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Phytosterol content of experimental diets differing in fatty acid composition

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Abstract

The goal of this study was to determine the relationship between phytosterol and fatty acid concentrations in experimental diets designed to have particular fatty acid profiles. Diet samples were collected during three National Heart, Lung and Blood Institute sponsored multi-center clinical feeding studies (DELTA and DASH programs). Phytosterols (β-sitosterol, campesterol, stigmasterol, campestanol, sitostanol, avenasterol and brassicasterol) were assayed in the saponified total lipid extracts of diet composites, as trimethylsilyl ether derivatives, by gas chromatography and gas chromatography/mass spectrometry. The predominant phytosterols (>84%) in all diets were β-sitosterol, campesterol and stigmasterol. Regression using a multiple linear model showed an inverse relationship between saturated fat (SFA) and total phytosterols ($\beta_1 = -2.55$; $p < 0.001$), a positive relationship between polyunsaturated fat (PUFA) and total phytosterols ($\beta_3 = 6.53$; $p < 0.03$), and no association between total phytosterols and monounsaturated fat (MUFA) ($\beta_2 = 0.55$, $p < 0.40$). The results suggest that dietary phytosterol content covaries with changes in PUFA and SFA. Total phytosterol content decreases with increasing SFA and is notably elevated by increasing PUFA. Further studies must elucidate the biological effects of varying phytosterol concentrations as components of different diets. However, clinicians should recognize the likely concurrent variance of phytosterol and fatty acid concentrations in experimental diets, since both have been shown to influence blood cholesterol levels.

1. Introduction

The fat content and fatty acid composition of diets have been associated with the risk of cardiovascular disease (Stone, 1990; Carleton et al., 1991; Mensink and Katan, 1992; Hegsted et al., 1993; Yu et al., 1995) and some types of cancer (Reddy et al., 1980; Miller, 1990). In intervention studies, dietary fatty acid concentrations are varied by altering the amounts and types of oils and fats or the proportion of animal and vegetable foods. However, changing the fat sources and/or foods in diets also could alter the concentrations of various lipid-soluble phytochemicals (e.g. carotenoids, flavonoids, tocopherols and phytosterols), which may also influence cardiovascular disease and cancer (Jenkins, 1995; Stone et al., 1996). The potential covariance of these biologically active microconstituents has rarely, if ever, been addressed in clinical and epidemiological studies of fat and fatty acids. In this manuscript we describe an evaluation of the concurrent variation in phytosterol and fatty acid concentrations in several experimental diets and provide a preliminary model of the extent to which such covariance could occur in dietary studies.

Phytosterols (plant sterols) are endogenous to all plants, and hypocholesterolemic and anticarcinogenic effects have been demonstrated for phytosterols *in vivo* (Raicht et al., 1980; Pollack, 1985; Rao and Janezic, 1992; Pelletier et al., 1995). Vegetable oils, nuts and seeds are prominent sources of phytosterols and unsaturated fatty acids. Animal fats are virtually devoid of phytosterols and contain primarily saturated fatty acids. Published data indicate that plant sources of saturated fatty acids have far lower phytosterol content than most commonly used unsaturated vegetable oils: for example, 91 mg/100 g in coconut oil and 952 mg/100 g in refined corn oil (Weihrauch and Gardner, 1978). Hydrogenation increases the saturated fatty acid content of vegetable oils while decreasing the phytosterol content by up to 50% (Weihrauch and Gardner, 1978). Consequently, exchanging fat sources to adjust dietary saturated fatty acid composition could result in inverse variation of dietary phytosterol content.

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Food composition data for phytosterols is limited and dated; the most recent compilation was published by Weihrauch and Gardner in 1978. It may not be possible therefore to calculate accurately the phytosterol content of foods and diets, and phytosterols are not routinely considered in the design or analysis of experimental diets. The goal of the present study was to evaluate the relationship between dietary phytosterol and saturated, monounsaturated and polyunsaturated fatty acid concentrations by retrospectively assaying phytosterols in experimental diets that were designed to have different fatty acid profiles. We hypothesized an inverse relationship between phytosterol and saturated fat content.

2. Materials and methods

2.1. Materials

Diet samples were collected during three National Heart, Lung and Blood Institute sponsored multi-center clinical feeding trials that were part of the DELTA (Dietary Effects on Lipoproteins and Thrombogenic Activity) and DASH (Dietary Approaches to Stop Hypertension) programs. The studies tested hypotheses about the effect of total fat and types of fatty acids on lipoproteins and thrombogenic activity (Ginsberg et al., 1998) or of dietary patterns and selected mineral concentrations on blood pressure (Appel et al., 1997). Sampling occurred at seven clinical sites: the Pennington Biomedical Research Center (DELTA and DASH studies), Pennsylvania State University, Columbia University, the University of Minnesota (DELTA), Brigham and Women's Hospital/Harvard Medical School, the Duke University Medical Center and Johns Hopkins University (DASH).

Nine different diets were analyzed. Two diet cycle composites per diet were assayed for phytosterols in the present study.) Diet design has been discussed elsewhere (Sacks et al., 1995; Dennis et al., 1998). Briefly, the DELTA diets differed in total fat and fatty acid concentrations and were developed by adding different fats and oils to low fat menus composed of the same foods and having the same cholesterol content (Dennis et al., 1998). The DASH diets had varying concentrations of total fat, fatty acids, sodium, potassium, calcium, magnesium, cholesterol and total dietary fiber. Nutrient levels were based on US national food consumption data and were designed to reflect nutrient intakes and food composition patterns that have been associated with lower blood pressure (Sacks et al., 1995). Table 1 summarizes the targeted and assayed nutrient profiles of the diets. The concentrations of total fat and fatty acids were within 3% energy of the goal in all cases, and the experimental diets for each feeding trial fully met the design criteria for the feeding experiments.

Dihydrocholesterol was purchased from Nu-Check Prep (Elysian, MN, USA), β-sitosterol and campesterol were from Sigma Chemical Co. (St Louis, MO, USA), stigmasterol and brassicasterol were from Matreya (Pleasant Gap, PA, USA), and cholesterol (SRM 911b) was from the National Institute of Standards and Technology (Gaithersburg, MD, USA). Pyridine (ACS, silation grade) and bis(trimethylsilyl)-trifluoroacetamide with 1% trimethylchlorosilane were purchased from Alltech (Deerfield, IL, USA). Pyrogallol (ACS) and dimethyldichlorosilane (used for siliconization of test tubes) were from Sigma Chemical Co., and potassium hydroxide (85%), chloroform (ACS) and methanol (ACS) were from Fisher Scientific (Pittsburgh, PA, USA). Cyclohexane (HPLC grade) was purchased from Aldrich-Sigma (Milwaukee, WI, USA). Absolute ethanol was obtained from AAPER Alcohol and Chemical Co. (Shelbyville, Kentucky, USA).

2.2. Methods

2.2.1. Sample collection, composite preparation and macronutrient analyses of diet samples

Total daily menus were collected individually and all foods were prepared and served as they were provided to subjects for consumption. Collected menus were frozen in airtight containers and shipped frozen to our laboratory. The menus were homogenized into 7-day (DASH) and 8-day (DELTA) diet cycle composites and assayed for total fat, moisture, protein, ash and fatty acids as described elsewhere (Dennis et al., 1998). Total saturated fatty acids comprised C10:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C22:0 and C24:0, total monounsaturated fatty acids consisted of C14:1, C16:1, C17:1, C18:1, C19:1, C20:1 and C22:1, and total polyunsaturated fatty acids was the sum of C18:2, C20:2, C20:3, C20:4, C22:6, C18:3$n-3$, C20:5$n-3$, C22:6$n-3$. Total energy was calculated from assayed total fat, moisture, protein and ash using conversion factors of 16.7360 kJ g^{-1} (4 kcal g^{-1}) for protein and carbohydrate and 37.6556 kJ g^{-1} (9 kcal g^{-1}) for fat.

2.2.2. Assay of phytosterols and cholesterol

Sterols were recovered from accurately weighed aliquots of the homogenized diet composites (5.0 ± 0.1 g) in the total lipid fraction extracted with chloroform/methanol/water, as described previously (Phillips et al., 1997). The total lipid extract was saponified, and trimethylsilyl derivatives of the nonsaponifiables were prepared and assayed using capillary gas chromatography by modification of the method of Thompson and Merola (1993). Briefly, in a siliconized test tube, a 10 ml aliquot of each total lipid extract was combined with 50 μg internal standard (2.5 ml of 20 μg ml^{-1}

Table 1
Target macronutrient concentrations for nine experimental diets from three clinical feeding studies and assayed concentrations in the diet composites sampled

Component	'Average American' diets[a]			Low saturated fat diets[b]		Step one diets[c]		Other diets[d]	
	AAD 1	AAD 2	AAD 3	Low sat. 1	Low sat. 2	Step one 1	Step one 2	High mono	Fruits and vegetables
Diet targets									
Total fat, %energy	37	37	37	26	27	30	30	37	37
SFA, %energy[e]	16	16	16	5	6	9	8	8	16
MUFA, %energy[e]	14	14	13	14	13	14	15	22	13
PUFA, %energy[e]	7	7	8	7	8	7	7	7	8
Protein, %energy	15	16	15	15	18	15	16	16	15
Total Carbohydrate, %energy	48	47	48	59	55	55	54	47	48
Assayed concentrations[f]									
Total fat, %energy	36	35 (1.6)	36 (1.4)	25 (0.4)	26 (1.2)	30 (0.4)	29	35 (0.2)	36 (0.3)
SFA, %energy[e]	16	15 (1.0)	16 (0.5)	6 (0.0)	8 (0.7)	10 (0.1)	8	8 (0.1)	13 (0.1)
MUFA, %energy[e]	14	14 (0.1)	14 (0.7)	12 (0.1)	10 (0.1)	13 (0.3)	15	20 (0.1)	14 (0.0)
PUFA, %energy[e]	6	6 (0.5)	7 (0.2)	7 (0.3)	8 (0.4)	7 (0.0)	5	6 (0.2)	8 (0.4)
Protein, %energy	16	16 (0.3)	15 (0.0)	16 (0.5)	18 (0.5)	15 (0.1)	17	16 (0.2)	15 (0.1)
Total Carbohydrate, %energy	48	49 (1.3)	50 (1.4)	58 (1.0)	56 (0.8)	55 (0.5)	54	49 (0.0)	50 (0.4)

[a] AAD 1: DELTA study, Protocol 1; AAD 2: DELTA study, Protocol 2; AAD 3: DASH study (control diet).
[b] Low sat. 1: DELTA study, Protocol 1; Low sat. 2: DASH study ('combination' diet).
[c] Step one 1: DELTA study, Protocol 1; Step one 2: DELTA study, Protocol 2.
[d] High mono: DELTA study, Protocol 2; Fruits and vegetables: DASH study.
[e] Normalized to total fat.
[f] Each value is the average for the two composites analyzed, with the SD shown in parentheses (except AAD 1 and Step one 2, $n=1$).
DELTA diets are described in Dennis et al. (1998) and DASH diets are described in Sacks et al. (1995).

dihydrocholesterol in absolute ethanol). Solvent was evaporated to dryness at 60°C under a stream of nitrogen (N-EVAP analytical evaporator, model 111, Organomation Associates, Inc. Berlin, MA, USA). The dried lipids were saponified (1.28% aqueous potassium hydroxide, 3% pyrogallol/ethanol) at 80°C with intermittent vigorous shaking; non-saponifiables were extracted from the cooled mixture with cyclohexane (20 ml). Approximately 16 ml of the cyclohexane solution was evaporated to dryness (60°C/N_2), and the trimethylsilyl ether derivatives of the free sterols were prepared by adding 0.25 ml of a 1:1 (v/v) solution of bis(trimethylsilyl)-trifluoroacetamide containing 1% trimethylchlorosilane in pyridine.

The trimethylsilyl ether derivatives were analyzed by gas–liquid chromatography using a Perkin Elmer Autosystem™ gas chromatograph with autosampler and flame ionization detector, interfaced with a Digital DECpc™ 433dxLP computer (Perkin Elmer, Norwalk, CT, USA). An RTx-5™ fused silica capillary column [60 m×0.25 mm ID, cross-bonded 5% diphenyl–95% dimethyl polysiloxane (0.1 μm film) (Restek Corp., Bellefonte, PA, USA)] was used. Data were acquired and processed by Turbochrom™ 4.0 software (PE Nelson, Cupertino, CA, USA). Operating conditions were as follows: helium carrier gas (32 psi; flow rate, 1 ml min^{-1}); injector temperature, 270°C; sample injection volume, 0.5 to 1.0 μl with a split ratio of 1:17; oven temperature, 270°C (isothermal); detector temperature, 300°C, with a mixture of hydrogen and air as the fuel source.

Peaks were identified by comparing retention times to those of authentic standards and also by gas chromatography–mass spectrometry of selected samples using a Hewlett Packard model 5709 chromatograph interfaced with a VG Analytical mass spectrometer (model 7070 E-HF) having a double focusing magnetic sector, a mass range of 16 000 amu, and maximal resolution of 20 000, using electron impact for ionization. Identification of analytes was based on comparison of the mass spectrum for the unknown with spectra of known standards.

The concentration of each individual phytosterol constituting >5% of the total non-cholesterol sterols (with total non-cholesterol sterols being the sum of all peaks eluting after the dihydrocholesterol internal standard) was determined from a standard response curve (analyte response/internal standard response vs analyte concentration/internal standard concentration). For phytosterols for which there were commercially available standards (β-sitosterol, campesterol, stigmasterol and brassicasterol), calibration curves were prepared using these standards. The purity of the commercially available phytosterol standards ranged from 96 to 99%, and the weight of the each standard in the calibration solutions was adjusted for the manufacturer's reported assayed purity of the specific lot used. The standard solutions were saponified and derivatized prior to chromatography, as described above. The standard response curve for each phytosterol was a linear regression fitted to triplicate values obtained at each of six concentrations. Phytosterols for which there were no commercially available standards were quantified from the calibration curves of a standard with the same total carbon number (i.e. β-sitosterol was used to quantify sitostanol and avenasterol; and campesterol was used to measure campestanol). 'Total phytosterols' comprised β-sitosterol, campesterol, stigmasterol, campestanol, sitostanol and avenasterol. Phytosterol and cholesterol concentrations in the diet composites were normalized to 8.37 MJ (2000 kcal).

The validity of the gas chromatography method employed for measurement of cholesterol was reported by Thompson and Merola (1993). The procedure used in the present study was tested for accurate and precise quantification of sterols other than cholesterol in diet and food composites (unpublished data). Details of these analyses will be reported elsewhere. A quality control material (diet composite comprising similar foods) was assayed with each batch of samples and ensured a lack of run-to-run analytical deviation.

2.2.3. Statistical analyses

The relationship between total phytosterols (PHYT) and fatty acids (saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA)) was evaluated by regression using the model $PHYT = \beta_0 + \beta_1(SFA) + \beta_2(MUFA) + \beta_3(PUFA) + \epsilon$. Statistical analyses were performed with the SAS® System for Windows™ (Release 6.12 TS020, 1996; SAS Institute,

Table 2
Assayed concentrations of phytosterols and cholesterol in diet composites[a]

Component (mg/8.37 MJ [f])	'Average American' diets[b]			Low saturated fat diets[c]		Step one diets[d]		Other diets[e]	
	AAD 1	AAD 2	AAD 3	Low sat. 1	Low sat. 2	Step one 1	Step one 2	High mono	Fruits and vegetables
Brassicasterol	n.d.	n.d.	n.d.	8 (0.1)	n.d.	n.d.	n.d.	n.d.	n.d.
Campesterol	24	29 (0.0)	24 (1.2)	35 (1.0)	32 (0.2)	31 (0.7)	33	29 (1.0)	29 (0.9)
Campestanol	7	7 (0.4)	5 (0.2)	8 (0.1)	8 (0.1)	8 (0.1)	10	7 (0.3)	7 (0.4)
Stigmasterol	15	18 (0.5)	18 (0.1)	18 (0.9)	22 (0.3)	17 (0.5)	17	17 (2.1)	21 (0.9)
β-Sitosterol	87	96 (1.7)	78 (1.5)	122 (4.3)	129 (4.4)	112 (5.1)	120	119 (1.5)	127 (1.4)
Sitostanol	8	9 (0.2)	7 (0.1)	10 (0.1)	10 (0.0)	9 (0.2)	13	10 (0.3)	9 (0.6)
Avenasterol	10	12 (0.6)	7 (0.2)	14 (0.0)	13 (0.3)	12 (0.6)	13	14 (0.7)	13 (0.2)
Cholesterol	273	277 (1.0)	223 (7.7)	271 (28.0)	132 (2.0)	264 (12.5)	274	269 (8.5)	166 (2.0)
Total phytosterols[g]	153	171 (2.4)	138 (2.3)	214 (4.2)	214 (5.2)	189 (7.1)	208	196 (4.6)	205 (1.2)

[a] Each value is the mean of the concentration assayed in each of two composites, with the SD shown in parentheses (except AAD 1 and Step one 2, $n = 1$).
[b] AAD 1: DELTA Protocol 1; AAD 2: DELTA Protocol 2; AAD 3: DASH (control diet).
[c] Low sat. 1: DELTA Protocol 1; Low sat. 2: DASH ('combination' diet).
[d] Step one 1: DELTA Protocol 1; Step one 2: DELTA Protocol 2.
[e] High mono: DELTA Protocol 2; Fruits and vegetables: DASH Study.
[f] mg/2000 kcal.
[g] Sum of brassicasterol, campesterol, campestanol, stigmasterol, β-sitosterol, sitostanol and avenasterol.
DELTA diets are described in Dennis et al. (1998), and DASH diets are described in Sacks et al. (1995).
n.d. = not detected (< 5 mg/8.37 MJ).

Inc., Cary, NC, USA) using the regression procedure (PROC REG); Pearson correlation coefficients were generated using the correlation procedure (PROC CORR).

3. Results and discussion

3.1. Phytosterol and cholesterol concentrations

Table 2 summarizes the assayed concentrations of phytosterols and cholesterol in the nine diets. The predominant individual phytosterols in all cases were β-sitosterol, campesterol and stigmasterol, which as a group constituted >84% of total phytosterols. Other phytosterols detected were campestanol, sitostanol (stigmastanol), avenasterol and brassicasterol. The concentration of phytosterols was highest (214 mg/8.37 MJ) in the low saturated fat diets and lowest (138 mg/8.37 MJ) in an average American diet. The major difference between diets was in β-sitosterol content (Table 2).

3.2. Relationship between phytosterol and fatty acid concentrations

Results of the multiple regression analysis ($R^2 = 0.72$, $p < 0.001$) demonstrated an inverse relationship between SFA and total phytosterols ($\beta_1 = -2.55$, $p < 0.001$) at constant MUFA and PUFA concentrations. In contrast, there was no significant relationship between MUFA and phytosterols ($\beta_2 = 0.55$, $p < 0.40$). Interestingly, despite the limited variation in dietary PUFA concentrations (Table 3), relatively small increases in PUFA were associated with a statistically significant elevation of total phytosterols ($\beta_3 = 6.53$; $p < 0.03$) in the context of this multiple regression model. Fig. 1 illustrates predicted changes in dietary phytosterols with varying SFA and PUFA concentrations using the regression model and parameter estimates generated from the data in Table 3. The variance inflation factors of nearly 1.0 for SFA, MUFA and PUFA in the regression model (1.08, 1.04 and 1.12, respectively) and the insignificant correlation coefficients (Table 4) suggest a lack of collinearity among the dependent variables (Myers, 1990). Table 3 summarizes the assayed concentrations of SFA, MUFA, PUFA and total phytosterols in the individual diet composites that were assayed. The standard deviation of the within-composite mean ($n = 2$) for SFA, MUFA and PUFA was <2.5% of the range of total phytosterol concentrations (82 mg/8.37 MJ) in all cases (except 9.9% for MUFA in one AAD-3 composite). Also, for each variable the average within-composite standard deviation was <3.4% of the range of values for that component. This information suggests that measurement error was negligible.

In the present study we found statistically significant relationships between phytosterol content and total saturated fat and total polyunsaturated fat concentrations in experimental diets that varied in fatty acid composition. The relationship was determined using diets

Table 3
Assayed concentrations of saturated fat (SFA), monounsaturated fat (MUFA), polyunsaturated fat (PUFA) and total phytosterols in individual diet composites (diet design nutrient targets and corresponding assayed composition are shown in Table 1)

Diet[a]	Composite	SFA[b] (g/8.37 MJ[c])	MUFA[b] (g/8.37 MJ)	PUFA[b] (g/8.37 MJ)	Total phytosterols[b] (mg/8.37 MJ)
AAD 1	1	30.7 (0.40)	26.1 (0.36)	12.5 (0.16)	153 (1.0)
AAD 2	1	31.1 (0.31)	30.2 (0.11)	11.5 (0.01)	172 (1.0)
	2	30.9 (0.14)	27.6 (0.36)	11.7 (0.02)	169 (2.1)
AAD 3	1	26.8 (0.76)	23.7 (0.10)	11.6 (0.14)	140 (0.8)
	2	26.8 (2.03)	23.1 (1.88)	11.6 (1.01)	136 (6.0)
Step one 1	1	18.9 (0.02)	26.1 (0.09)	13.7 (0.02)	194 (1.6)
	2	19.7 (0.04)	27.5 (0.09)	14.1 (0.22)	184 (1.1)
Step one 2	1	16.3 (0.14)	30.2 (0.08)	10.6 (0.02)	208 (3.6)
Low sat. 1	1	12.3 (0.28)	24.4 (0.05)	13.1 (0.20)	217 (0.8)
	2	12.1 (0.10)	24.6 (0.35)	13.9 (0.28)	211 (1.4)
Low sat. 2	1	13.3 (0.20)	19.6 (0.25)	14.1 (0.08)	210 (4.4)
	2	15.1 (0.41)	19.5 (0.32)	15.1 (0.29)	218 (1.5)
High mono	1	17.2 (0.08)	42.6 (0.96)	13.3 (0.17)	192 (8.9)
	2	17.4 (0.18)	42.0 (1.18)	12.5 (0.35)	199 (0.9)
Fruits and vegetables	1	25.0 (0.16)	27.9 (0.41)	16.5 (0.08)	206 (0.4)
	2	25.2 (0.05)	27.9 (0.02)	15.4 (0.04)	204 (0.1)

[a] See Table 1 and Table 2.
[b] Each value is the mean of two analyses, with SD in parentheses.
[c] g/2000 kcal. Each value is the mean of two analyses, with SD in parentheses.

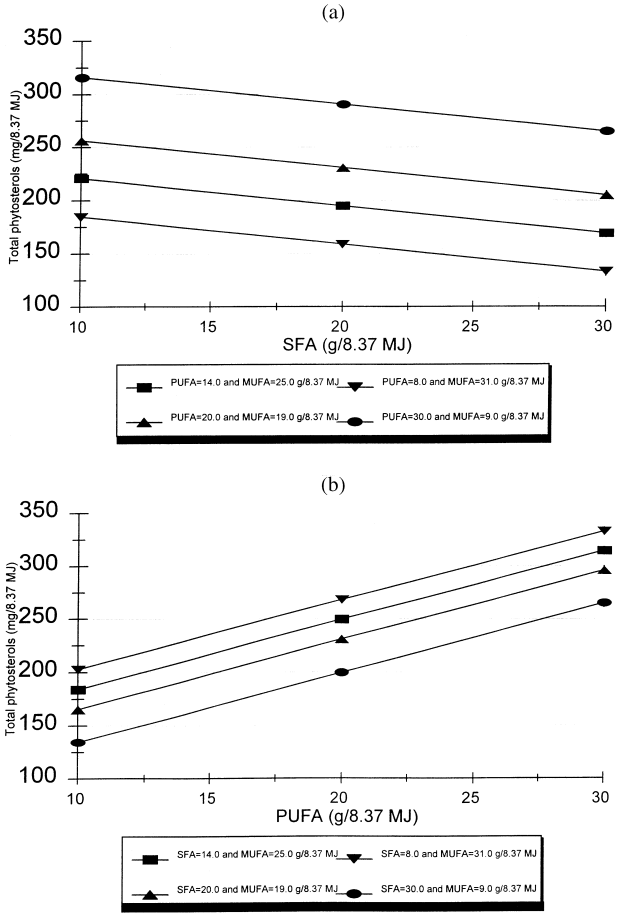

Fig. 1. Predicted total phytosterol concentrations (PHYT) in diets relative to changes in saturated fatty acid (SFA) and polyunsaturated fatty acid (PUFA) concentrations, generated from phytosterol and fatty acid data from assayed diets (Table 3) using the regression model, $PHYT = \beta_0 + \beta_1(SFA) + \beta_2(MUFA) + \beta_3(PUFA) + \epsilon$. (a): PHYT vs SFA at fixed concentrations of PUFA and MUFA; $\beta_1 = -2.55$ ($p < 0.001$). (b): PHYT vs PUFA at fixed concentrations of SFA and MUFA; $\beta_3 = 6.53$ ($p < 0.03$). The relationship between monounsaturated fat (MUFA) and PHYT was insignificant ($\beta_2 = 0.55$, $p < 0.40$) and is not illustrated; $\beta_0 = 140.66$ ($p < 0.01$).

from multiple feeding studies which differed in the foods and fats used to achieve particular fatty acid profiles.

There was an inverse relationship between phytosterol and SFA concentrations (Fig. 1(a)), which was consistent with our initial hypothesis. However, a statistically significant large positive relationship between PUFA and total phytosterols (Fig. 1(b)) was also revealed. This result was particularly notable given the limited range of PUFA concentrations (5.9 g/8.37 MJ) in the diets studied (Table 3). The magnitude of the PUFA regression coefficient (β_3, 6.53) suggests that diets with more divergent PUFA concentrations would have even larger differences in phytosterol content. The findings also suggest that the sources of PUFA in the diets were rich in phytosterols. Further studies will

Table 4
Pearson correlation coefficients for SFA, MUFA and PUFA in the multiple linear model, with p values in parentheses

	SFA	MUFA	PUFA
SFA	1.0000 (0.000)	0.0170 (0.950)	−0.2778 (0.298)
MUFA	0.0170 (0.950)	1.0000 (0.000)	−0.1816 (0.501)
PUFA	−0.2778 (0.298)	−0.1816 (0.501)	1.0000 (0.000)

evaluate these hypotheses more comprehensively, since the results of the present evaluation are based on a limited range of fatty acid concentrations and retrospective analyses that were constrained by the design of the diets for the feeding trials.

It is interesting that there was no significant relationship between phytosterol and MUFA concentrations, since plant foods are a primary source of MUFA as well as PUFA. The reason for this result is unknown. However, if the food sources of MUFA had relatively low phytosterol concentrations, the relationship may have been undetected since the diets did not vary widely in MUFA content (Table 3).

The diets studied contained a variety of common SFA sources (e.g. meats, dairy products and coconut oil) and PUFA sources (e.g. margarines, vegetable oils, nuts and mayonnaise). Vegetable fats high in SFA (e.g. palm kernel oil and coconut oil) tend to have low phytosterol concentrations (< 150 mg/100 g) relative to most vegetable oils, which are rich in PUFA (e.g. corn, rapeseed and wheat germ oils) (Weihrauch and Gardner, 1978). High-PUFA oils vary widely in phytosterol content, however. For example, concentrations of 494 mg/100 g and 1390 mg/100 g total phytosterols in safflower oil and unrefined corn oil, respectively, have been reported (Weihrauch and Gardner, 1978). Refining and hydrogenation markedly decrease the total phytosterol content of vegetable oils, so the extent of processing will also affect the phytosterol content of specific oil and fat samples. Further analyses of individual foods and fat sources, currently underway, will elucidate sources of variation in the phytosterol composition of diets.

Some of the diets also differed in the proportion of fruits and vegetables, despite having similar fatty acid profiles (Sacks et al., 1995). Newer analytical methods to recover phytosterol glycosides (Jonker et al., 1985) are being modified and validated in our laboratory for the analysis of a variety of foods and diets. As shown by Jonker et al. (1985), the phytosterol content of some foods, particularly fruits, vegetables and whole grains, may be higher than determined by standard methodology.

It has been suggested that the dietary phytosterol to cholesterol ratio may be more significant than the

absolute concentrations of these components in influencing blood cholesterol levels (Pelletier et al., 1995) and carcinogenesis (Nair et al., 1984). In the present study, it was not possible to conduct an unbiased evaluation of the phytosterol to cholesterol ratio, however, since the dietary cholesterol content in some of the studies had been artificially adjusted by adding a concentrated cholesterol source (dried egg yolk) (Dennis et al., 1998).

The clinical relevance of absolute differences in dietary phytosterol concentration remains to be determined (Farquhar, 1995; Jenkins, 1995). There has been considerable controversy over the role of phytosterols in the observed hypocholesterolemic effect of vegetable oils first suggested by Beveridge and coworkers (Beveridge et al., 1964). Most studies of dietary phytosterols have involved several grams per day, given as supplements. However, in a study by Vanhanen et al. (1993), a control diet in which 50 g dietary fat was replaced with 50 g rapeseed oil containing 360 mg phytosterols, serum total and LDL cholesterol were reduced by 1.2% and 1.1%, respectively, and serum cholesterol precursors (lathosterol, desmosterol and Δ8-cholestenol) increased. Other studies have suggested possible interaction between PUFA and phytosterols (Pollack and Kritchevsky, 1981), and timing of phytosterol intake relative to cholesterol may also impact the efficacy of phytosterols on cholesterol metabolism (Mattson et al., 1982). Dietary phytosterols may also inhibit low-density lipoprotein oxidation, which is also known to affect cardiovascular disease (Steinberg et al., 1989). Aviram and Eias (1993) found increased sitosterol and decreased oxidation in low-density lipoproteins after olive oil feeding. More research is needed to ascertain the role of dietary phytosterols at different levels and as an integral part of diets.

The most important finding of the present study is the definitive relationships between phytosterol, SFA and PUFA concentrations. These results provide one example of the covariance of biologically active dietary microconstituents with recognized macronutrients (SFA, PUFA) in the experimental diets of well-designed, tightly controlled clinical feeding studies. The relationship between these components is particularly noteworthy because the primary sources of the fatty acids targeted in the diet design are also the major food sources of phytosterols. Consequently, the correlation can be expected when the only difference between diets is the fats, oils and foods used to adjust fatty acid concentrations. Such a relationship was detected among experimental diets that differed only in the amounts and types of oils and fats (DELTA study) as well as diets comprising different foods (DASH study). Similar trends could exist for other plant food constituents (e.g. tocopherols, tocotrienols, flavonoids and carotenoids) from differences in, for example, whole vs refined grains, unprocessed/unpeeled vs processed/peeled fruits and vegetables, refined vs crude vegetable oils, hydrogenated vs unhydrogenated oils and spinach vs iceberg lettuce. Differences in dietary phytochemical concentrations resulting from between-study diversity in the specific foods and fats used to achieve target nutrient profiles should be considered as a possible contributor to variance in the clinical results of separate feeding trials. Further research should elucidate the role and possible interactions, if any, of microconstituents in the beneficial effects attributed to particular dietary macronutrients and dietary patterns. Meanwhile, phytosterol concentrations should be monitored in studies of dietary fatty acids and cholesterol metabolism.

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