptoxanthin, Lycopene, and α- and β-Carotene Concentrations and Their Ratios to Total Cholesterol in Sterol and Stanol Ester–Enriched and Control Diets (N = 15)

Carotenoids	Sterol Ester	Stanol Ester	Control
Lutein (μmol/L)‡			
Day 0	2.18 ± 3.02	1.78 ± 2.21	1.96 ± 1.82
Day 21	2.41 ± 2.59	2.55 ± 2.48	2.71 ± 2.55
% change*	10.5	43.2	38.5
α-cryptoxanthin (μmol/L)			
Day 0	0.61 ± 0.54	0.61 ± 0.42	0.54 ± 0.23
Day 21	0.70 ± 0.50	0.71 ± 0.46	0.81 ± 0.46
% change	14.5	15.2	49.3
β-cryptoxanthin (μmol/L)‡			
Day 0	2.05 ± 1.12	2.02 ± 1.47	2.31 ± 1.55
Day 21	3.41 ± 1.31	3.85 ± 2.52	3.82 ± 1.51
% change	66.6	90.7	65.5
Lycopene (μmol/L)			
Day 0	1.06 ± 0.54	1.16 ± 0.50	1.21 ± 0.62
Day 21	1.13 ± 0.39	1.15 ± 0.62	1.38 ± 0.54
% change	7.2	−0.9	13.6
α-carotene (μmol/L)‡			
Day 0	1.41 ± 0.89	1.15 ± 0.69	1.30 ± 0.69
Day 21	2.15 ± 1.04	1.82 ± 1.24	2.23 ± 1.35
% change	53.0	58.2	71.3
α-carotene: TC†			
Day 21	0.40 ± 0.23	0.32 ± 0.19	0.38 ± 0.19
% change	157	108	145
β-carotene (μmol/L)			
Day 0	2.83 ± 1.97	3.09 ± 3.33	3.02 ± 1.74
Day 21	3.28 ± 1.59	3.26 ± 2.40	3.82 ± 2.05
% change	16.2	5.7	26.6
β-carotene:TC†			
Day 21	0.61 ± 0.31	0.58 ± 0.46	0.65 ± 0.35
% change	67.8	69.7	81.9

*Percent change relative to day 0.
†μmol/mmol.
‡Overall time effect ($P \leq .05$).

nificant differences were observed among the diets (Table 4). There was no association between carotenoid and total cholesterol level concentrations.

DISCUSSION

To our knowledge, this study is the first to compare effects of diets enriched with sterol and stanol ester on serum fat-soluble vitamin and carotenoid concentrations in male hypercholesterolemic subjects using a crossover design with precisely controlled diets. Overall, serum fat-soluble vitamin and carotenoid concentrations did not differ between enriched sterol and stanol ester and control diets. We found no effect of sterol and stanol ester diets on serum retinol, α- and γ-tocopherol, vitamin D and K concentrations, or their changes relative to baseline. Similar results were found by Gylling and Miettinen[12] for serum vitamin D and retinol concentrations after consumption of stanol ester in margarine for 6 weeks. Serum initial and final concentrations for vitamins A, D, and K, and for α- and γ-tocopherol, and standardized values for α- and γ-tocopherol, were not significantly different between the sterol and stanol ester and control diets in our study. Similar to our findings, Hendriks et al[11] found no effect of plant sterol ester consumption on vitamin D and K, and the α-tocopherol to total cholesterol ratio, while significant reductions were seen in α-tocopherol levels; however, subjects self-selected their own diets.

There was no effect of sterol or stanol ester on serum carotenoid concentrations. However, most of the serum carotenoid concentrations increased over time. The increase of serum carotenoid concentrations, relative to baseline, might be due to healthier food consumption patterns during the trial compared with typical eating patterns of volunteers. Evidently, significant improvement in overall lipid profiles in subjects on the control diet was observed. Similar findings were reported by Jones et al using controlled study diets.[5] Noncholesterol sterols and α-tocopherol and α- and γ-carotenes are transported by lipoproteins, mainly LDL, in serum; therefore, their ratios to total cholesterol are good indicators of the level of absorption inhibition caused by plant sterols and stanols. Our findings show that these ratios remained unchanged or increased. The effect of sterol and stanol esters on the status of serum lutein and α- and β-cryptoxanthin concentrations was not significant; it is the first such time these data have been reported. Contrary to our findings, Gylling and Miettinen[12] found reductions in α- and γ-carotenes. Similarly, Hallikainen and Uusitupa[14] found reduction in serum γ-carotene with diets enriched in plant stanol esters, perhaps due to higher plant stanol intakes (>2.5 g/d) compared with the present investigation (1.76 g/d). Hendriks et al[11] and Weststrate and Meijer[15] also found significant reductions in serum lycopene and α- and β-carotene concentrations with plant sterol esters consumption.

According to Hendriks et al,[11] the level of plant sterol consumption in a dietary trial should be optimized to efficiently block the cholesterol absorption, while having minimal effect on other fat-soluble compounds. When hypercholesterolemic men and women consumed varying levels of stanol esters (0.8, 1.6, 2.4, and 3.2 g/d), serum α- and β-tocopherol concentrations were suppressed in a dose-dependent manner compared to a control diet.[7] In this case, the higher the dose of stanol esters, the greater the suppression of serum tocopherol concentrations. In the present study, consumption of 1.92 g/d of sterol and 1.76 g/d stanol esters in margarine increased or did not alter serum lipid-soluble carotenoid concentrations (Table 4). Our findings show that the present dose of stanol ester did not block serum fat-soluble vitamin and carotenoid absorption. Also, consumption of sterol ester in this study had no adverse effects on any of serum fat-soluble vitamin concentrations. These findings in comparison to previous studies suggest that consumption of about 2 g/d sterol or stanol esters effectively reduces serum cholesterol, while not perturbing serum fat-soluble vitamin and carotenoid concentrations. In conclusion, consumption of moderate levels of plant sterols or stanols with a healthy diet does not appear to have a negative physiologic action with respect to serum fat-soluble vitamin and carotenoid concentrations.

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