# High dietary intake of phytosterol esters decreases carotenoids and increases plasma plant sterol levels with no additional cholesterol lowering

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**Abstract** **The objective of this study was to measure the effects on serum lipids and plasma phytosterols of 6.6 g/day phytosterols from three foods (bread, breakfast cereal, and spread) consumed for 12 weeks compared with a diet that was not enriched with phytosterols. Thirty-five subjects undertook a nonrandomized, single-blind study consisting of a 2 week baseline period, 6 weeks on high-phytosterol intake, 6 weeks on high-phytosterol intake plus increased fruit and vegetable intake, and a final 2 week washout period. Serum total cholesterol decreased by 8.3% from 6.59 to 6.04 mmol/l, and LDL cholesterol decreased by 12.6% from 4.44 to 3.88 mmol/l. Plasma phytosterol levels increased by 45% (sitosterol) and 105% (campesterol). Cholesterol-adjusted plasma α- and β-carotene levels decreased by 19–23%, lutein by 14%, and lycopene by 11%. Levels of α-carotene and lutein increased with extra fruit and vegetables. Only lycopene failed to increase during the washout phase. There were no significant changes in biochemical parameters. Serum LDL cholesterol lowering with 6.6 g/day ingested phytosterols was in the range seen with 1.6–3.2 g/day phytosterols. Lowering of plasma carotenoids was greater than that seen with lower phytosterol intake and was partially reversed by increased fruit and vegetable intake.**—Clifton, P. M., M. Noakes, D. Ross, A. Fassoulakis, M. Cehun, and P. Nestel. **High dietary intake of phytosterol esters decreases carotenoids and increases plasma plant sterol levels with no additional cholesterol lowering.** ***J. Lipid Res.*** **2004.** 45: **1493–1499.**

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There are extensive data confirming the effectiveness of esterified phytosterols in margarines, with LDL cholesterol lowering of 10–15% with a dose of 1.6–2.4 g/day sterol (1–6). There are few published data (7, 8) on the short-term use of higher amounts of plant sterols (8.6–9 g/day) in margarines, and there is some evidence that carotenoid lowering is greater on a high dose of phytosterol without enhancement of cholesterol lowering (7). It is not known if increased fruit and vegetables can restore the plasma level of carotenoids after a higher intake of phytosterols, although they can achieve this with a more usual intake of phytosterols (4). The aim of this study was to examine the effect of 6.6 g/day phytosterols in bread, breakfast cereals, and margarine over a 12 week period, to examine the role of extra fruit and vegetables in ameliorating the decrease in carotenoid concentration, and to examine the changes in carotenoid concentrations after cessation of phytosterol ingestion.

## METHODS

### Subjects

Thirty-five mildly hypercholesterolemic men and women were recruited and entered the study. The study was conducted at two clinical research centers: the Commonwealth Scientific and Industrial Research Organization (CSIRO) Division of Health Sciences and Nutrition in Adelaide and the Baker Medical Research Institute in Melbourne. Subjects were screened on the basis of the following inclusion criteria: age, 20–75 years; body mass index (BMI), <31; total serum cholesterol, >5.0 mmol/l and <7.5 mmol/l; and serum triglycerides, <4.5 mmol/l. No lipid-lowering medication was permitted, nor was medication likely to affect lipid metabolism. Subjects were not diabetic, had normal thyroid status, and had no metabolic disorder other than hyperlipidemia. Subjects with a strong aversion or known allergies/intolerances to the foods involved were excluded. The study was approved by the CSIRO Committee for Human Experimentation and the Baker Medical Centre Ethics Committee, and all subjects gave informed consent.

### Study design

All subjects, at both centers, undertook dietary interventions in a nonrandomized manner. The study was single blind, and

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foods were appropriately coded. Subjects were advised not to consume self-purchased phytosterol-enriched products at any time during the trial. Dietary interventions are listed in **Table 1**.

### Food requirements

When consumed at full compliance, the food requirements were designed to contribute a total of 6.6 g/day phytosterols in the ester form, with contributions as follows: 3 × 5 g (15 g) reduced fat spread (2.1 g/day); three slices of white bread (2.4 g/day); and one serving (60 g) of cereal (muesli) (2.1 g/day).

### Measurements

The following measurements were made during the study.

Dietary intake was monitored using Victorian Anti-cancer Foundation diet assessment books (food frequency) during each intervention to determine compliance and assess micronutrient intake. Compliance was assessed by a daily record of the consumption of the supplied foods and the number of servings of fruit and vegetables consumed during the supplementation period. Food was supplied every 2 weeks at each visit.

The weight and height of subjects were determined at entry to the study. Subsequently, weight was measured at each visit to the clinic, which occurred every 2 weeks. Subjects were provided with the opportunity to report adverse events, if any, at each visit.

Subjects were requested to complete a daily checklist of foods consumed during the interventions.

Serum lipids (total cholesterol, HDL cholesterol, triglycerides) were determined on two consecutive days at the end of each period (weeks 2, 8, 14, and 16). LDL cholesterol levels were calculated.

Plasma carotenoids, plasma fat-soluble vitamins (A, D, and E), and plasma phytosterols were measured at the end of each period (weeks 2, 8, 14, and 16).

Biochemical and hematological parameters (used as indicators of the short-term safety of the high-phytosterol intake) were determined at the end of weeks 2, 8, 14, and 16. These parameters were full blood count, electrolytes, glucose, urea and creatinine, calcium, phosphate, liver function tests, clotting tests (prothrombin, partial thromboplastin test), and routine urinalysis.

### Analyses

*Serum lipids.* Venous blood samples (20 ml) were collected in plain tubes after subjects fasted overnight (12 h). Serum was separated by low-speed centrifugation at 600 *g* for 10 min at 5°C (GS-6R centrifuge; Beckman, Fullerton, CA) and frozen at −20°C. At the end of the study, all samples from each subject were analyzed within the same analytic run. Total cholesterol and triacylglycerols were measured on a Cobas-Bio centrifugal analyzer (Roche Diagnostica, Basel, Switzerland) using enzymatic kits (Hofmann-La Roche Diagnostica, Basel, Switzerland) and standard control sera. Plasma HDL cholesterol concentrations were measured after precipitation of apolipoprotein B-containing lipoproteins by PEG 6000. The following modification of the Friedewald equation for molar concentrations was used to calculate LDL cholesterol (in mmol/l): LDL cholesterol = total cholesterol − (triacylglycerol/2.18) − HDL cholesterol. All other routine biochemistry and hematology was done at an accredited local pathology laboratory.

*Plasma phytosterols.* Plasma phytosterols were determined by gas chromatography based on a modification of the method described by Wolthers et al. (9). Briefly, 400 μl of plasma sample was saponified with 400 μl of 33% KOH at 60°C for 30 min, cooled, and extracted with hexane. The extract was evaporated to dryness with a stream of nitrogen, and the phytosterols were derivatized by treatment with 150 μl of SyLON BTZ (Supelco) for 30 min at 80°C. The silyl derivatives of the phytosterols were extracted into hexane and concentrated with a stream of nitrogen to 50 μl, and a 1 μl aliquot was injected onto the GC column (split ratio of 1:10). The gas chromatograph consisted of a DANI 6500 instrument equipped with a split/splitless injector and a flame ionization detector coupled to a DELTA computerized chromatography data system. The injector, detector, and oven temperatures were set at 275, 275, and 280°C, respectively. The capillary column used was a 60 m × 0.22 mm BPX5 (SGE Australia P/l). Plasma phytosterol concentrations were calculated from the standard curves using the ratio of the phytosterol peak area to the peak area of the internal standard (5β-cholestan-3α-ol). The pure internal standard and lathosterol, campesterol, and sitosterol reference samples were obtained from Sigma Chemical Co. (St. Louis, MO).

*Plasma carotenoids and vitamins A and E.* After subjects fasted overnight, blood samples were collected using EDTA as an anticoagulant. The plasma was separated by low-speed centrifugation, frozen immediately in liquid nitrogen, and then stored at −80°C until analysis. Plasma extractions and HPLC were performed according to the method of Yang and Lee (10). Minor modifications to this method were derived from Khachik et al. (11).

*Sample preparation and analysis.* Only a small number of samples were processed at any one time to minimize the exposure to laboratory conditions. The lighting was minimal throughout sample preparation, and amber vials were used for the final extract storage. Samples had the internal standard added and an equal volume of ethanol. Vitamins and carotenoids were extracted with hexane, and the extract was evaporated to dryness under nitrogen. Extracts were then stored at −20°C. Mobile

TABLE 1. Dietary interventions

| Time Period | Intervention |
|---|---|
| Baseline (weeks 1–2), control period (2 weeks) | Usual diet plus phytosterol-free foods (bread, breakfast cereal, and spread) at the same quantities as in the next two periods |
| Period 1 (weeks 3–8), sterol-enriched food period (6 weeks) | Usual diet plus phytosterol-enriched foods (bread, breakfast cereal, and spread) contributing 6.6 g/day phytosterols |
| Period 2 (weeks 9–14), sterol-enriched food period with additional fruit and vegetables (6 weeks) | Usual diet plus phytosterol-enriched foods (bread, breakfast cereal, and spread) contributing 6.6 g/day phytosterols with additional vegetable and/or fruit intake[a] |
| Period 3 (weeks 15–16), free-living period (sterol washout period) (2 weeks) | Usual diet plus phytosterol-free foods (bread, breakfast cereal, and spread) in the same quantities as in the previous two periods; this period was to monitor whether measured parameters returned to baseline levels |

[a] During this period, dietary advice was given to consume at least five servings of fruit and vegetables every day, with at least one serving from the following: pumpkin, sweet potato, carrot, tomato, apricot, broccoli, and spinach (one serving = 0.5 cup or 100 ml).

TABLE 2. Mean nutrient content of the diet during the intervention periods

| Nutrient | Baseline | Period 1 | Period 2 | Period 3 |
|---|---|---|---|---|
| Energy (kJ/day) | 8,038 ± 2,957 | 8,424 ± 2,037 | 8,328 ± 2,132 | 8,334 ± 2,104 |
| Protein (% energy) | 18.6 ± 1.9 | 17.2 ± 1.3 | 16.6 ± 1.2 | 17.2 ± 1.3 |
| Carbohydrate (% energy) | 47.6 ± 2.9 | 46.8 ± 1.9 | 49.2 ± 2.1 | 47.2 ± 2.1 |
| Fat (% energy) | 33.5 ± 3.7 | 35.7 ± 2.9 | 34.0 ± 2.9 | 35.3 ± 3.0 |
| Saturated fat (% energy) | 12.7 ± 1.6 | 12.5 ± 1.2 | 12.1 ± 1.2 | 12.5 ± 1.3 |
| Polyunsaturated fat (% energy) | 5.5 ± 0.7 | 7.5 ± 0.5 | 6.9 ± 0.5 | 7.0 ± 0.4 |
| Monounsaturated fat (% energy) | 12.2 ± 1.4 | 12.6 ± 1.2 | 11.9 ± 1.2 | 12.7 ± 1.3 |
| Cholesterol (mg/day) | 253 ± 111 | 233 ± 93 | 218 ± 86 | 231 ± 90 |
| Fiber (g/day) | 24.7 ± 7.8 | 23.6 ± 6.1 | 26.9 ± 7.8 | 25.2 ± 7.3 |
| β-Carotene (mg/day) | 2.43 ± 1.3 | 2.42 ± 0.8 | 2.98 ± 1.5 | 2.82 ± 1.2 |

Values shown are means ± SD.

phase was used to redissolve the samples ready for HPLC analysis. All samples from each volunteer were extracted in duplicate and analyzed in one run by HPLC to minimize the effect of day-to-day variation.

*Quality control.* A standard reference material (National Institute of Standards and Technology product 968b) was initially tested after preparation of the standards. All vitamins and carotenoids at the high, medium, and low levels fell within the certified ranges. A quality control (QC) plasma was prepared for this study by pooling ~20 ml of plasma that was mixed thoroughly, and 500 μl aliquots were transferred into storage vials and run with each batch of samples. QC plasma was stored at −80°C.

A Shimadzu LC 10 HPLC device fitted with a refrigerated autosampler and a SPD-M10Avp photodiode array detector with a class LC 10 chromatography workstation were used for analysis of the prepared samples. Isocratic separations of the fat-soluble vitamins and carotenoids were carried out on a Rainin (4.6 mm inner diameter × 250 mm length) C18 (5 μm spherical particles) reverse-phase column. The mobile phase was a mixture of acetonitrile (55%), methanol (22%), hexane (11.5%), and dichloromethane (11.5%) at a flow rate of 1.0 ml/min. Ammonium acetate (0.01%, w/v) was added to the mobile phase for stabilization of the carotenoids. Wavelengths of 292 nm (α-tocopherol and α-tocopherol acetate), 325 nm (retinol), 450 nm, and 472 nm (carotenoids) were monitored throughout each run.

Standards (*trans*-α- and -β-carotene, lycopene, lutein, retinol, α-tocopherol, and α-tocopherol acetate) were obtained from Sigma Chemical Co. Solvents (hexane, methanol, acetonitrile, and dichloromethane) were all analytical HPLC grade, and the ethanol was 99.5% Univar absolute ethanol.

### Statistical analysis

Repeated-measures ANOVA was calculated with treatment period as the within-subject factor and gender as the between-subject factor. Age, baseline LDL cholesterol and BMI, and change in weight were inserted into the model as covariates. Where there was a significant treatment effect detected by repeated measures, post hoc tests were used to locate differences using a Bonferroni correction to make allowance for the large number of tests performed. Analyses were performed with SPSS 10.0 for Windows (SPSS Inc., Chicago, IL). Significance was set at $P < 0.05$.

## RESULTS

### Subjects

All thirty-five subjects recruited (23 women, 12 men) completed this 16 week study. The average age was 55.3 years, and the average BMI was 27.8. The average weight increased by 0.21 kg ($P > 0.05$) over the 16 week period.

### Compliance

Dietary compliance was excellent. Compliance scores (percentage eaten of all supplements required to be consumed) were as follows: bread, 98, 98, 97, and 86%; cereal, 99, 99, 98, and 88%; and margarine, 98, 97, 96, and 90% for the baseline period, period 1, period 2, and period 3, respectively. Additional fruit and vegetable intake (five total for each day) had an 84% compliance over the 6 weeks of period 2.

### Dietary data

There were no significant changes between the four dietary periods for total dietary fat, saturated fat, or energy (**Table 2**). β-Carotene intake increased by 24% ($P < 0.001$) and fiber intake increased by 3.3 g/day ($P < 0.001$) from period 1 to period 2 (the increased fruit and vegetable period).

### Serum lipids

High intakes of phytosterols decreased total serum cholesterol by 8.5% averaged over period 1 and period 2, or 0.55 ± 0.65 mmol/l, with a range of responses from an increase of 0.38 mmol/l to a decrease of 1.83 mmol/l (**Table 3**). The mean change was exactly the same for period

TABLE 3. Effect of diets containing 6.6 g/day phytosterols on serum lipids

| Lipid | Baseline Period (2 Weeks) | Period 1 (6 Weeks) | Period 2 (6 Weeks) | Period 3 (Washout) (2 Weeks) |
|---|---|---|---|---|
| Total cholesterol | 6.59 ± 1.01[a] | 6.04 ± 0.73[b] | 6.03 ± 0.84[b] | 6.42 ± 0.97[a] |
| HDL cholesterol | 1.35 ± 0.38[a] | 1.38 ± 0.38[a] | 1.40 ± 0.40[a] | 1.39 ± 0.41[a] |
| Triglycerides | 1.81 ± 0.96[a] | 1.71 ± 0.87[a] | 1.67 ± 0.93[a] | 1.62 ± 0.99[a] |
| LDL cholesterol | 4.44 ± 0.92[a] | 3.89 ± 0.87[b] | 3.87 ± 0.71[b] | 4.31 ± 0.91[a] |

Values shown are means ± SD (mmol/l).
[a,b] Values with different superscripts are significantly different ($P < 0.05$) from each other.

TABLE 4. Effect of diets containing 6.6 g/day phytosterols on plasma sterol levels and adjusted sterol levels (sterol/total cholesterol)

| Sterol | Baseline | Period 1 | Period 2 | Period 3 |
|---|---|---|---|---|
| Lathosterol (mg/L) | 3.11 ± 1.16[a] | 3.52 ± 1.08[b] | 3.61 ± 1.34[b] | 3.11 ± 1.26[a] |
| Adjusted (mg/mmol) | 0.47 ± 0.16[a] | 0.58 ± 0.17[b] | 0.60 ± 0.20[b] | 0.48 ± 0.16[a] |
| Campesterol (mg/L) | 3.14 ± 1.53[a] | 6.26 ± 2.20[b] | 6.62 ± 2.48[b] | 4.20 ± 1.89[c] |
| Adjusted (mg/mmol) | 0.48 ± 0.19[a] | 1.04 ± 0.34[b] | 1.10 ± 0.34[b] | 0.65 ± 0.25[c] |
| Sitosterol (mg/L) | 3.32 ± 1.47[a] | 4.65 ± 1.82[b] | 5.00 ± 1.86[b] | 3.53 ± 1.48[c] |
| Adjusted (mg/mmol) | 0.50 ± 0.20[a] | 0.77 ± 0.29[b] | 0.83 ± 0.34[b] | 0.70 ± 0.20[a] |

Values shown are means ± SD (μg/ml and mg/mmol; n = 35).
[a,b,c] Values with different superscripts are significantly different ($P < 0.05$) from each other.

1 and period 2, so the addition of extra fruit and vegetables had no effect on total cholesterol. With withdrawal of phytosterols, plasma cholesterol increased by 0.39 ± 0.65 mmol/l, with a much wider range of responses from a decrease of 2.05 mmol/l to an increase of 1.64 mmol/l. Although the cholesterol did not return to the control level, the two values were not statistically different. Five subjects had no decrease in total cholesterol during either period 1 or period 2, and three subjects (two of whom appeared to have a response to phytosterols in the first two periods) had no increase in cholesterol in period 3. LDL cholesterol decreased by 12.6% over period 1 and period 2, or 0.56 ± 0.57 mmol/l, ranging from an increase of 1.05 mmol/l to a decrease of 1.64 mmol/l.

### Plasma phytosterols

**Table 4** shows the results for plasma phytosterols and lathosterol (as an indicator of cholesterol synthesis). Plasma campesterol increased by 105% averaged over period 1 and period 2, whereas sitosterol increased by 45%. During the washout, campesterol was still significantly increased (by 34% compared with baseline), whereas sitosterol, although still slightly increased, was not statistically different from baseline. Lathosterol increased significantly by 15% and had decreased to the baseline level during the washout period.

Using the plasma lathosterol levels and plasma phytosterol levels as predictors of total cholesterol response in a multiple regression analysis, the average total cholesterol level in period 1 and period 2 was positively related to the total cholesterol level at baseline ($P < 0.001$) and to baseline lathosterol levels ($P = 0.004$). Together, these account for 80% of the variance in total serum cholesterol during periods 1 and 2. Thus, the greater the cholesterol synthesis and concomitantly the lower the cholesterol absorption (although the phytosterols were eliminated from the regression model by lathosterol) at baseline, the higher the serum cholesterol in periods 1 and 2 (i.e., less response to phytosterol). If lipid-adjusted values were used, the same observations were made.

### Plasma carotenoids

Plasma carotenoids adjusted for total cholesterol and unadjusted are shown in **Table 5**. The data for α- and β-carotene were highly skewed and kurtotic and required nonparametric analysis. The 26% decrease in adjusted β-carotene levels and the 20% decrease in adjusted α-carotene levels were significant ($P < 0.05$). Only the α-carotene levels increased significantly with extra fruit and vegetable consumption. The β-carotene levels increased upon the withdrawal of phytosterols to levels equivalent to those measured during the baseline period. Lutein levels decreased by 14% and also increased with extra fruit and vegetable consumption to levels equivalent to those observed during the baseline and washout periods. α-Tocopherol levels were only lower than baseline and washout levels during the period when extra fruit and vegetables were consumed. Lycopene levels decreased by 11% in period 1 and by 22% in period 2, suggesting either a time delay in the response to phytosterols or an adverse response to the increased fiber. During the washout period, the levels increased back toward the baseline value.

TABLE 5. Effects of diets containing 6.6 g/day phytosterols, with and without additional dietary fruit and vegetables, on plasma levels of carotenoids and fat-soluble vitamins and adjusted levels

| Period | Lutein | Retinol | α-Tocopherol | Vitamin D | Lycopene | α-Carotene | β-Carotene |
|---|---|---|---|---|---|---|---|
| Baseline | 0.50 ± 0.21[a] | 2.34 ± 0.42[a] | 39.9 ± 10.4[a] | 0.056 ± 0.14[a] | 0.87 ± 0.40[a] | 0.15 ± 0.15[a,b] | 0.69 ± 0.58[a,c] |
| Adjusted | 0.077 ± 0.034[a] | 6.03 ± 0.99[a] | | | 0.13 ± 0.06[a] | 0.024 ± 0.025[a] | 0.105 ± 0.091[a,c] |
| Period 1 (phytosterol) | 0.40 ± 0.18[a] | 2.39 ± 0.54[a] | 35.4 ± 7.9[a] | 0.057 ± 0.18[a] | 0.71 ± 0.35[a] | 0.12 ± 0.08[a] | 0.49 ± 0.34[b] |
| Adjusted | 0.067 ± 0.030[b] (−14%) | 5.85 ± 0.97[a] (−3%) | | | 0.12 ± 0.06[a,b] (−11%) | 0.020 ± 0.014[b] (−20%) | 0.082 ± 0.057[b] (−26%) |
| Period 2 (phytosterol + FV) | 0.43 ± 0.18[a] | 2.35 ± 0.44[a] | 34.5 ± 8.4[b] | 0.055 ± 0.17[a] | 0.63 ± 0.30[a] | 0.14 ± 0.08[a,b] | 0.50 ± 0.31[a,b] |
| Adjusted | 0.073 ± 0.031[a,b] (−6%) | 5.68 ± 0.84[b] (−6%) | | | 0.11 ± 0.05[b] (−22%) | 0.023 ± 0.013[a] (−5%) | 0.083 ± 0.051[b,c] (−21%) |
| Period 3 (washout) | 0.47 ± 0.20[a] | 2.36 ± 0.53[a] | 39.1 ± 10.9[a] | 0.057 ± 0.17[a] | 0.74 ± 0.28[a] | 0.14 ± 0.08[b] | 0.58 ± 0.36[c] |
| Adjusted | 0.075 ± 0.034[a] (−3%) | 6.07 ± 1.22[a] (0%) | | | 0.12 ± 0.05[a,b] (−11%) | 0.023 ± 0.013[a] (−4%) | 0.092 ± 0.059[a] (−13%) |

FV, extra fruit and vegetables. Values shown are means ± SD and are expressed as μmol/l (carotenoids), μmol/l (fat-soluble vitamins), and μmol/mmol (adjusted levels).
[a,b,c] Values with different superscripts are significantly different ($P < 0.05$) from each other.

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No changes attributable to high-dose phytosterol levels could be discerned in routine laboratory tests (data not shown).

## DISCUSSION

There have been very few recent studies with high intakes of phytosterols. The decrease in LDL cholesterol levels of 12.6% seen with ingestion of 6.6 g/day phytosterols in bread, margarine, and breakfast cereal was similar in magnitude to those reported in other studies, in which 1.6–3.2 g/day phytosterols and stanols in spreads were consumed (1–6). Davidson et al. (7) fed 3, 6, and 9 g/day phytosterol esters in low-fat dressings and spreads in a parallel study and found no significant changes in LDL cholesterol compared with controls. Ayesh et al. (8) fed 24 men and women 8.6 g/day phytosterol esters in spread for 21–28 days and found serum LDL cholesterol was decreased by 23%. The change in LDL cholesterol in the current study was no greater than that seen with 1.6 g/day in milk in a previous study (12) but was twice as great as the decrease seen with yogurt, bread, and cereal. Clearly, taking a high dose of sterol even in spreads does not necessarily produce a larger LDL cholesterol-lowering effect than does a standard serving of 1.6 g/day. The cholesterol-lowering effect of sterols appears to be persistent for at least 12 months (13).

Despite a clear increase, the plasma phytosterol levels after dietary supplementation with phytosterols are still very similar to the range seen in normal subjects. In a study by Stalenhoef, Hectors, and Demacker (14) of 33 healthy control subjects, fasting plasma campesterol levels on their normal diet ranged from 1.5 to 9.7 μg/ml (mean, 5.2 μg/ml) and plasma sitosterol ranged from 0.8 to 6.6 μg/ml (mean, 3.6 μg/ml). In this group while taking phytosterols, the range of plasma phytosterols was 3–10 μg/ml (mean, 6.2 μg/ml) for campesterol and 2–10 μg/ml (mean, 5.0 μg/ml) for sitosterol. The increase in plasma sitosterol and campesterol with the phytosterols is ~50% greater compared with the 39% and 71% increases reported by Weststrate and Meijer (3), but the intake used in that study (3.3 g/day) was approximately half of the intake used in this study. The phytosterol level is also 4-fold greater than that seen in our low-dose studies with 1.6 g/day (4, 12), so clearly the sterols in bread and cereals are bioavailable. Similarly, lathosterol is increased by 4-fold.

Plasma campesterol increased to a greater degree than sitosterol and was still higher than baseline after 2 weeks of washout despite there being a much lower amount of campesterol in the food. This suggests that campesterol kinetics are different from those of sitosterol, and indeed there is direct evidence now that this is so (15).

Although plasma plant sterol levels have been associated with coronary artery disease even after adjustment for total cholesterol levels, the effect size is very small (1%), and the authors themselves interpreted this as an association between cholesterol absorption and risk rather than a direct effect of the plant sterols (16), although this is still an open question.

The decrease in carotenoid levels when 6.6 g/day phytosterols was consumed was greater than with 1.6 g/day phytosterols consumed in milk in a previous study (12), with the difference ranging from 50% higher for β-carotene to 400% higher for lycopene despite the decrease in LDL cholesterol being similar. However, the decrease in plasma carotenoid levels with 6.6 g/day phytosterols was no different from that reported in the literature for much lower levels (1.6–3.2 g/day) of phytosterol consumption in spreads (5, 17). Even as little as 1 g/d phytostanols has been reported to decrease lipid-standardized β-carotene levels by 14.4% (18), which is very similar to the results seen in this study with a much larger amount. If one compares the 10 subjects who participated in both this trial with 6.6 g/day sterols and the previous trial with 1.6 d/day sterols, there is a clear dose-response effect, with 2- to 4-fold greater changes in lutein, lycopene, and α-carotene.

Over all studies, the magnitude of the change in β-carotene, on average, is on the order of 10% after adjustment. This is negligible compared with seasonal changes of 70% for α- and β-carotene (19). Dietary advice to eat five servings per day of fruit and vegetables (including one that is carotenoid-rich) can increase α-carotene by 32% in 4 weeks (4).

With the exception of α-carotene and lutein, the carotenoid levels did not return completely to baseline levels with advice to increase fruit and vegetable consumption, but they did recover upon withdrawal of phytosterols from the diet. This may have been attributable to increases in fiber, especially for lycopene, which is less efficiently transferred from the fat phase to the micellar phase than lutein (20).

The lack of response to extra fruit and vegetables by plasma β-carotene levels in this study may be accounted for by the failure of the subjects to increase their intake of β-carotene-rich vegetables. However, the observed high compliance of the subjects and the fact that α-carotene levels increased suggest that this was not the case. In contrast, a previous study (4) using 1.6 g/day phytosterols showed that the decrease of β-carotene could be reversed by consumption of extra servings of fruit and vegetables. A much greater increase in dietary β-carotene might be required to reverse the apparent depression of plasma β-carotene levels caused by the higher phytosterol intake used in this study.

Low serum levels of β-carotene have been associated with increased risk of cancer and cardiovascular disease (21, 22). However, in clinical intervention trials, supplementation of the diet with β-carotene either had no benefit or caused harm (23). Furthermore, there is a suggestion that low β-carotene concentrations may be a consequence of an underlying inflammatory process rather than being involved as a causative factor (24). Another interpretation from these studies is that β-carotene itself is not protective and that carotenoid-rich fruit and vegetables may contain other protective factors or that an increased intake of β-carotene may merely be a marker of a healthy lifestyle.

Clearly, high intakes of phytosterols that decrease all carotenoids and increase plant sterol levels could potentially nullify some of the effects of LDL cholesterol, but it is difficult to quantify exactly. From cohort studies and clinical intervention trials, the cholesterol-lowering effect of phytosterol-enriched spreads can be translated into cardiovascular disease risk conservatively of ~15–20% (25). From observational studies, the estimated benefit of consuming fruit and vegetables at the 90th percentile level is a reduction by 15% of the risk of heart disease compared with consumption at the 10th percentile level, so the decrease in carotenoids induced by plant sterols might potentially increase cardiac risk by 5% (26). Increased plasma plant sterols might increase the risk by 2% based on data from Rajaratnam, Gylling, and Miettinen (16), so on balance, the coronary risk might still be reduced by 8–13%. Animal studies with very high doses of phytosterols have shown beneficial effects on atherosclerosis (27). Levels of α- and β-carotene measured in the Dutch population were 20% lower than the baseline levels in this study (3, 5), whereas the plasma lycopene levels have been reported to vary between 26 and 60% of Australian mean levels. This provides an appropriate framework for considering the magnitude of any public health risk given the changes in plasma carotenoids associated with the ingestion of phytosterols at the levels used in this study.

## Conclusions

There appears to be no advantage gained by increasing total intake of phytosterols above 3.2 g/day with respect to any additional cholesterol lowering, but if an intake of 6.6 g/day was achieved on a regular basis, no adverse effects other than a modest decrease of plasma carotenoids and increase of plasma plant sterols would be seen, which potentially might reduce the protective effects of a decrease in cholesterol. Any long-term effects on the promotion of cancer would not be expected, especially given the experimental evidence that plant sterols may have growth-inhibitory effects (28), although no protection from colorectal cancer has been found with dietary sterols (29).

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