Cholesterol-lowering efficacy of a sitostanol-containing phytosterol mixture with a prudent diet in hyperlipidemic men[1–3]

Peter JH Jones, Fady Y Ntanios, Mahmoud Raeini-Sarjaz, and Catherine A Vanstone

ABSTRACT

Background: Dietary plant sterols (phytosterols) have been shown to lower plasma lipid concentrations in animals and humans. However, the effect of phytosterol intake from tall oil on cholesterol and phytosterol metabolism has not been assessed in subjects fed precisely controlled diets.

Objective: Our objective was to examine the effects of sitostanol-containing phytosterols on plasma lipid and phytosterol concentrations and de novo cholesterol synthesis rate in the context of a controlled diet.

Design: Thirty-two hypercholesterolemic men were fed either a diet of prepared foods alone or a diet containing 1.7 g phytosterols/d for 30 d in a parallel study design.

Results: No overall effects of diet on total cholesterol concentrations were observed, although concentrations were lower with the phytosterol-enriched than with the control diet on day 30 ($P < 0.05$). LDL-cholesterol concentrations on day 30 had decreased by 8.9% ($P < 0.01$) and 24.4% ($P < 0.001$) with the control and phytosterol-enriched diets, respectively. HDL-cholesterol and triacylglycerol concentrations did not change significantly. Moreover, changes in circulating campesterol and β-sitosterol concentrations were not significantly different between phytosterol-fed and control subjects. In addition, there were no significant differences in fractional (0.091 ± 0.028 and 0.091 ± 0.026 pool/d, respectively) or absolute (0.61 ± 0.24 and 0.65 ± 0.23 g/d, respectively) synthesis rates of cholesterol observed between control and phytosterol-fed subjects.

Conclusion: Addition of blended phytosterols to a prudent North American diet improved plasma LDL-cholesterol concentrations by mechanisms that did not result in significant changes in endogenous cholesterol synthesis in hypercholesterolemic men. *Am J Clin Nutr* 1999;69:1144–50.

KEY WORDS Phytosterols, plant sterols, plasma cholesterol, low-density lipoprotein, LDL, high-density lipoprotein, HDL, sitostanol, humans, men, hyperlipidemia, tall oil

INTRODUCTION

Increasingly, dietary approaches to lowering heart disease risk are finding appeal over pharmacologic alternatives in the general population. One such approach has been to use naturally occurring plant sterols (phytosterols) as cholesterol-lowering adjuncts in foods (1–6). Sitostanol, the saturated derivative of the most common phytosterol, β-sitosterol, has successfully lowered circulating cholesterol concentrations in most human feeding trials. Decreases in total and LDL-cholesterol concentrations of 5–15% have been observed in studies lasting as long as 12 mo (7–16), although lack of efficacy has also been observed (17).

Although most studies using pure sitostanol or sitostanol ester have shown lowering of cholesterol in humans, results across studies show considerable variability that is likely due, in large part, to differences in study design and the method of administration of the phytosterol material (7–17). To date, no experiment has been conducted in which the sitostanol-containing phytosterols were administered over the 3 daily meals of a precisely controlled metabolic diet. How the addition of sitostanol-containing phytosterols alters plasma cholesterol concentrations in a dietary setting in which meal timing, composition, and quantity are rigorously maintained has not been established.

Both sitostanol and β-sitosterol are believed to reduce plasma cholesterol concentrations extrinsically by competitively blocking cholesterol absorption from the intestinal lumen (18, 19), displacing cholesterol from bile salt micelles (20), increasing bile salt excretion (21), or hindering the cholesterol esterification rate in the intestinal mucosa (22, 23). Additional intrinsic actions of phytosterols may include modification of hepatic acetyl-CoA carboxylase (24) and cholesterol 7-α hydroxylase enzyme activities (25) in animals and humans.

Whether the cholesterol-lowering ability of sitostanol-rich phytosterol mixtures influences cholesterogenesis has not been fully addressed. β-Sitosterol has been shown to lower plasma cholesterol concentrations while simultaneously stimulating (26, 27), inhibiting (28), or exerting (29) no effect on cholesterol synthesis in animals and humans. Previous reports of studies that examined the effect of phytosterols on cholesterogenesis in humans determined synthesis rates indirectly (14, 26, 30). However, none of those studies examined the effect of a precisely

[1] From the School of Dietetics and Human Nutrition, Faculty of Agricultural and Environmental Sciences, McGill University, Montreal.

[2] Supported by a grant from Forbes Medi-Tech Inc, Vancouver, Canada.

[3] Address reprint requests to PJH Jones, School of Dietetics and Human Nutrition, 21111 Lakeshore Road, Ste Anne de Bellevue, Quebec H9X 3V9 Canada. E-mail: Jonesp@agradm.lan.mcgill.ca.

Received April 10, 1998.

Accepted for publication December 14, 1998.

controlled, prudent North American diet with simultaneous administration of tall oil phytosterols on cholesterogenesis measured directly by the deuterium uptake method. Tall oil is the fat-soluble fraction of the hydrolysate obtained from trees during the pulping process.

Our objective was to examine whether tall oil phytosterol consumption alters lipoprotein-cholesterol concentrations and sterol metabolism in hyperlipidemic men when provided as a food supplement suspended in margarine. The hypothesis tested was that when sitostanol-containing phytosterols are provided to hyperlipidemic men over the 3 meals of a prudent, fixed-food North American diet, lipoprotein cholesterol profiles, plasma phytosterol concentrations, and de novo cholesterol synthesis rates will be different from when the diet is provided alone over 30 d.

SUBJECTS AND METHODS

Subjects

Thirty-two men (aged 25–60 y) with primary hypercholesterolemia were selected. Subjects were screened, after 12 h of fasting, for total cholesterol and triacylglycerol concentrations 1 and 2 wk before the start of the study. Criteria for acceptance were plasma total cholesterol concentrations between 6.5 and 10 mmol/L, total circulating triacylglycerol concentrations <3.5 mmol/L, and a body mass index (BMI; in kg/m^2) >18 or <37. Individuals who reported having diabetes, heart disease, or hypothyroidism or who had stopped using medication for hypercholesterolemia for <4 wk were excluded. Subjects were randomly assigned into the 2 treatment groups on the basis of plasma total cholesterol concentrations.

Protocol and diet

The study was a randomized, double-blind clinical trial. All subjects were provided for 30 d with a North American diet considered to be healthy in terms of macronutrient and fat content. The control diet group (n = 16) received a diet of prepared food alone; the phytosterol-enriched diet group (n = 16) received a diet with sitostanol-containing phytosterols [22 mg/kg body wt (1.5 g/70-kg man)] suspended in the margarine component of the diet. The diet was formulated to meet Canadian recommended nutrient intakes and to provide fat, fiber, and carbohydrate subcomponents consistent with recommendations of Health and Welfare Canada (31). Dietary protein, carbohydrate, and fat made up 15%, 50%, and 35% of ingested energy, respectively. The dietary fat was composed of 11%, 10%, and 14% of energy as saturated, polyunsaturated, and monounsaturated fats, with a blend of butter, corn oil, olive oil, and canola oil–based margarine.

Diets, fed in amounts determined to maintain body weight throughout the 30-d trial (32), were provided under supervision as 3 meals of equal energy each day. A 3-d rotating cycle was used, each cycle having similar macro- and micronutrient contents. Meals were prepared at the Clinical Nutrition Research Unit for consumption on site or, in certain cases, for takeout, as described previously (16). During meal preparation, foods were weighed precisely to the nearest 0.5 g. Subjects were instructed not to consume any foods or beverages other than those provided by the diet. Alcoholic and caffeinated beverage consumption was strictly prohibited over the course of the trial. Subjects were provided with decaffeinated, energy-free carbonated beverages to drink between meals.

Phytosterols were prepared from tall oil by solvent extraction and purification through repeated crystallization. Sitostanol made up ≈20% of the mixture by weight. The remaining phytosterols were mostly sitosterol and campesterol. The phytosterols were administered by suspending them in 30 g prewarmed margarine each day, providing a ratio of margarine to phytosterol of ≈20:1 (by wt). The 30 g margarine was divided equally among the 3 meals and mixed directly with food ingredients during preparation before cooking. When fluctuations in body weight occurred, adjustments to energy intakes were made during the initial 10-d period of the trial only. There were no changes to subjects' diets thereafter.

On day 29 of the trial, subjects were given orally 1.2 g D_2O (99.8% atom percent excess) per kg body weight at 0800. Deuterium uptake into cholesterol was measured over the following 24 h. Blood samples were collected just before and 24 h after deuterium dosing for red blood cell free cholesterol and water deuterium enrichment measurements.

Subjects underwent routine physical examinations and detailed blood chemistry analyses before and on day 30 of the study. A physician was on call continually throughout the trial for subjects to contact in case they experienced discomfort.

Analyses

Lipid and phytosterol analyses

Blood samples were collected from subjects before breakfast on days 0, 10, 20, 29, and 30 of the trial. Plasma was obtained after 20 min of centrifugation at 520 × g at 4°C. Plasma total, and LDL- and HDL-cholesterol and triacylglycerol concentrations were then determined. In addition, samples were collected from subjects on days 40 and 50, after the end of the diet.

Plasma total cholesterol, HDL-cholesterol, and triacylglycerol concentrations were measured in duplicate by using a VP autoanalyzer and commercial enzymatic kits (Abbott Laboratories, North Chicago, IL). HDL-cholesterol concentrations were measured in plasma after precipitation of apolipoprotein B lipoproteins with dextran sulfate and magnesium chloride (33). The concentration of LDL cholesterol was calculated according to the methods of Friedewald et al (34). CVs for replicate analyses of total cholesterol, HDL-cholesterol, and triacylglycerol concentrations were 2.74%, 6.53%, and 1.93%, respectively.

Plasma phytosterol concentrations were determined in duplicate by gas-liquid chromatography from the nonsaponifiable material of plasma lipid as reported previously (35). Briefly, 0.5-mL plasma samples were saponified with 0.5 mol methanolic KOH/L for 1 h at 100°C and the nonsaponifiable materials were extracted with petroleum ether. 5-α Cholestane was used as an internal standard. Samples were injected into a gas-liquid chromatograph equipped with a flame ionization detector (HP 5890 Series II; Hewlett Packard, Palo Alto, CA) and with a 30-m capillary column (SAC-5; Supelco, Bellefonte, PA). Detector and injector temperatures were 310 and 300°C, respectively. Duplicate samples were run isothermically at 285°C. Phytosterol peaks were identified by comparison with authenticated standards (Supelco).

De novo cholesterol synthesis determination

Cholesterol biosynthesis was determined as the rate of incorporation of deuterium from body water into free sterol over 24 h. Labeled water equilibrates quickly with intra- and extracellular water body pools and permits direct determination of choles-

TABLE 1
Mean and percentage changes in subjects' body weights between days 0 and 30 in the control and phytosterol-enriched diet groups[1]

	Control	Phytosterol
Body weight (kg)		
Day 0	79.8 ± 9.6	87.8 ± 15.2
Day 30	78.9 ± 6.5	86.7 ± 15.1
Body weight change (%)	−1.1 ± 1.1	−1.2 ± 1.4

[1] $\bar{x}$ ± SD; n = 16 men per group. There was no significant difference in body weight between groups on days 0 or 30.

terol synthesis rates (36). Deuterium enrichment was measured in red blood cell free cholesterol and plasma water as reported previously (37–39).

The fractional synthesis rate (FSR) of cholesterol was determined as incorporation of precursor deuterium into plasma total cholesterol relative to the maximum theoretic enrichment by using the linear regression model described previously (37, 39). The absolute synthesis rate (ASR = FSR × M_1 pool) was calculated according to the model of Goodman et al (40) as follows:

$$M_1 \text{ pool} = 0.287 \text{ wt (kg)} + 0.0358 \text{ plasma total cholesterol (mmol/L)} - 2.40 \text{ TGGP} \quad (1)$$

where TGGP is a variable that is equal to 1, 2, or 3 depending on the serum triacylglycerol concentration: <2.267, 2.267–3.401, or >3.401 mmol/L, respectively.

Statistical methods

Plasma lipid concentration data were evaluated by using a two-factor repeated- measures analysis of variance (ANOVA) procedure with tests for time and diet effects, and time-by-diet interactions. When the time-by-diet interaction was $P < 0.10$, repeated measures one-way ANOVA procedures were used. Wilk's lambda test was used to analyze time effects, whereas Student-Neuman-Keuls' post hoc tests were used to identify significant effects of diet at particular times (41). For total cholesterol, consistent with our initial hypothesis, percentage changes between days 0 and 30 were compared by using ANOVA. When main time effects were significant, a quadratic model was fitted to individual data of each treatment to determine whether the pattern of the decline was different. Slopes of the different diets were tested by using unpaired Student's t tests as were the effects of tall oil–derived phytosterols on FSR and ASR values. The relation between plasma total cholesterol concentrations, phytosterols, ASR, and FSR were determined by using Pearson product-moment correlation coefficients. The accepted level of significance was $P < 0.05$.

RESULTS

Thirty-three subjects started the feeding trial; 32 subjects completed the entire study. All subjects tolerated the experimental diet without any reported adverse effects. In addition, results of blood chemistry and urine tests were normal at the start of the diet period and throughout the duration of the trial. Screening checkups conducted at each 10-d time point of the trial suggested no clinical irregularities. Overall, subjects maintained excellent health throughout the duration of the experiment, except for one subject in the phytosterol group who reported diarrhea considered to be associated with a bout of influenza over the final 4 study days.

The sitostanol-containing phytosterol mixture was found to be inert in that subjects reported no particular abnormal or atypical smell, taste, aftertaste, or mouth-feel of meals during either diet. Subjects were not able to identify which diet they were consuming. There were no reported abnormalities of stool consistency or color, with the exception noted above.

Mean body weight, age, and BMI did not differ between control and phytosterol-enriched diet groups (**Table 1**). There was no significant change in the mean body weight of subjects across the 30-d feeding period. Seven study subjects had their energy intake altered by 10–20% over the first week of each trial. Three subjects either lost weight or reported lack of satiation, and thus their energy intake was increased. Four subjects reported feeling overfull and thus their energy intakes were reduced. In instances in which energy intakes were adjusted, relatively steady weight was achieved over the remainder of the period.

Mean total cholesterol concentrations over the 50-d study are shown in **Figure 1**. Mean plasma lipid profiles during the 30-d feeding period are presented in **Table 2**. Total cholesterol concentrations measured over the 30-d feeding period showed substantial variation in pattern between subjects. For the control diet group, the tertile (n = 5) of subjects showing the greatest response to diet had a mean 23% decline from day 0 to day 30 ($P < 0.05$), whereas the tertile (n = 5) with the least response showed an average 1% increase in total cholesterol concentrations (NS; data not shown). The variability in cholesterol concentrations during the phytosterol-enriched diet was similar to that observed during the control diet. The tertile of subjects showing the greatest response to diet had a mean decline in cholesterol concentrations of 31% ($P < 0.05$), whereas the tertile with the least response showed an average decline in total cholesterol concentrations of 6% (NS; data not shown). Variance in response was not associated with the initial circulating lipid concentration, change in body weight, or number of meals consumed away from the Clinical Nutrition Research Unit.

There was a significant main effect of time on total cholesterol concentration (Table 2). For effects of diet, with use of the two-factor ANOVA model, there was no interaction between

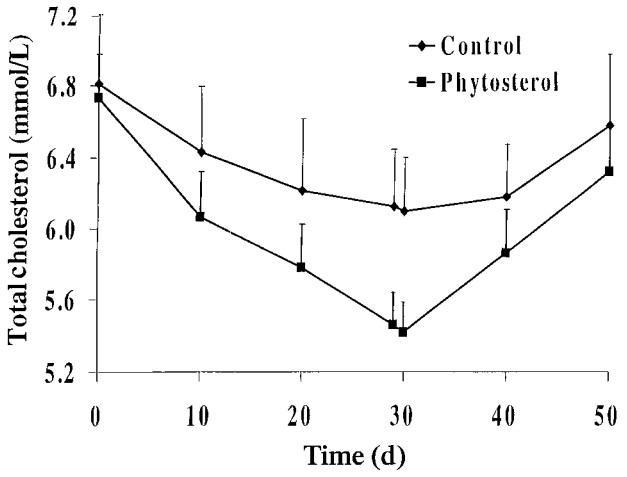

FIGURE 1. Effect of a phytosterol-enriched diet over time on mean (±SE) total cholesterol concentrations of hypercholesterolemic men.

TABLE 2
Plasma lipid concentrations in the control and phytosterol-enriched diet groups[1]

Plasma lipid and study day	Control	Phytosterol
	mmol/L	
Total cholesterol[2]		
Day 0	6.81 ± 1.30	6.73 ± 1.15
Day 10	6.43 ± 1.39	6.06 ± 1.05
Day 20	6.22 ± 1.60	5.78 ± 1.19
Day 30	6.10 ± 1.45	5.42 ± 0.92
LDL cholesterol[3]		
Day 0	5.00 ± 1.27	4.45 ± 1.37
Day 10	4.87 ± 1.30	3.94 ± 0.95[4,5]
Day 20	4.59 ± 1.42[6]	3.66 ± 0.84[4,5]
Day 30	4.56 ± 1.35[6]	3.37 ± 0.94[5,7]
HDL cholesterol		
Day 0	0.64 ± 0.18	0.75 ± 0.24
Day 10	0.64 ± 0.20	0.71 ± 0.26
Day 20	0.63 ± 0.20	0.71 ± 0.25
Day 30	0.60 ± 0.18	0.67 ± 0.18
Total triacylglycerols[8]		
Day 0	2.55 ± 1.20	3.33 ± 1.23
Day 10	2.00 ± 0.60	2.97 ± 1.09
Day 20	2.18 ± 0.90	3.08 ± 1.14
Day 30	2.02 ± 0.66	3.00 ± 1.60

[1] $\bar{x}$ ± SD; n = 16 men per group.
[2] Significant main effect of time, $P = 0.0001$.
[3] Significant main effects of diet, $P = 0.009$, and time, $P = 0.0001$.
[4,6,7] Significantly different from day 0 within study group: [4] $P < 0.01$, [6] $P < 0.05$, [7] $P < 0.001$.
[5] Significantly different from control, $P < 0.05$.
[8] Significant main effect of diet, $P = 0.013$.

time and dietary treatment for mean circulating total cholesterol concentrations, indicating no overall effect of diet. When total cholesterol concentrations were expressed as the difference between the mean of day 29 and 30 values compared with the concentration on day 0, a 10.4% decline was observed for the control diet. For the phytosterol-enriched diet, the decline was 19.5%. When a specific comparison was made between days 0 and 30 for total cholesterol concentrations, a significant difference was observed between diet groups ($P < 0.05$).

The individual mean total cholesterol data for days 0–30 with the control and phytosterol-enriched diets were fitted by using quadratic models. The quadratic term for total cholesterol with the control diet was significant ($r = 0.999$, $P = 0.012$), whereas that with the phytosterol-enriched diet was not ($P = 0.162$). The slope of the decline in total cholesterol during the phytosterol-enriched diet was linear ($r = 0.984$, $P = 0.001$).

The LDL-cholesterol concentrations over the 50-d study are shown in **Figure 2**. Despite dietary control, substantial variations in pattern between subjects were observed for LDL-cholesterol concentrations measured over the 30-d feeding period. With the control diet, the tertile ($n = 5$) of individuals showing the greatest response to diet had an average 24% decline ($P < 0.05$), whereas the tertile ($n = 5$) with the least response showed an average 4% drop in LDL-cholesterol concentrations (NS; data not shown). The variability was similar with the phytosterol-enriched diet. In the present study, the tertile of individuals showing the greatest response to diet had an average 37% decline ($P < 0.05$), whereas the tertile with the least response showed an average 8% drop in LDL-cholesterol concentrations (NS; data not shown).

Both the control and phytosterol-enriched diets caused a progressive decline in LDL-cholesterol concentrations over time, with a trend toward resumption of prediet concentrations over days 40 and 50 (Figure 2). Significant main effects of time and diet treatment were shown for LDL-cholesterol concentrations ($P < 0.05$; Table 2). A marginally significant ($P < 0.1$) interaction between time and dietary treatment was observed for LDL-cholesterol concentrations. LDL-cholesterol concentrations expressed as a percentage difference between the mean of days 29 and 30 compared with day 0 differed significantly ($P < 0.01$) between the control (8.9%) and phytosterol-enriched (24.4%) diets. On days 10, 20, and 30, mean plasma LDL-cholesterol concentrations were significantly lower ($P < 0.05$), by 8.7%, 9.0%, and 15.5%, respectively, in the group consuming the phytosterol-enriched diet compared with the control diet.

The quadratic terms of LDL-cholesterol curves for the control and phytosterol-enriched diets were not significant, but the slopes of the 2 dietary treatment lines were significantly different ($P = 0.041$, Student's unpaired t test). The decline in LDL-cholesterol concentrations with the phytosterol-enriched diet was steeper (−0.036 mmol/d, $r = 0.989$) than that with the control diet (−0.016 mmol/d, $r = 0.969$).

There was no significant difference at the start between the group mean HDL-cholesterol concentration in those consuming the phytosterol-enriched diet and those consuming the control diet, although the mean for the latter group was 12% lower than that of the phytosterol-enriched diet group (Table 2). Neither time nor diet showed significant effects on HDL-cholesterol concentrations in subjects over the duration of the trial.

For the plasma triacylglycerol concentration there was a significant main effect of diet but no time effect or time-by-diet interaction. The triacylglycerol concentration in the phytosterol-enriched diet group was initially higher than that in the control diet group and this difference was maintained throughout the experiment (Table 2).

Mean plasma campesterol and β-sitosterol concentrations in the control diet group did not vary across diet or time from those in the group given phytosterols (**Table 3**). Correction for variations in total cholesterol concentrations and expression of the values of β-sitosterol per mol cholesterol did not result in any significant diet or time effects or a time-by-diet interaction (**Table 4**). For the campesterol-cholesterol ratio, main effects of diet and time were significant, but there was no time-by-diet interaction (Table 4). The campesterol-cholesterol ratio in the phytosterol-enriched diet group was initially higher than that in the control group and this difference was maintained throughout the experiment.

The mean FSR did not differ significantly between the control (0.0911 ± 0.0280 pool/d; range: 0.0408–0.1420) and phytosterol-enriched (0.0914 ± 0.0250 pool/d; range: 0.0487–0.1320) diet groups. In addition, no significant difference was noted in the ASR between the control (0.613 ± 0.243 g/d; range: 0.200–1.10) and phytosterol-enriched (0.647 ± 0.234 g/d; range: 0.274–1.060) diet groups. Significant correlations between circulating phytosterol concentrations and various indexes such as total cholesterol, FSR, ASR, body weight, and BMI were observed. Plasma campesterol concentrations (day 30) correlated with total cholesterol concentrations in the control group ($r = 0.62$, $P = 0.011$), but not in the phytosterol-enriched diet

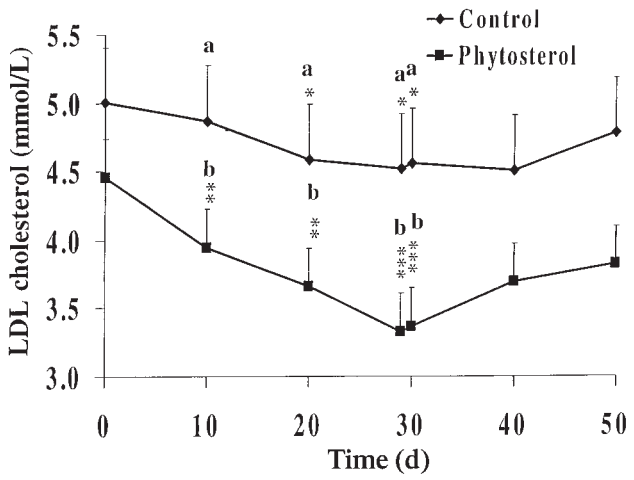

FIGURE 2. Effect of a phytosterol-enriched diet over time on mean (±SE) LDL-cholesterol concentrations of hypercholesterolemic men. *, **, ***Significantly different from day 0: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Between diets, time points with different letters are significantly different, $P < 0.05$.

group. Conversely, the correlation of percentage change in plasma campesterol concentrations with percentage change in total cholesterol concentrations was not significant across treatments. Circulating campesterol concentrations were negatively correlated with M_1 pool size on day 30 in the control ($r = -0.68$, $P = 0.004$) and phytosterol-enriched ($r = -0.48$, $P = 0.05$) diet groups. The FSR was negatively correlated with campesterol ($r = -0.4$, $P = 0.027$), β-sitosterol ($r = -0.39$, $P = 0.03$), the ratio of campesterol to cholesterol ($r = -0.37$, $P = 0.038$), and the ratio of β-sitosterol to cholesterol ($r = -0.34$, $P = 0.05$) concentrations. A negative correlation ($r = -0.36$, $P = 0.045$) was also found between the ASR and total cholesterol concentrations for pooled data of the 2 groups. Finally, BMI correlated with plasma total cholesterol ($r = 0.34$, $P = 0.04$), campesterol ($r = -0.41$, $P = 0.02$), and β-sitosterol ($r = -0.452$, $P = 0.009$) concentrations, and with the percentage change in campesterol on day 30 ($r = 0.425$, $P = 0.015$) in all subjects.

DISCUSSION

To our knowledge, this study is the first in which hypercholesterolemic subjects consumed precisely controlled diets enriched in phytosterols; previous studies used self-selected diets (5, 10, 11, 15, 17). Supplementation with 22 mg tall oil phytosterols•kg body wt^{-1}•d^{-1} was effective in lowering circulating LDL-cholesterol concentrations in these subjects without changing either endogenous cholesterol synthesis or phytosterol concentrations. Although the reduction in LDL-cholesterol concentrations observed in this study agrees with that found by other investigators (9, 14), the observation of no change in cholesterogenesis does not agree with previous reports (26, 42).

The efficacy of phytosterols in lowering circulating lipid concentrations was shown previously for both unsaturated (1, 2, 5, 15) and saturated (8–16) phytosterols either esterified or nonesterified to fatty acids. Use of both pure unsaturated and saturated phytosterols has drawbacks, however. Unsaturated phytosterols must generally be consumed in amounts >4 g/d to be effective (1, 3, 4), although there are reports of efficacy with low doses of unsaturated phytosterols (5) or phytosterol esters (15). Similarly, sitostanol, although more effective than sitosterol in cholesterol-lowering ability, has limitations in that the material must be prepared chemically through hydrogenation from β-sitosterol, then modified to produce sitostanol ester.

In contrast, the present study showed the efficacy of a mixture of unsaturated and saturated phytosterols in lowering LDL cholesterol. After 30 d, our mixture decreased LDL-cholesterol concentrations by 15.5% over and above the action of diet alone. This degree of reduction was similar to that achieved with comparable doses of fully saturated stanol esters given over longer periods (10). When used in conjunction with a prudent North American diet, the extent of cholesterol lowering was approximately twice that attributable to phytosterols alone; LDL-cholesterol concentrations declined almost 25% in our hyperlipidemic subjects. Our mixture of phytosterols was obtained from tall oil without hydrogenation or chemical manipulation after extraction, and the tall oil starting material is available worldwide in abundant quantity.

Independent of the present observation of efficacy of this phytosterol mixture in reducing LDL-cholesterol concentrations is the observation that the full effect of this material is likely attained after more prolonged use. Data for LDL-cholesterol concentrations suggested a steeper decline over time in the group fed phytosterols than that in the control group. For total cholesterol concentrations, the curves differed in shape between the control and phytosterol-fed groups. Phytosterols may thus be acting on lipids in a manner that is mechanistically distinct from that of diet alone. The present data are consistent with those of Miettinen et al (10), who showed that only 80% of the eventual plateau in cholesterol lowering had been achieved at 60 d in mildly hypercholesterolemic subjects consuming self-regulated diets. The final plateau was obtained at about 6 mo (10). In both our study and that of Miettinen et al (10), discontinuation of phytosterols resulted in a rapid return of lipid concentrations to prestudy values (Figures 1 and 2).

Our results contrast with those of Denke (17), who provided 3 g unesterified sitostanol/d to hyperlipidemic subjects and found no significant effect on circulating lipid concentrations. The absence of action observed in the study by Denke may as likely have been a result of study design as of the biological inefficacy of free sitostanol. The present study differed from that of Denke in several ways. First, Denke's subjects were not consuming prepared diets fixed in composition, as in the present experiment. Second, sitostanol in the previous study was provided in capsules, not blended into the fat of each meal as in the present

TABLE 3
Plasma plant sterol concentrations in the control and phytosterol-enriched diet groups[1]

	Campesterol		β-Sitosterol	
Study day	Control	Phytosterol	Control	Phytosterol
	μmol/L			
0	21.2 ± 8.0	22.1 ± 9.7	7.5 ± 2.8	5.4 ± 2.4
10	22.8 ± 8.1	25.7 ± 9.3	7.2 ± 3.5	5.9 ± 2.0
20	21.5 ± 9.0	28.3 ± 17.9	6.4 ± 3.2	6.4 ± 2.9
30	26.4 ± 12.4	27.5 ± 11.7	6.1 ± 5.2	4.4 ± 1.8

[1] $\bar{x}$ ± SD; $n = 16$ men per group. There were no significant main effects of diet or time and no significant time-by-diet interactions.

TABLE 4
Plasma plant sterol concentrations according to cholesterol in the control and phytosterol-enriched diet groups[1]

	Campesterol[2]		β-Sitosterol	
Study day	Control	Phytosterol	Control	Phytosterol
	mmol/mol cholesterol			
0	3.03 ± 0.83	3.39 ± 1.65	1.07 ± 0.31	0.89 ± 0.40
10	3.58 ± 1.17	4.25 ± 1.38	1.09 ± 0.31	0.98 ± 0.31
20	3.44 ± 1.15	4.75 ± 2.29	1.00 ± 0.39	1.14 ± 0.56
30	4.28 ± 1.68	5.18 ± 2.22	0.96 ± 0.62	0.85 ± 0.46

[1] $\bar{x}$ ± SD; n = 16 men per group.
[2] Significant main effects of diet (P = 0.049) and time (P = 0.0008).

study. Capsular phytosterols may not fully disperse or solubilize in the gut digesta before absorption, limiting their ability to reduce cholesterol absorption. Third, in the study by Denke, compliance was monitored by pill count, not by visual confirmation as in the present study. Thus, the previous study may have not completely confirmed compliance with phytosterol consumption. Also, distribution of the phytosterol intake across the 3 daily meals in the present study may have improved efficacy over more intermittent capsular administration.

The test diet alone produced a notable cholesterol-lowering effect without the addition of phytosterols. Although the diet's fat content was not exceptionally lower than that typically consumed by North Americans (43), several features may have been responsible for this improved lipid profile. First, the control diet was relatively high in mono- and polyunsaturated fats, which may have replaced the saturated fats typically found in subjects' habitual intakes. Second, this diet had a lower cholesterol content as a result of the unsaturated fat. Third, the diet was fed to avoid a positive energy balance, a metabolic state associated with increased circulating insulin concentrations and cholesterogenesis (44). In addition, lower circulating total cholesterol and LDL-cholesterol concentrations may have been due to elevated fiber intakes and no alcohol consumption. It is surprising that a distinct lipid-lowering effect was not observed in the control group of the longer-term sitostanol ester feeding study of Miettinen et al (10) because the rapeseed oil would have contributed unsaturated fats to the subjects' diets.

Plasma campesterol concentrations did not change during the study in either the control or phytosterol-enriched diet groups. Phytosterol concentrations were comparable with those reported previously in hypercholesterolemic subjects (10, 45, 46) and are consistent with results reporting that >8 times the normal intake (250–500 mg/d) of dietary β-sitosterol is required to substantially modify plasma sitosterol concentrations (21). Similarly, Lees et al (2) showed that daily administration of 3 g phytosterols, containing largely β-sitosterol, to hypercholesterolemic patients for 1 mo failed to increase plasma phytosterol concentrations.

Although plasma total and LDL-cholesterol concentrations declined with tall oil phytosterol feeding in this study, no compensatory increase in endogenous cholesterol synthesis was suggested by our data. A possible reason for our failure to detect differences in cholesterogenesis between groups was that cholesterol synthesis was compared on day 30 of the trial and not between days 0 and 30. Thus, the lack of response in the FSR and ASR of cholesterol between groups on day 30 may have been obscured by the effect of the control diet itself. Although the correlation between the ASR and plasma total cholesterol in all subjects suggests that endogenous cholesterol synthesis varies with plasma total cholesterol concentrations, this observed association provides insufficient evidence that cholesterol synthesis was different because of phytosterol supplementation.

In summary, the results of the present study show the efficacy of a widely available phytosterol mixture in lowering LDL cholesterol and altering the pattern of response in total cholesterol concentrations in hyperlipidemic men when provided in conjunction with a prudent diet. These changes in lipid profiles were similar in magnitude to those reported in a recent study in which subjects consumed ≈2 g wood- or vegetable-derived saturated stanol esters per day for 8 wk (47). We conclude that sitostanol-containing blends of unsaturated phytosterols have the potential to lower plasma lipid concentrations, which are a risk factor in the development of heart disease in susceptible populations.

We acknowledge the excellent technical assistance of Nathalie Bouthot, Jane Rop, and Chris Vanstone and the clinical expertise of William Parsons. We also thank the staff of the Mary Emily Clinical Nutrition Research Unit of McGill University for preparing the meals.

REFERENCES

1. Farquhar JW, Smith RE, Dempsey ME. The effect of β-sitosterol on the serum lipids of young men with arteriosclerotic heart disease. Circulation 1956;14:77–82.
2. Lees AM, Mok HY, Lees RS, McCluskey MA, Grundy SM. Plant sterols as cholesterol-lowering agents: clinical trials in patients with hypercholesterolemia and studies of sterol balance. Atherosclerosis 1977;28:325–38.
3. Schlierf G, Oster P, Heuck CC, Raetzer H, Schellenberg B. Sitosterol in juvenile type II hyperlipoproteinemia. Atherosclerosis 1978;30:245–8.
4. Pollak OJ, Kritchevsky D. Sitosterol. In: Clarkson TB, Kritchevsky D, Pollak OJ, eds. Monographs on atherosclerosis. 10th ed. Basal, Switzerland: Karger, 1981.
5. Pelletier X, Belbraouet S, Mirabel D, et al. A diet moderately enriched in phytosterols lowers plasma cholesterol concentrations in normocholesterolemic humans. Ann Nutr Metab 1995;39:291–5.
6. Becker M, Staab D, von Bergmann K. Long-term treatment of severe familial hypercholesterolemia in children: effect of sitosterol and bezafibrate. Pediatrics 1992;89:138–42.
7. Vanhanen HT, Miettinen TA. Effects of unsaturated and saturated dietary plant sterols on their serum contents. Clin Chim Acta 1992;205:97–107.
8. Heinemann T, Kullak-Ublick GA, Pietruck B, von Bergmann K. Mechanisms of action of plant sterols on inhibition of cholesterol absorption. Comparison of sitosterol and sitostanol. Eur J Clin Pharmacol 1991;40:S59–63.
9. Becker M, Staab D, von Bergmann K. Treatment of severe familial hypercholesterolemia in childhood with sitosterol and sitostanol. J Pediatr 1993;122:292–6.
10. Miettinen TA, Puska P, Gylling H, Vanhanen HT, Vartiainen E. Reduction of serum cholesterol with sitostanol-ester margarine in a mildly hypercholesterolemic population. N Engl J Med 1995;333:1308–12.
11. Gylling H, Siimes MA, Miettinen TA. Sitostanol ester margarine in dietary treatment of children with familial hypercholesterolemia. J Lipid Res 1995;36:1807–12.
12. Vanhanen HT, Kajander J, Lehtovirta H, Miettinen TA. Serum levels, absorption efficiency, faecal elimination and synthesis of cholesterol during increasing doses of dietary sitostanol esters in hypercholesterolaemic subjects. Clin Sci 1994;87:61–7.

13. Gylling H, Miettinen TA. Serum cholesterol and cholesterol and lipoprotein metabolism in hypercholesterolaemic NIDDM patients before and during sitostanol ester-margarine treatment. Diabetologia 1994;37:773–80.
14. Miettinen TA, Vanhanen HT. Dietary sitostanol related to absorption, synthesis and serum level of cholesterol in different apolipoprotein E phenotypes. Atherosclerosis 1994;105:217–26.
15. Weststrate JA, Meijer GW. Plant sterol-enriched margarines and reduction of plasma total- and LDL-cholesterol concentrations in normocholesterolaemic and mildly hypercholesterolaemic subjects. Eur J Clin Nutr 1998;52:334–43.
16. Jones PJH, Howell T, MacDougall DE, Feng JY, Parsons W. Short-term administration of tall oil phytosterols improves plasma lipid profiles in subjects with different cholesterol levels. Metabolism 1998;47:751–6.
17. Denke MA. Lack of efficacy of low-dose sitostanol therapy as an adjunct to a cholesterol-lowering diet in men with moderate hypercholesterolemia. Am J Clin Nutr 1995;61:392–6.
18. Heinemann T, Axtmann G, von Bergmann K. Comparison of intestinal absorption of cholesterol with different plant sterols in man. Eur J Clin Invest 1993;23:827–31.
19. Heinemann T, Leiss O, von Bergmann K. Effect of low-dose sitostanol on serum cholesterol in patients with hypercholesterolemia. Atherosclerosis 1986;61:219–23.
20. Child P, Kuksis A. Investigation of the role of micellar phospholipid in the preferential uptake of cholesterol over sitosterol by dispersed rat jejunal villus cells. Biochem Cell Biol 1986;64:847–53.
21. Salen G, Ahrens EH Jr, Grundy SM. Metabolism of β-sitosterol in man. J Clin Invest 1970;49:952–67.
22. Child P, Kuksis A. Critical role of ring structure in the differential uptake of cholesterol and plant sterols by membrane preparations in vitro. J Lipid Res 1983;24:1196–209.
23. Ikeda I, Sugano M. Some aspects of mechanism of inhibition of cholesterol absorption by β-sitosterol. Biochim Biophys Acta 1983;732:651–8.
24. Laraki L, Pelletier X, Mourot J, Debry G. Effects of dietary phytosterols on liver lipids and lipid metabolism enzymes. Ann Nutr Metab 1993;37:129–33.
25. Shefer S, Salen G, Bullock J, et al. The effect of increased hepatic sitosterol on the regulation of 3-hydroxy-3-methylglutaryl-coenzyme A reductase and cholesterol 7 α-hydroxylase in the rat and sitosterolemic homozygotes. Hepatology 1994;20:213–9.
26. Grundy SM, Ahrens EH Jr, Davignon J. The interaction of cholesterol absorption and cholesterol synthesis in man. J Lipid Res 1969;10:304–15.
27. Konlande JE, Fisher H. Evidence for a nonabsorptive antihypercholesterolemic action of phytosterols in the chicken. J Nutr 1969;98:435–42.
28. Kakis G, Kuksis A. Effect of intravenous infusion of intralipid, cholesterol, and plant sterols on hepatic cholesterogenesis. Can J Biochem Cell Biol 1984;62:1–10.
29. Subbiah MT, Kuksis A. Differences in metabolism of cholesterol and sitosterol following intravenous injection in rats. Biochim Biophys Acta 1973;306:95–105.
30. Miettinen TA, Tilvis RS, Kesaniemi YA. Serum plant sterols and cholesterol precursors reflect cholesterol absorption and synthesis in volunteers of a randomly selected male population. Am J Epidemiol 1990;131:20–31.
31. Health and Welfare Canada. Recommended nutrient intakes for Canadians. Ottawa: Department of National Health and Welfare, 1983.
32. Mifflin MD, St Jeor ST, Hill LA, Scott BJ, Daugherty SA, Koh YO. A new predictive equation for resting energy expenditure in healthy individuals. Am J Clin Nutr 1990;51:241–7.
33. Warnick GR, Benderson J, Albers JJ. Dextran sulfate-Mg^{2+} precipitation procedure for quantitation of high-density-lipoprotein cholesterol. Clin Chem 1982;28:1379–88.
34. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499–502.
35. Ntanios F, Jones PJH. Effects of variable dietary sitostanol concentrations on plasma lipid profile and phytosterol metabolism in hamsters. Biochim Biophys Acta 1998;1390:237–44.
36. Jeske DJ, Dietschy JM. Regulation of rates of cholesterol synthesis in vivo in the liver and carcass of the rat measured using ^{3}H water. J Lipid Res 1980;21:364–76.
37. Jones PJH. Use of deuterated water for measurement of short-term cholesterol synthesis in humans. Can J Physiol Pharmacol 1990;68:955–9.
38. Jones PJH, Leitch CA, Li ZC, Connor WE. Human cholesterol synthesis measurement using deuterated water. Theoretical and procedural considerations. Arterioscler Thromb Vasc Biol 1993;13:247–53.
39. Jones PJH, Scanu AM, Schoeller DA. Plasma cholesterol synthesis using deuterated water in humans: effect of short-term food restriction. J Lab Clin Med 1988;111:627–33.
40. Goodman DS, Smith FR, Seplowitz AH, Ramakrishnan R, Dell RB. Prediction of the parameters of whole body cholesterol metabolism in humans. J Lipid Res 1980;21:699–713.
41. SAS Institute Inc. SAS procedure guide, version 6.12. Cary, NC: SAS Institute Inc, 1994.
42. Vanhanen HT, Blomqvist S, Ehnholm C, et al. Serum cholesterol, cholesterol precursors, and plant sterols in hypercholesterolemic subjects with different apoE phenotypes during dietary sitostanol ester treatment. J Lipid Res 1993;34:1535–44.
43. Posner BM, Cupples LA, Franz MM, Gagnon DR. Diet and heart disease risk factors in adult American men and women: the Framingham Offspring-Spouse nutrition studies. Int J Epidemiol 1993;22:1014–25.
44. Jones PJH. Regulation of cholesterol biosynthesis by diet in humans. Am J Clin Nutr 1997;66:438–46.
45. Miettinen TA, Vanhanen HT, Ojala J-P, Tikkanen MJ. Non-cholesterol sterols and faecal elimination of cholesterol during statin and fibrate treatment. Atherosclerosis 1992;97:S73–80.
46. Vanhanen HT. Cholesterol malabsorption caused by sitostanol ester feeding and neomycin in pravastatin-treated hypercholesterolaemic patients. Eur J Clin Pharmacol 1994;47:169–76.
47. Hallikainen MA, Uusitupa MIJ. Effects of 2 low-fat stanol ester–containing margarines on serum cholesterol concentrations as part of a low-fat diet in hypercholesterolemic subjects. Am J Clin Nutr 1999;69:403–10.