MEMORANDUM OF CALL
Between Dr. Gilbert Leveille (McNeil Consumer Healthcare)
and Dr. Sharon Ross (DSNP, ONPLDS, CFSAN, FDA)
May 1, 2000 (3:05 pm)
Re: Petition for Health Claim: Plant Stanol Esters

A call was placed to Gilbert Leveille by Sharon Ross to ask that McNeil submit three recently published studies as a supplement to their health claim petition. The three studies are: (1) Cholesterol-lowering efficacy of a sitostanol-containing phytosterol mixture with a prudent diet in hyperlipidemic men. Jones PJH, Nyanios FY, Raeini-Sarjaz M, and Vanstone CA. Am J Clin Nutr (1999) 69:1144-1150, (2) Lack of efficacy of low-dose sitostanol therapy as an adjunct to a cholesterol-lowering diet in men with moderate hypercholesterolemia. Denke MA. Am J Clin Nutr (1995) 61:392-6., and (3) Plant sterol and stanol margarines and health. Law M. BMJ (2000) 320:861-864. Gilbert Leveille stated that he will follow-up on this request by sending the papers to the FDA by the end of the week.

00P-1276

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McNeil

McNeil Consumer Healthcare, 7050 Camp Hill Road, Fort Washington, PA 19034-2299 (215) 273-7000

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Sharon A. Ross, PhD
Food & Drug Administration
Office of Nutritional Products,
Labeling and Dietary Supplements
HFS-830
Center for Food Safety and Applied Nutrition
200 C Street, SW
Washington, DC 20204

RE: Plant Stanol Esters Health Claim Petition - Supplement

Dear Dr. Ross:

Per today's conversation and at FDA's request we are submitting as a supplement to the February 15, 2000 Plant Stanol Esters Health Claim Petition the following three journal articles:

Denke, Margo A., "Lack of Efficacy of Low-Dose Sitostanol Therapy as an Adjunct to a Cholesterol-Lowering Diet in Men with Moderate Hypercholesterolemia," *American Journal of Clinical Nutrition,* 1995;61:392-6.

Jones, Peter J. H., et al, "Cholesterol-Lowering Efficacy of a Sitostanol-Containing Phytosterol Mixture with a Prudent Diet in Hyperlipidemic Men," *American Journal of Clinical Nutrition*, 1999;69: 1144-50.

Law, Malcolm, "Plant Sterol and Stanol Margarines and Health," *British Medical Journal*, 2000;320:861-4.

Should you have any questions, please do not hesitate to contact me at 215/273-8194.

Sincerely,
MCNEIL CONSUMER HEALTHCARE

Kimberly F. Upcum for

Gilbert A. Leveille
Worldwide Vice President,
Regulatory and Scientific Affairs

enc.

cc: L. A. Larsen, PhD (HFS-830)

Lack of efficacy of low-dose sitostanol therapy as an adjunct to a cholesterol-lowering diet in men with moderate hypercholesterolemia[1–3]

Margo A Denke

ABSTRACT Plant sterols have been shown to reduce dietary cholesterol absorption and hence, total and low-density-lipoprotein (LDL)-cholesterol concentrations in humans. In this study the cholesterol-lowering effects of dietary supplementation with the hydrogenated plant sterol sitostanol (3 g/d) were tested in 33 men with moderate hypercholesterolemia who were consuming an outpatient diet in which dietary cholesterol was restricted to <200 mg/d. Sitostanol therapy did not significantly lower LDL cholesterol compared with the diet alone. Similarly, sitostanol therapy in conjunction with a cholesterol-lowering regimen of diet and 8 g cholestyramine did not significantly lower LDL-cholesterol concentrations. Hence, although previous reports have suggested that low-dose sitostanol therapy is an effective means of reducing LDL-cholesterol concentrations, its effectiveness may be attenuated when the diet is low in cholesterol. *Am J Clin Nutr* 1994;61:392–6

KEY WORDS Cholesterol-lowering diet; plant sterols, sitostanol

Introduction

Dietary cholesterol accounts for a significant part of the cholesterol-raising effects of a typical American diet, and, consequently, dietary cholesterol intake should be restricted in a cholesterol-lowering diet (1). Besides restricting dietary cholesterol intake, an additional way to reduce the effects of dietary cholesterol on serum cholesterol is to block its absorption. Plant sterols have the capacity to reduce cholesterol absorption (2). By this mechanism, plant sterols found in membrane extracts and in the unsaponifiable fraction of vegetable oils can lower serum cholesterol concentrations (3).

Plant sterol extracts were one of the first hypolipidemic agents developed for the treatment of hypercholesterolemia (4). Several investigations have demonstrated that plant sterols can lower total and low-density-lipoprotein (LDL)-cholesterol concentrations 10–20% in subjects consuming diets rich in dietary cholesterol (5–7). Earlier investigations evaluated the efficacy of high doses of plant sterols (10–20 g/d) (6), but more recent studies (5,7) have shown that relatively low doses (1.5–3.0 g/d) also produce significant reductions in cholesterol concentrations. However, because dietary cholesterol restriction is a component of a cholesterol-lowering diet (8–10), and whether plant sterols will be equally effective during even more restricted dietary cholesterol intakes needs to be investigated.

The use of low doses of plant sterols as cholesterol-lowering agents is attractive. Except in patients with sitosterolemia (11), plant sterols are poorly absorbed and have been associated with no known toxicity (12). Even if plant sterols do not achieve as great a reduction in LDL-cholesterol concentrations as does conventional drug therapy, their lack of systemic effects supports their use in patients who do not quite achieve target LDL-cholesterol concentrations with usual dietary therapy alone. Included among such individuals would be those at low immediate risk for coronary heart disease (CHD), eg, premenopausal women and children.

The current study was conducted to explore the utility of plant sterols as a dietary adjunct for diets low in dietary cholesterol. A low dose of the hydrogenated plant sterol sitostanol was administered to men with moderate hypercholesterolemia who were following a cholesterol-lowering diet with dietary cholesterol intakes <200 mg/d. To further explore the benefits of sitostanol in this high-risk population, the combination of low-dose cholestyramine and low-dose sitostanol was also evaluated.

Subjects and methods

Subjects

Thirty-three men aged 31–70 y ($\bar{x}$ = 56 y) with a body mass index (BMI, kg/m^2) of 26.2 ± 0.3 and with moderate hypercholesterolemia [mean LDL-cholesterol concentrations of 4.52 mmol/L after a Step 1 Diet (13)] were recruited for study. All men had fasting triglycerides <2.82 mmol/L. Eighteen men had documented CHD but were asymptomatic at the time of study. None had xanthelasma, corneal arcus, or tendon xantho-

[1] From the Center for Human Nutrition, Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, and the Veterans' Affairs Medical Center, Dallas.

[2] Supported by the Veterans' Administration, the Southwestern Medical Foundation, and the Moss Heart Foundation, Dallas; grants from the National Institutes of Health (HL-29252 and M01-RR-00633) and the US Public Health Service (5-M01-RR00633); and the NIH-NHLBI Clinical Investigator Award (NIH5-K08-HL02456).

[3] Address reprint requests to MA Denke, Center for Human Nutrition, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75235–9052.

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Am J Clin Nutr 1995;61:392–6. Printed in USA. © 1995 American Society for Clinical Nutrition

mata. These men had participated in a larger study which evaluated dietary responsiveness to a cholesterol-lowering diet (14), and agreed to further testing of their response to other cholesterol-lowering modalities besides diet. Each of the sequential test periods of sitostanol alone, cholestyramine alone, or a combination of sitostanol and cholestyramine, was 3 mo in duration. A 1-mo washout period of the Step 1 Diet alone separated each of the treatment periods.

Methods

Beta-sitosterol was harvested from tall oil (a resinous extract from wood pulp of pine trees) and was hydrogenated forming sitostanol. The sitostanol was then suspended in safflower oil and packed into gelatin capsules, each containing 250 mg sitostanol and 1 g safflower oil (Delande, Köln, Germany). Subjects were instructed to continue the Step 1 Diet, while adding four capsules of sitostanol to each meal. Hence, during sitostanol administration, subjects were to consume a total of 12 capsules (3000 mg) in three divided doses during three meals.

Because cholestyramine is additive in its action with other lipid-lowering agents (15), the effect of adding sitostanol to 8 g cholestyramine/d was also evaluated and compared with the effects of cholestyramine alone. Cholestyramine was supplied as a flavored, edible bar (Cholybar; Parke Davis, Morris Plains, NJ). Each bar contained 4 g cholestyramine. The effects of cholestyramine alone were previously reported (16), but will be presented again to allow for an evaluation of potential synergism between sitostanol and cholestyramine. During the combination therapy, subjects were instructed to consume the sitostanol capsules with each meal, and the cholestyramine 2 h after the morning and evening meals.

Compliance with sitostanol therapy was assessed by capsule count and monthly interviews, and compliance with dietary therapy was assessed by an initial 7-d dietary record obtained during the first Step 1 Diet only period and by monthly 24-h dietary recalls obtained throughout the study. Blood was drawn for lipid and lipoprotein concentrations monthly, and on 5 separate days during the last 2 wk of the 3-mo supplementation period and during each Step 1 Diet period that occurred between test periods to measure drift and any carryover effects.

Measurements

Plasma concentrations of total cholesterol and lipoprotein cholesterol were measured enzymatically; total cholesterol was determined on whole plasma (17). High-density-lipoprotein (HDL) cholesterol was measured after precipitation of apolipoprotein B–containing lipoproteins in whole plasma with heparin manganese (18). Another aliquot of plasma was used to isolate very-low-density lipoproteins (VLDLs) (density <1.006 kg/L) by ultracentrifugation. Cholesterol was determined in the isolated 1.006-kg/L supranate and plasma infranate. Corrections were made for total recoveries that were consistently >95%. LDL cholesterol was calculated as the difference between 1.006-kg/L infranate cholesterol and HDL cholesterol.

Statistical analysis

The mean of the five lipid and lipoprotein cholesterol values drawn during the last 2 wk of each treatment period or washout period was used to define the lipid and lipoprotein response for that individual. To evaluate the effects of sitostanol as an adjunct to dietary therapy, a repeated-measures analysis of variance (ANOVA) was used to compare lipid concentrations during the Step 1 Diet, sitostanol plus the Step 1 Diet, and subsequent Step 1 Diet washout. If significant differences were found, two-tailed t tests were performed. To compare the effects of sitostanol as an adjunct to cholestyramine therapy, a two-tailed t test was used to compare lipid concentrations during the Step 1 Diet plus cholestyramine those during the Step 1 Diet plus cholestyramine plus sitostanol. For a power >80% and an $\alpha < 0.05$, a sample size of 18–20 subjects would be required to detect a difference of 10% in LDL-cholesterol concentrations.

Results

Compliance with the sitostanol therapy, assessed by pill count, was 94%. Most missed capsules were related to missed meals; on days of a missed meal, subjects often consumed only 2 g sitostanol. Compliance with diet, as assessed by 24-h recall, showed little drift throughout the study. Self-reported intake of dietary cholesterol from monthly 24-h recalls averaged 188 mg/d, and with an average of 30% of energy from fat and 10% of energy from saturated fatty acids. Note that most subjects did not consume egg yolks, but derived most of their dietary cholesterol from meats. Compliance with cholestyramine therapy, assessed by bar count, was 90%. Ten subjects reported an improvement in constipation when sitostanol therapy was added to cholestyramine therapy.

Table 1 shows the lipid and lipoprotein concentrations obtained during each of the treatment phases. Sitostanol added to a Step 1 Diet had no significant effect on LDL-cholesterol concentrations compared with the Step 1 Diet alone. LDL-cholesterol concentrations during the sitostanol period were statistically indistinguishable from the concentrations during the other diet-only periods. Similarly, when sitostanol plus cholestyramine was compared cholestyramine therapy alone, the addition of sitostanol did not significantly affect the LDL-cholesterol concentration.

To determine whether there were subpopulations that achieved definite LDL lowering with sitostanol, the change in LDL-cholesterol concentrations between the Step 1 Diet and the sitostanol supplementation period was graphed as shown in **Figure 1**. Response to therapy in individual subjects showed a high degree of variability. Whereas 13 of 30 subjects had ≥ a 0.13 mmol/L (5 mg/dL) decrease in LDL values (Fig 1), the majority of subjects had no LDL reduction with sitostanol therapy.

To determine whether the effects of sitostanol were small but consistent, a comparison of the lowering achieved during the two sitostanol treatment periods is plotted in the top panel of **Figure 2**. The change in LDL observed in each individual between a Step 1 Diet alone minus the Step 1 Diet plus sitostanol (x axis) and the change in LDL observed between a Step 1 Diet and cholestyramine minus the Step 1 Diet and cholestyramine plus sitostanol (y axis) is plotted. The absence of correlation between these two periods is notable ($r = -0.02$, $P = 0.89$).

The consistency with which subjects responded to cholestyramine therapy is displayed in the bottom panel of Figure

TABLE 1
Effects of sitostanol, cholestyramine, and the combination of sitostanol and cholestyramine on lipid and lipoprotein concentrations, by period[1]

	Step 1 Diet	Step 1 Diet + sitostanol	Step 1 Diet	Step 1 Diet + cholestyramine	Step 1 Diet	Step 1 Diet + cholestyramine and sitostanol	Step 1 Diet
				mmol/L			
Total cholesterol	6.18 ± 0.75	6.15 ± 0.80	6.30 ± 0.75	5.74 ± 0.72[2]	6.38 ± 0.72	5.63 ± 0.72[3]	6.28 ± 0.93
Total triglycerides	1.65 ± 0.62	1.67 ± 0.54	1.77 ± 0.63	1.77 ± 0.58	1.76 ± 0.72	1.73 ± 0.53	1.63 ± 0.44
VLDL cholesterol	0.65 ± 0.26	0.65 ± 0.26	0.72 ± 0.31	0.72 ± 0.31	0.75 ± 0.41	0.70 ± 0.31	0.65 ± 0.26
LDL cholesterol	4.52 ± 0.67	4.44 ± 0.80	4.67 ± 0.78	3.95 ± 0.67[2]	4.63 ± 0.70	3.85 ± 0.70[3]	4.60 ± 0.80
HDL cholesterol	1.01 ± 0.28	1.06 ± 0.31	1.01 ± 0.28	1.06 ± 0.34	1.01 ± 0.31	1.06 ± 0.26	1.03 ± 0.28

[1] $\bar{x}$ ± SD; n = 33.
[2] Significantly different from preceding and subsequent Step 1 Diet alone and Step 1 Diet + sitostanol, $P < 0.001$ (repeated-measures ANOVA).
[3] Significantly different from preceding and subsequent Step 1 Diet alone periods and Step 1 Diet plus sitostanol, $P < 0.001$ (repeated-measures ANOVA).

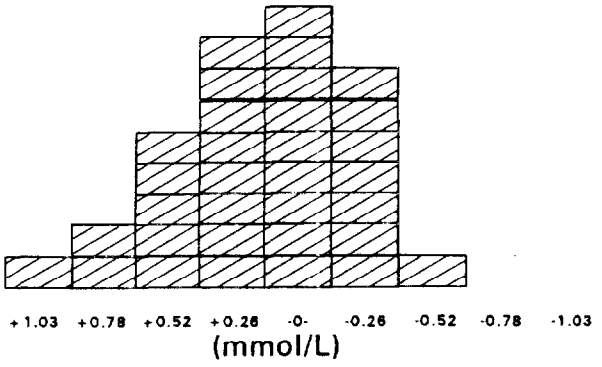

FIGURE 1. Distribution of changes in low-density-lipoprotein (LDL) concentrations (Step 1 Diet LDL-cholesterol concentration minus Step 1 Diet plus sitostanol LDL-cholesterol concentration) obtained for each individual. The x axis denotes the midpoint value of response. Each block represents one subject.

2. LDL values from the Step 1 Diet alone minus LDL values on cholestyramine therapy alone (x axis) and the Step 1 Diet minus cholestyramine plus sitostanol (y axis) are plotted for each individual. The excellent correlation of LDL response is notable (r = 0.82, $P < 0.00001$).

Discussion

The National Cholesterol Education Program (19) recommends dietary therapy as the first step in the management of individuals with hypercholesterolemia. Whereas dietary therapy alone may be sufficient for some individuals to achieve target LDL-cholesterol concentrations (14,20), other individuals' concentrations will remain high despite dietary therapy. Agents with mild cholesterol-lowering properties that have little toxicity could provide an important additional therapy for patients whose LDL-cholesterol concentrations are not high enough with dietary therapy to warrant treatment with the usual cholesterol-lowering drugs. Such agents could be considered adjuncts to the cholesterol-lowering diet, particularly if the mechanism of action is interference with the absorption of cholesterol or other cholesterol-raising nutrients.

Dietary cholesterol and saturated fatty acids have different absorption characteristics that have been perturbed by dietary adjuncts. Whereas saturated fatty acids are 94–98% absorbed, dietary cholesterol is only 40–60% absorbed. Fatty acid absorption is not easily disrupted; we showed previously that excess dietary calcium can reduce apparent saturated fatty acid absorption by 3–5%, resulting in significant reductions in LDL-cholesterol concentrations (21). Dietary cholesterol absorption is more easily disrupted; under certain conditions, plant sterols can efficiently reduce dietary cholesterol absorption on a 1:1 molar basis, resulting in a significant reduction in LDL-cholesterol concentrations (22).

The current study evaluated the effectiveness of a hydrogenated plant sterol as a dietary adjunct in men with moderate elevations in LDL-cholesterol concentration despite dietary therapy. A 3 000-mg dose of sitostanol was chosen, which should have been more than sufficient to block most of dietary cholesterol absorption, and some cholesterol lowering should occur. Hegsted et al (23) initially suggested that both saturated fatty acid and dietary cholesterol intakes are linearly related to serum cholesterol concentrations. The change in serum cholesterol was 6.77 (change in decigrams dietary cholesterol/11 087 J). Keys et al (24) suggested that saturated fatty acids produce a linear increase in cholesterol concentrations, dietary cholesterol produces an exponential effect: change in cholesterol = 1.5 ($x^{1/2} - y^{1/2}$), where x and y are mg dietary cholesterol/418 J. Both investigators reevaluated their predictive equations after including studies from other laboratories. Although Hegsted's modified equation (25) again described a linear relationship: change in cholesterol = 0.097 (change in dietary cholesterol mg/418 J), Keys confirmed his initial prediction of an exponential effect (26). Using these equations to predict the cholesterol lowering expected in this study, if dietary cholesterol intake was reduced from 200 to 0 mg/d, Keys would predict a 0.23 mmol/L (9 mg/dL) lowering whereas Hegsted would predict a 0.36 mmol/L (14 mg/dL) lowering using his initial equation, or 0.21 mmol/L (8 mg/dL) using the later, modified equation. Additionally, even greater serum cholesterol lowering might occur if plant sterols could interfere with reabsorption of cholesterol secreted into the intestine with the bile (27). Despite these favorable predictions, only a nonsignificant 0.03–0.10 mmol/L (1–4 mg/dL) reduction in LDL-cholesterol concentrations was actually observed.

We can only speculate as to why a greater cholesterol-lowering effect was not observed with sitostanol therapy. Potential explanations could relate to the variability of respon-

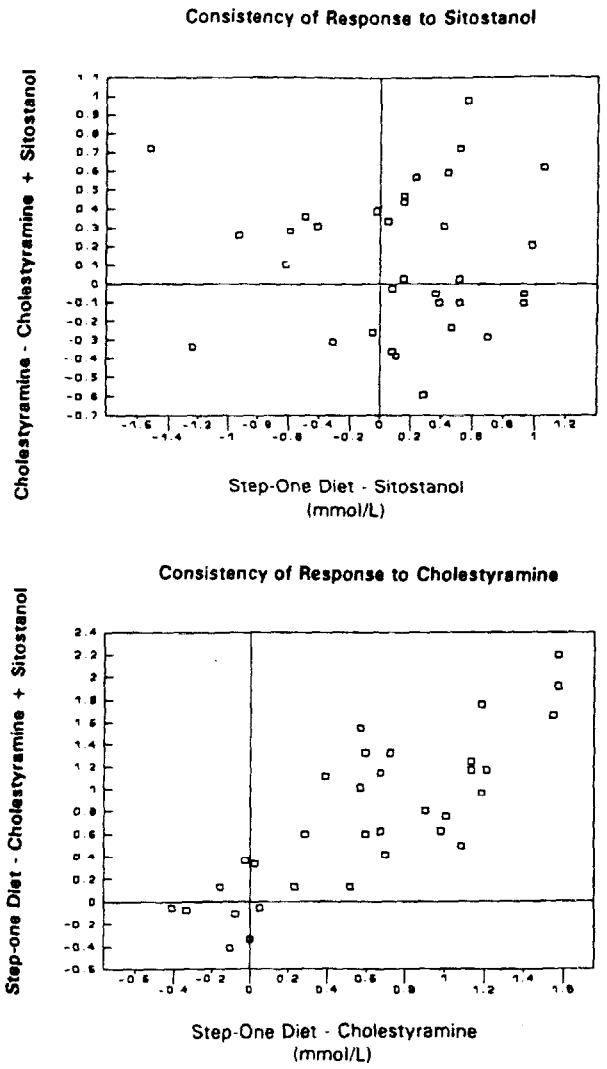

FIGURE 2. Comparison of individual low-density-lipoprotein- (LDL) cholesterol responses to the two phases that used sitostanol therapy (top) and the two phases that used cholestyramine therapy (bottom). Top: Correlation between changes in LDL from a Step 1 Diet alone to a Step 1 Diet plus sitostanol (x axis) and from cholestyramine alone to cholestyramine plus sitostanol (y axis). This comparison evaluates the correlation between the two periods using sitostanol. No correlation was observed. Bottom: Correlation between changes in LDL from a Step 1 Diet alone to a Step 1 Diet plus cholestyramine (x axis) and from a Step 1 Diet alone to cholestyramine plus sitostanol (y axis). This comparison evaluates the correlation between the two periods using cholestyramine. A highly significant correlation was observed ($r = 0.82$, $P < 0.00001$).

siveness to dietary cholesterol, the study population, the method of sitostanol administration, or the baseline dietary intake of the subjects.

Several previous investigations failed to show that dietary cholesterol raises serum cholesterol concentrations (28–30). The exact mechanism explaining the lack of response to dietary cholesterol in these studies remains unclear (31). If the study populations were predominantly nonresponders to dietary cholesterol (32), whether or not dietary cholesterol absorption was blocked should have little bearing on serum cholesterol concentrations. This explanation seems unlikely for this investigation because these subjects are responsive to a cholesterol-lowering diet (14), and the response to saturated fatty acids appears congruent with the response to dietary cholesterol (33). Thirteen of 30 subjects achieved > a 0.13 mmol/L (5 mg/dL) reduction in LDL cholesterol with sitostanol therapy (Fig 1); however, this reduction was not reproduced when bile acid resins were added to the regimen.

Second, many previous investigations of plant sterol therapy studied individuals with familial hypercholesterolemia (4,34). Those with familial hypercholesterolemia have lower rates of bile acid synthesis (35), with a resultant enrichment of the cholesterol content of bile. If blocking reabsorption of biliary cholesterol enhances the cholesterol-lowering properties of plant sterols, subjects with familial hypercholesterolemia may receive added benefit from plant sterol therapy. This study examined men with polygenic hypercholesterolemia, who may respond less favorably to plant sterol therapy than do individuals with familial hypercholesterolemia. Support for this notion is seen in two studies in which subjects with moderate hypercholesterolemia had no (36) or a modest reduction of 0.15 mmol/L (6 mg/dL) in LDL-cholesterol concentrations (37).

Another reason why the expected cholesterol lowering was not achieved could be because of a difference in the particular sitostanol preparation used: if the sitostanol preparation did not mix with intestinal contents, dietary cholesterol absorption could not be blocked. The sitostanol in the current preparation should have been easily dispersed in the sunflower oil, and subjects were counseled to take their capsules with meals. Sitostanol has been shown to be more effective than sitosterol at blocking cholesterol absorption (38). Therefore, this explanation also seems unlikely.

Last, the lack of response to sitostanol could be attributed to the baseline study diet. This study population was consuming a diet already low in dietary cholesterol with average intakes of 192 mg cholesterol-/d. The dietary cholesterol was derived mainly from meats. Previous studies that have demonstrated cholesterol lowering with sitostanol therapy have used subjects who ingested larger quantities of egg yolk cholesterol. Because the efficacy of sitostanol relies on a reduction in dietary cholesterol absorption, if dietary cholesterol from meat is more poorly absorbed than dietary cholesterol from egg yolks, sitostanol added to a meat-based diet may result in smaller reductions in serum cholesterol concentrations than will sitostanol added to an egg yolk–based diet (39).

This study suggests that sitostanol added to a diet low in dietary cholesterol produces only a mild cholesterol lowering—an effect no greater than that observed with water-soluble fiber (40). The results do not exclude the use of sitostanol as an agent to block dietary cholesterol during meals of high cholesterol content (eg, egg dishes) or the use of sitostanol as a weak adjunct to a cholesterol-lowering diet. Further work is needed to fully define the utility of plant sterols as an adjunct to cholesterol-lowering dietary therapy.

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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

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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

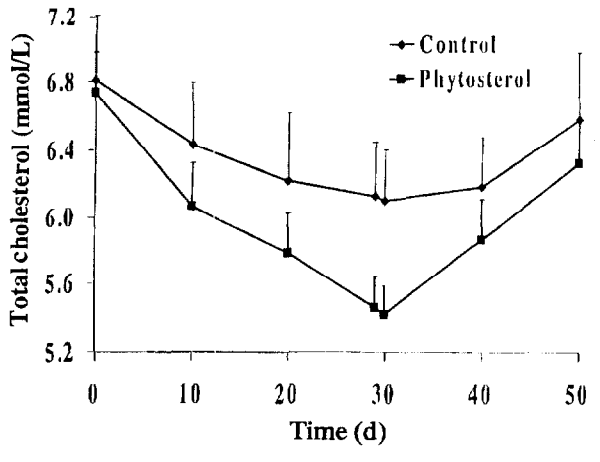

FIGURE 1. Effect of a phytosterol-enriched diet over time on mean (±SE) total cholesterol concentrations of hypercholesterolemic men.

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

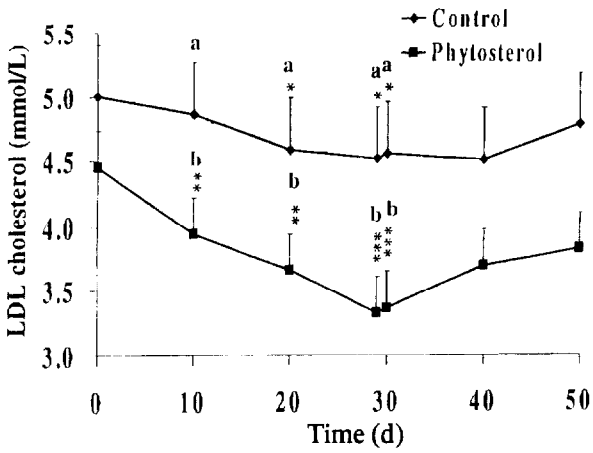

FIGURE 2. Effect of a phytosterol-enriched diet over time on mean (±SE) LDL-cholesterol concentrations of hypercholesterolemic men. *, **, ***Significantly different from day 0: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Between diets, time points with different letters are significantly different, $P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

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.

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hypogonadism can be clearly shown, treatment with sildenafil citrate (with appropriate warnings about cardiovascular risks and drug interactions with nitrites) is likely to be safer and more efficacious than injections of testosterone esters.

To conclude, I really do not find the analogy of the female menopause helpful in understanding or trying to manage the problems of senescence in men. Moreover, the endocrinology of ageing is much broader than that the term suggests. As Lamberts, van den Beld, and van der Lely have pointed out,[16] while the fragility of elderly people might be related to a gonadopause, an adrenopause (the age related fall of dehydroepiandrosterone sulphate concentrations), or a somatopause (the decline in secretion of growth hormone and insulin like growth factor), actually in old people the commonest endocrine disorders are diabetes mellitus and hypothyroidism. These conditions are definitely treatable.

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Plant sterol and stanol margarines and health

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A new polyunsaturated margarine with added plant stanols, Benecol, was introduced in several European countries last year, and a similar margarine with added plant sterols will be introduced under the Flora label later this year. These products lower serum concentrations of cholesterol, but they are expensive.[1-14] In Great Britain the cost is about £2.50 ($4.00) for a 250 g tub compared with 60p for ordinary polyunsaturated margarine and 90p for butter. This article considers quantitatively the health aspects of adding plant sterols and stanols to margarines and other foods.

Summary points

Plant sterols and stanols reduce the absorption of cholesterol from the gut and so lower serum concentrations of cholesterol

Plant sterols or stanols that have been esterified to increase their lipid solubility can be incorporated into foods

The 2 g of plant sterol or stanol added to an average daily portion of margarine reduces serum concentrations of low density lipoprotein cholesterol by an average of 0.54 mmol/l in people aged 50-59, 0.43 mmol/l in those aged 40-49, and 0.33 mmol/l in those aged 30-39

A reduction in the risk of heart disease of about 25% would be expected for this reduction in low density lipoprotein cholesterol; this is larger than the effect that could be expected to be achieved by people reducing their intake of saturated fat

The added costs of £70 per person per year will limit consumption; however, if stanols and sterols become cheaper, their introduction into the food chain will make them an important innovation in the primary prevention of heart disease

Methods

Randomised trials included in this review were identified by a Medline search using the term "plant sterols." Additional trials were identified from citations in these papers and in review articles. Other trials in children with familial hypercholesterolaemia were not included.

Plant sterols and stanols

Sterols are an essential component of cell membranes, and both animals and plants produce them. The sterol ring is common to all sterols; the differences are in the side chain. Cholesterol is exclusively an animal sterol. Over 40 plant sterols (or phytosterols) have been identified but β-sitosterol (especially), campesterol, and stigmasterol are the most abundant. These three sterols are structurally similar to cholesterol: they are all 4-desmethyl sterols (containing no methyl groups at carbon atom 4).

Stanols are saturated sterols (they have no double bonds in the sterol ring). Stanols are less abundant in nature than sterols. Plant stanols are produced by hydrogenating sterols. The term sterol is sometimes used as a generic term to include unsaturated sterols

and saturated stanols, but it is used here to refer specifically to the unsaturated compounds.

It was recognised in the 1950s that plant sterols lower serum concentrations of cholesterol[5]; they do this by reducing the absorption of cholesterol from the gut by competing for the limited space for cholesterol in mixed micelles (the "packages" in the intestinal lumen that deliver mixtures of lipids for absorption into the mucosal cells).[6 11 16-18] In Europe the average consumption of butter or margarine is 25 g per person each day, and the fortified margarines contain 2 g of plant sterols or stanols per daily portion. About 0.25 g of plant sterols and 0.3 g of cholesterol occur naturally in the daily diet; the amount of plant sterols consumed daily is twice as high in a vegetarian diet. The added plant sterols or stanols in fortified margarine reduce the absorption of cholesterol in the gut—both dietary and endogenous (that is, excreted in bile)—by about half, from the normal proportion of about half the total cholesterol to one quarter. This reduced absorption lowers serum cholesterol despite the compensatory increase in cholesterol synthesis which occurs in the liver and other tissues.[6 11] Plant sterols are potentially atherogenic like cholesterol[10] but atherogenesis does not occur because so little of the plant sterols are absorbed (for example, about 5% of β-sitosterol, 15% of campesterol, and less than 1% of dietary stanols are absorbed).[16] The use of plant sterols as cholesterol lowering drugs has been limited: initially the market was small and later the greater efficacy of statins was evident. By the 1980s, however, it was recognised that as naturally occurring substances plant sterols and stanols could be added to foods. Because fats are needed to solubilise sterols, margarines are an ideal vehicle for them, although cream cheese, salad dressing, and yoghurt are also used. Esterification of the plant sterols and stanols with long chain fatty acids increases their lipid solubility and facilitates their incorporation into these foods. Benecol was the first fortified margarine, and stanols were added because the evidence suggested that they had greater potential to lower cholesterol than sterols and the amount absorbed from the gut is negligible.[16 18 20 21]

Benefits of plant sterols and stanols

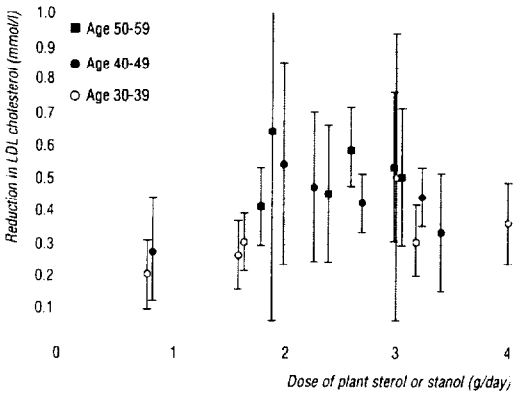
The table summarises the results of randomised double blind trials in adults that compared the ability of polyunsaturated margarines with and without added plant sterols to lower cholesterol. The effect of selection for comparatively high concentrations of serum cholesterol in some trials was modest and, with the exception of one small trial,[3] mean serum concentrations of low density lipoprotein (LDL) cholesterol in the control groups ranged from 3.0 mmol/l to 4.5 mmol/l (median 3.8 mmol/l), close to the age-specific mean found in the Western world. The randomised comparisons in three trials suggested that there was little difference in the extent to which sterols or stanols lower cholesterol concentrations (although the confidence intervals are consistent with the evidence above that stanols are better).[12 14] The table shows the reduction in LDL cholesterol in each trial; the reductions in total cholesterol concentrations were similar and there was little change in serum concentrations of high density lipoprotein cholesterol or triglyceride.

The figure shows the reduction in concentration of LDL cholesterol achieved in each trial and the dose of plant sterol or stanol. The reduction in the concentration of LDL cholesterol at each dose is significantly greater in older people than in younger people. The dose-response relation is continuous up to a dose of about 2 g of plant sterol or stanol per day. At higher doses no further reduction in LDL cholesterol is apparent, confirming the evidence of a plateau identified by earlier non-randomised studies.[17] At doses of ≥2 g per day the average reduction in serum LDL cholesterol was 0.54 mmol/l (14%; 95% confidence

Randomised double blind trials that reported the difference in serum cholesterol obtained from using polyunsaturated margarines with and without added plant sterols or stanols. Trials were parallel group trials unless indicated otherwise

Trial (place)	No of participants in treatment group/placebo group	Mean age (years)	Duration of trial (weeks)	Type	Average daily dose (g)	Placebo adjusted reduction in serum low density lipoprotein cholesterol (mmol/l)
Westrate et al[1] (Netherlands)	80*	45	3.5	Stanol	2.7	0.42 (0.33 to 0.51)
				Sterol	3.2	0.44 (0.35 to 0.53)
Hendriks et al[2] (Netherlands)	80*	37	3.5	Sterol	0.8	0.20 (0.10 to 0.31)
				Sterol	1.6	0.26 (0.15 to 0.36)
				Sterol	3.2	0.30 (0.20 to 0.41)
Miettinen et al[3] (Helsinki)	51/51	50	52	Stanol	1.8	0.41 (0.29 to 0.53)
	51/51			Stanol	2.6	0.59 (0.47 to 0.71)
Hallikainen et al[4] (Finland)	38/17	43	8	Stanol	2.3	0.47 (0.24 to 0.70)
Vanhanen et al[5] (Helsinki)	34/33	46	6	Stanol	3.4†	0.33 (0.15 to 0.51)
Gylling et al[6] (Helsinki)	22*	51	7	Stanol	3.0	0.53 (0.30 to 0.76)
Jones et al[7] (Montreal)	22*	35	1.4	Sterol	1.7‡	0.30 (0.21 to 0.39)
Gylling et al[8] (Helsinki)	21*	53	5	Stanol	2.4§	0.45 (0.24 to 0.66)
Jones et al[9] (Montreal)	16/16	about 50	4	Stanol	1.9	0.64 (0.06 to 1.22)
Niinikoski et al[10] (Finland)	12/12	37	5	Stanol	3.0	0.50 (0.06 to 0.94)
Gylling et al[11] (Helsinki)	11*	58	6	Stanol	3.0	0.50 (0.29 to 0.71)
Miettinen et al[12] (Helsinki)	9/8	45	9	Stanol	1.0†	0.28 (0.01 to 0.55)¶
	7/8			Sterol	0.8†	0.26 (−0.05 to 0.57)¶
Vanhanen et al[13] (Helsinki)	7/8	47	6	Stanol	0.8†	0.28 (0.0 to 0.56)¶
	7/8			Stanol	2.0†	0.54 (0.23 to 0.85)
Plat et al[14] (Netherlands)	70/42	33	8	Stanol	4.0	0.36 (0.23 to 0.49)

*Crossover trial. †In mayonnaise. ‡In olive oil. §In butter. ¶Data from these small trials which tested low doses are combined in the figure.



Results of randomised double blind trials of margarines with and without added plant sterols or stanols showing the reduction in serum concentrations of low density lipoprotein (LDL) cholesterol (95% confidence intervals) plotted against the daily dose. The data are from the table

interval 0.46 to 0.63 mmol/l) for participants aged 50-59,[6 8 11] 0.43 mmol/l (9%; 0.37 to 0.47 mmol/l) in participants aged 40-49,[1 5 13] and 0.33 mmol/l (11%; 0.25 to 0.40 mmol/l) for those aged 30-39[1 2 5 10]; this trend was statistically significant (P = 0.005). At a dose of 2 g per day (the amount added to an average daily portion of fortified margarine) the reduction in LDL cholesterol is likely to be at least 0.5 mmol/l for those aged 50-59 and 0.4 mmol/l for those aged 40-49.

Data from observational studies and randomised trials indicate that in people aged 50-59 the reduction in LDL cholesterol of about 0.5 mmol/l would reduce the risk of heart disease by about 25% after about two years.[22] In younger people the proportionate reduction in risk would be similar (the reduction in cholesterol concentrations is smaller but the association between cholesterol and heart disease is stronger).[22] Trials of six different interventions to lower serum cholesterol have all found a reduction in the incidence of heart disease (these interventions include four pharmacologically unrelated drugs, a reduction in dietary saturated fat, and ileal bypass surgery).[22 23] Nothing except a reduction in cholesterol is common to the six interventions, and for each intervention the proportionate reduction in mortality from heart disease is commensurate with the reduction in cholesterol concentration.[17 18] Margarines with plant sterols or stanols thus reduce the risk of heart disease by one quarter: this is the reduction expected from the decrease in serum cholesterol.

This is an impressive result for a dietary change that, price apart, is modest. It is larger than the effect that could be expected to occur if people ate less animal fat, and it is all the more impressive in light of the fact that despite the extensive promotion of healthy eating there has been little reduction in average serum cholesterol concentrations in many countries. Recent surveys in England found that mean cholesterol concentrations are only 1-2% lower than those of 25 years ago.[24 25] For a person replacing butter with a plant sterol margarine the reduction in cholesterol would be even greater. Replacing butter with ordinary polyunsaturated margarines lowers total serum and LDL cholesterol by about 0.3 mmol/l,[26 27] so the overall reduction would be about 0.7 mmol/l, or as much as any cholesterol lowering drug except statins.

Efficacy in combination with low fat diets

One non-randomised study found only a small average reduction in LDL cholesterol concentrations (0.16 mmol/l) despite participants taking 3 g of plant stanols daily.[28] The participants were on a low fat and low cholesterol diet, and the result was interpreted as suggesting that plant sterols are ineffective when dietary fat, dietary cholesterol, or LDL cholesterol concentrations are low. This is unlikely. In two recent randomised trials of stanol margarines in which participants were on low fat, low cholesterol diets, the reductions in serum concentrations of LDL cholesterol were similar to those found in other trials in which the intake of dietary fat was higher.[1 9] Plant stanols were equally effective in patients taking statins who had mean LDL cholesterol concentrations of only 2.9 mmol/l.[6] Other explanations for the discrepancy are more plausible: chance (at the upper confidence interval of the result, an LDL cholesterol reduction of 0.43 mmol/l is what might be expected) or the fact that the stanol was administered in capsules and not esterified and blended into the fat of a meal. (Sterols administered in capsules may not disperse fully or dissolve in the gut, limiting their ability to reduce the absorption of cholesterol.[2])

Safety

The most important concern about plant sterols is that they reduce the absorption of some fat soluble vitamins. Randomised trials have shown that plant sterols and stanols lower blood concentrations of β carotene by about 25%, concentrations of α carotene by 10%, and concentrations of vitamin E by 8%.[1 2 8 29] Since these vitamins protect LDL cholesterol from oxidation, and sterols and stanols reduce the amount of LDL cholesterol, the changes in blood concentrations of the vitamins were adjusted in the trials for the lower concentrations of LDL cholesterol. With this adjustment concentrations of vitamin E were not lower but concentrations of β carotene were reduced by between 8% and 19%.[1 2 8 29] There was no benefit to increasing the blood concentrations of β carotene and vitamin E by greater proportions than these,[30 31] although we do not know whether this is the case for other carotenes. Eating more fruit and vegetables would counter the decrease in absorption. The blood concentration of vitamin D is unaffected.[2] No other side effects or biochemical anomalies were evident in the randomised trials of plant sterol or stanol margarines (one of which lasted a year[3]), in earlier studies testing doses as high as 3 g/day for three years, or in animal studies testing proportionately higher doses.[16 17 32] Stanol margarines have been sold in Finland for three years without evidence of hazard, and a tenth of the amount of plant sterols found in these margarines occurs naturally in a normal diet. Plant sterols or stanols do not adversely affect the taste or consistency of margarines.[3 10]

The place of sterol and stanol margarines in the diet

The excess cost per person of margarines containing added plant sterols or stanols is about 20p per day or £70 per year. Affluent people may willingly pay this to reduce their risk of death from heart disease by a quarter but poorer people, who are at higher risk of heart disease, will tend to be dissuaded from buying the product. The cost reflects the large amount of raw material needed (about 2500 parts to extract one part sterol). Moreover, supplies are limited. The present sources—extracted from a byproduct in the refining of vegetable oils or from the oil obtained from pinewood pulp in papermaking—can supply only about 10% of the people in the West. In the foreseeable future, the product will be used only by a minority of people. However, in many countries there is also a legal obstacle: no health claim can be made in the advertising of these margarines because they are a food, not a drug. More people might buy the product if they were aware of the size of the health benefit.

Plant sterol and stanol margarines may appeal to patients with ischaemic heart disease but they should not replace statins because the reduction in the concentration of LDL cholesterol is greater with statins. Both could be taken together however, since the cholesterol lowering effects of the two are additive.[8] The overall costs of the two are equivalent: statins cost about three times as much as plant sterol margarines but they lower serum cholesterol by three times as much.

In the longer term, the addition of plant sterols and stanols to foods could be an important public health policy if new technology and economies of scale can lower the cost and enable a greater demand to be met. The serum cholesterol of the average older adult in Western countries is high (6.0 to 6.5 mmol/l),[34] with a correspondingly high lifetime risk of death from heart disease (about 25%). Introducing plant sterols into the food chain would lower the average serum cholesterol concentration in Western countries, with the added advantage of "demedicalising" the reduction (that is, one would not have to become a patient to benefit). There is a precedent for such fortification: in the United States folic acid has been added to flour since 1997. In addition to the expected reduction in the incidence of neural tube defects, there has also been a significant reduction in the average serum concentration of homocysteine,[33] which is likely to reduce mortality from heart disease.

The launch of margarines containing plant sterols and stanols is a welcome first step in what may become an important innovation in the primary prevention of ischaemic heart disease. It is to be hoped that in the longer term plant sterols and stanols will become cheap and plentiful and so will be able to be added to foods eaten by the majority of the population.

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