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Effect of plant sterols in combination with other cholesterol-lowering foods

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Abstract

The National Cholesterol Education Program Adult Treatment Panel III guidelines advocate effective combinations of cholesterol-lowering dietary components. This approach (dietary portfolio) produces large reductions in serum cholesterol, but the contribution of individual components remains to be established. We therefore assessed the effect of eliminating one out of the 4 dietary portfolio components. Plant sterols were selected because at 2 g/d, they have been reported to reduce low-density lipoprotein cholesterol (LDL-C) by 9% to 14%. Forty-two hyperlipidemic subjects were prescribed diets high in soy protein (22.5 g/1000 kcal), viscous fibers (10 g/1000 kcal), and almonds (23 g/1000 kcal) for 80 weeks. Subjects were instructed to take these together with plant sterols (1.0 g/1000 kcal) except between weeks 52 and 62. While taking the full dietary portfolio, including plant sterols, mean LDL-C reduction from baseline was 15.4% ± 1.6% ($P < .001$). After sterol elimination, mean LDL-C reduction was 9.0% ± 1.5% ($P < .001$). Comparable LDL-C reductions were also seen for the 18 subjects with a complete data set: on plant sterols, 16.7% ± 3.1% ($P < .001$) and off plant sterols, 10.3% ± 2.6% ($P < .001$), resulting in a 6.3% ± 2.0% ($P = .005$) difference attributable to plant sterols. Compliance in this group of 18 was 67.0% ± 5.9% for plant sterols and 61.9% ± 4.8% for the other components. In combination with other cholesterol-lowering foods and against the background of a low–saturated fat diet, plant sterols contributed over one third of the LDL-C reduction seen with the dietary portfolio after 1 year of following dietary advice.

1. Introduction

The National Cholesterol Education Program Adult Treatment Panel III (ATP III) guidelines have emphasized the principle of maximal therapy for the management of low-density lipoprotein cholesterol (LDL-C) through adding viscous fibers and plant sterols to the original advice on reduction of saturated fat, dietary cholesterol, and body weight as the dietary prescription [1]. With these dietary changes, it has been estimated that 25% to 35% reductions in LDL-C can be achieved [2,3]. The importance of maximizing cholesterol reduction has been further stressed in the ATP III update that, based on the success of statin trials in reducing coronary heart disease (CHD), advocates treating to a new optional target for LDL-C of less than 100 mg/dL (1.8 mmol/L) in high-risk subjects [4]. The United States

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Food and Drug Administration has also encouraged the development of cholesterol-lowering functional foods by allowing CHD risk reduction health claims for products containing plant sterols, viscous fibers, soy protein, and nuts [5-9]. Diets containing these elements have confirmed predicted lipid-lowering effects of 28% to 35% in LDL-C when they are combined in the same diet in what has been termed a *dietary portfolio* [10-13].

However, it is not clear what cholesterol reduction each functional food component contributes to the overall cholesterol reduction observed and whether all ingredients have to be present.

We have therefore taken the opportunity to remove one element, plant sterols, from the diets of individuals who have been taking the combination diet (dietary portfolio) for 1 year to determine its effects on the previously observed reduction in serum cholesterol. Plant sterols were selected because they represent the component with possibly the largest effect, with 2 g resulting in 9% to 14% reduction in LDL-C [14], and for which more data are available than the other components.

2. Methods

2.1. Participants

Fifty-five subjects completed a 1-year ad libitum dietary portfolio study and were asked to forgo consumption of plant sterol–enriched margarine for 10 weeks, after which they were asked to continue for a further 18 weeks with plant sterol–enriched margarine reinstated in their diets. Forty-nine subjects were enrolled in the present study. Forty-two

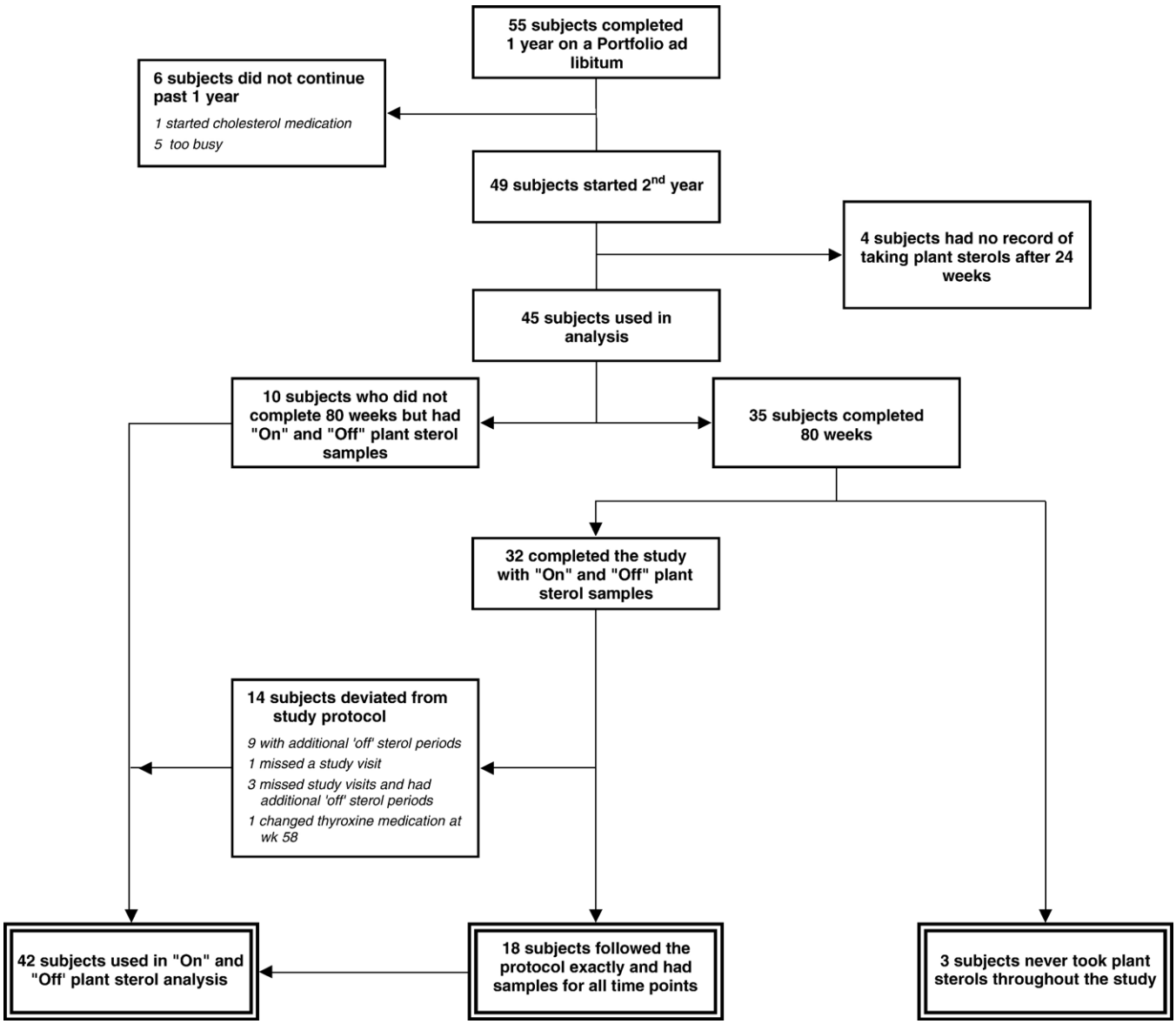

Fig. 1. Flow diagram showing progress of participants through the trial.

provided data on and off plant sterols, of whom 18 followed the protocol with no deviation in terms of complete attendance and diet records. Data on 3 subjects who had taken the dietary portfolio without plant sterols for the length of the study are shown separately. Data on 4 subjects who had taken plant sterols inconsistently as part of overall poor compliance and had no on–plant sterol data during the 32- to 80-week period of the study were excluded (Fig. 1). The 42 subjects, 19 men and 23 postmenopausal women, with on– and off–plant sterol data who were included in the analysis had a mean (±SE) age of 59.7 ± 1.5 years (range, 32-86 years), body mass index (BMI) of 27.3 ± 0.6 kg/m^2 (range, 19.1-36.8 kg/m^2), and LDL-C of 4.48 ± 0.11 mmol/L (range, 3.25-5.60 mmol/L). The 3 individuals who consumed no plant sterol margarine from baseline had a mean (±SE) age of 66.3 ± 0.9 years (range, 65-68 years), BMI of 26.5 ± 0.5 kg/m^2 (range, 25.6-27.3 kg/m^2), and LDL-C of 4.75 ± 0.31 mmol/L (range, 4.19-5.24 mmol/L) (Table 1). One-year data on the 42 subjects have been published [13]. All participants had previously raised LDL-C levels (>4.1 mmol/L) [1]. No participants had a history of cardiovascular disease, diabetes, or renal or liver disease. None were taking medications known to influence serum lipids apart from 5 women who were on stable doses of thyroxine and had normal thyroid-stimulating hormone levels and also one woman who was taking a stable dose of estrogen replacement therapy. One woman had decreased her thyroxine levels during the study, from 0.088 to 0.075 mg/d, 3 weeks before the week-58 sample; and therefore, only her last 3 values on a stable dose were used. Fifteen participants had been prescribed cholesterol-lowering medications as part of their usual care but had discontinued them at least 2 weeks before the original start of the dietary portfolio, 1 year before the current intervention. Six participants were taking antihypertensive medications at a constant dose during the study. Two participants had previously changed their blood pressure medications, and one started blood pressure medication before the study. During the study period, one subject failed to take her calcium channel blocker before the week-72 sample; and a further subject increased her dosage of angiotensin-converting enzyme inhibitor at week 80.

Table 1
Baseline characteristics of study subjects with (n = 42) and without (n = 3) plant sterol consumption

	Plant sterol–consumption group		Plant sterol–free group	
	Mean ± SE	Range	Mean ± SE	Range
Age (y)	59.7 ± 1.5	(32-86)	66.3 ± 0.9	(65-68)
Weight (kg)	74.1 ± 1.9	(48.8-97.7)	66.6 ± 2.1	(63.1-70.3)
BMI (kg/m^2)	27.3 ± 0.6	(19.1-36.8)	26.5 ± 0.5	(25.6-27.3)
TC	6.70 ± 0.12	(5.00-8.47)	6.63 ± 0.31	(6.09-7.16)
LDL-C (mmol/L)	4.48 ± 0.11	(3.25-5.60)	4.75 ± 0.31	(4.19-5.24)
HDL-C (mmol/L)	1.31 ± 0.06	(0.87-2.76)	1.21 ± 0.04	(1.16-1.29)
Triglycerides (mmol/L)	2.02 ± 0.12	(0.68-4.32)	1.48 ± 0.09	(1.35-1.64)
Glucose (mmol/L)	5.1 ± 0.1	(4.3-6.1)	5.5 ± 0.3	(5.1-6.0)
Systolic blood pressure (mm Hg)	121 ± 2	(98-161)	152 ± 10	(132-164)
Diastolic blood pressure (mm Hg)	73 ± 1	(59-96)	78 ± 6	(69-90)

To convert cholesterol and triglycerides to milligrams per deciliter, multiply by 38.67 and 88.57, respectively. TC indicates total cholesterol.

2.2. Study protocol

The intervention was a single-phase, 80-week, open-label study of a self-selected (ad libitum) dietary portfolio of cholesterol-lowering foods, with plant sterols in the form of an enriched margarine removed from the diet between weeks 52 and 62. All subjects had been instructed to follow a low–saturated fat (<7% of energy intake), low-cholesterol (<200 mg/d) diet for 2 months before commencing the long-term dietary portfolio study. During the study, participants were seen at weeks 0, 2, 4, 8, 12, 24, 32, 42, 52, 58, 62, 72, and 80. Only data for weeks 0 and 32 onward were used in this analysis, providing 20 and 18 weeks, respectively, before and after the 10 weeks of plant sterol elimination from the diet. At each visit, fasting body weights were checked and blood samples were obtained after 12-hour overnight fasts. Blood pressure was measured using a mercury sphygmomanometer by the same observer on 3 successive occasions in the nondominant arm while the subject was seated. Seven-day diet records were obtained for the week before the clinic visit and checked by the dietitian. The records were discussed with the dietitian, and suggestions were made to enhance compliance. The previous week's exercise was also recorded, and the dietitian encouraged the participant to hold this constant over the study period.

Previously published LDL-C data for weeks 0 and 32 to 52 are also presented here, for comparative purposes, from the first year in which subjects participated [13].

The Ethics Committees of the University of Toronto, St. Michael's Hospital, the Drug Directorate of Health Canada, and the Natural Health Products Directorate of Health Canada approved the study. Written informed consent was obtained from the participants.

2.3. Diets

Before the original study, participants ate their routine therapeutic low-fat diets with mean macronutrient profiles that were close to current ATP III guidelines (≤7% energy from saturated fat and <200 mg/d dietary cholesterol) [1] (Table 2). Between weeks 52 to 62, participants were asked to stop their plant sterol–enriched margarine and take a similar margarine without added plant sterols. They were asked to change no other aspect of their diet. After week 62, they were asked to resume consumption of their plant sterol–enriched margarine to week 80 (Fig. 2).

The dietary advice for the 80-week study was based on the consumption goals for the same 4 dietary components that had been emphasized in previous metabolic dietary

Table 2
Absolute mean (±SE) body weight, lipid, and blood pressure measurements across weeks 32 to 80 while on and off plant sterols during ad libitum portfolio (n = 42)

	Body weight (kg)	TC (mmol/L)	LDL-C (mmol/L)	HDL-C (mmol/L)	Triglyceride (mmol/L)	TC/HDL-C	LDL-C/HDL-C	SBP[a] (mm Hg)	DBP[a] (mm Hg)
Baseline	74.1 (1.9)	6.70 (0.12)	4.48 (0.11)	1.31 (0.06)	2.02 (0.12)	5.44 (0.19)	3.66 (0.15)	119.8 (2.3)	73.7 (1.2)
On plant sterols[b]	73.9 (1.9)	5.97 (0.10)	3.76 (0.08)	1.36 (0.07)	1.87 (0.12)	4.71 (0.17)	2.99 (0.13)	116.2 (1.7)	71.9 (1.0)
Off plant sterols[c]	73.4 (1.9)	6.25 (0.13)	4.07 (0.12)	1.35 (0.06)	1.83 (0.11)	4.96 (0.19)	3.26 (0.15)	114.6 (1.6)	71.5 (1.0)
Treatment difference	−0.5 (0.2)	0.28 (0.06)	0.31 (0.06)	−0.02 (0.01)	−0.04 (0.06)	0.24 (0.07)	0.27 (0.06)	−1.7 (0.9)	−0.6 (0.6)
P	.034	<.001	<.001	.268	.509	.001	<.001	.073	.347

[a] n = 38 subjects.
[b] Twenty-three subjects had less than the planned 5 samples on plant sterol margarine over the study period.
[c] Seventeen subjects had additional blood samples (ie, in addition to weeks 58 and 62) obtained while off plant sterol margarine; 4 subjects had only one sample obtained while off plant sterol margarine.

portfolio studies [10-12]. Foods were bought by subjects from supermarkets and health food stores with the exception of the bread, which was obtained at cost from a baker, and the margarine, which was unobtainable in Canada and provided to all but 3 subjects who did not take plant sterol supplements during the study. Subjects were instructed on how to achieve the 4 primary aims of the dietary portfolio of 1.0 g plant sterols per 1000 kcal of diet in a plant sterol ester–enriched margarine; approximately 10 g viscous fibers per 1000 kcal of diet from oats, barley, psyllium, and the vegetables okra and eggplant; 22.5 g soy protein per 1000 kcal as soy milk, tofu, and soy meat analogues; and 23 g whole almonds per 1000 kcal of diet in addition to their ongoing low-fat diet. All of these 4 diet components have been recognized to lower serum cholesterol [5-9]. Participants were also instructed to consume additional sources of plant protein and fiber in the form of dried legumes and to take the recommended 5 to 10 daily servings of fruit and vegetables. To the extent acceptable to participants, advice was given to take a vegetarian diet without the use of dairy foods, eggs, or meats.

Self-taring electronic scales (Salter Housewares, Kent, England) were provided to all participants. They were asked to weigh and record all food items consumed in the week before clinic visits.

Compliance with the self-selected dietary portfolio was assessed from the completed 7-day food records. These diet records were reviewed by the dietitian at each visit. Consumption of the 4 primary components of the diet (soy protein foods, viscous fibers, almonds, and plant sterol–enriched margarine) was estimated from the food record and expressed as a percentage of amount recommended for that individual's energy requirement.

2.4. Analyses

Serum was analyzed according to the Lipid Research Clinics protocol [15] for total cholesterol, triglyceride, and high-density lipoprotein cholesterol (HDL-C) after dextran sulphate–magnesium chloride precipitation [16]. The LDL-C was calculated by the method of Friedewald et al [17] (LDL-C = total cholesterol − [triglyceride/2.2 + HDL-C]).

Diets were analyzed for macronutrients, fatty acids, cholesterol, and fiber using a computer program based on the United States Department of Agriculture data [18] (Table 3).

2.5. Statistical analysis

The results are expressed as means ± SE. Participants were instructed to take no plant sterols for a 10-week

Table 3
Nutritional profiles (mean ± SEM) of ad libitum portfolio while on and off plant sterols from weeks 32 to 80 (n = 40)

	Baseline (wk 0)	On plant sterols	Off plant sterols	Treatment difference	*P*
Energy (kcal/d)	1602.8 ± 79.6	1698.4 ± 72.7	1687.7 ± 76.1	−10.7 ± 36.0	.767
Total protein (% cal)	19.0 ± 0.6	19.6 ± 0.5	19.9 ± 0.5	0.2 ± 0.5	.652
Vegetable protein (% cal)	7.6 ± 0.3	14.2 ± 0.6	14.0 ± 0.6	−0.2 ± 0.3	.563
Animal protein (% cal)	11.3 ± 0.6	5.5 ± 0.6	5.9 ± 0.5	0.4 ± 0.5	.408
Available carbohydrates (% cal)	55.2 ± 1.1	50.2 ± 0.8	50.0 ± 0.7	−0.2 ± 0.5	.777
Total dietary fiber (g/1000 kcal)	17.8 ± 1.1	25.9 ± 1.0	24.4 ± 1.0	−1.4 ± 0.6	.026
Total fat (% cal)	24.4 ± 1.1	28.9 ± 0.6	29.1 ± 0.7	0.2 ± 0.6	.775
SFA (% cal)	6.9 ± 0.4	5.7 ± 0.2	5.9 ± 0.3	0.1 ± 0.2	.561
MUFA (% cal)	9.8 ± 0.5	12.2 ± 0.4	12.8 ± 0.4	0.6 ± 0.3	.102
PUFA (% cal)	5.3 ± 0.4	9.1 ± 0.3	8.4 ± 0.3	−0.8 ± 0.2	.001
Dietary cholesterol (mg/1000 kcal)	91.7 ± 7.8	46.5 ± 5.7	57.3 ± 6.6	10.8 ± 3.3	.002
Alcohol (% cal)	1.4 ± 0.4	1.2 ± 0.3	1.0 ± 0.3	−0.1 ± 0.2	.417

SFA indicates saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Table 4
Proportionate intake (mean ± SEM) of the 4 key components of the dietary portfolio while on and off plant sterols from weeks 32 to 80 (n = 18)

	Soy intake out of 25[a] (22.5 g/1000 kcal)	Viscous fiber intake out of 25[a] (10 g/1000 kcal)	Almond intake out of 25[a] (23 g/1000 kcal)	Plant sterol intake out of 25[a] (1 g/1000 kcal)	Total portfolio intake out of 100
Pre (on plant sterols)	15.7 ± 1.6	13.9 ± 1.6	17.4 ± 1.6	17.1 ± 1.6	64.2% ± 5.1%
Off plant sterols	15.7 ± 1.7	13.0 ± 1.4	16.5 ± 2.1	0.0 ± 0.0	45.2% ± 4.2%
Post (on plant sterols)	15.4 ± 1.5	13.1 ± 1.3	16.9 ± 1.3	16.2 ± 1.4	61.6% ± 4.8%

[a] Prescribed intake of the 4 dietary portfolio components was 22.5 g/1000 kcal for soy, 10 g/1000 kcal for viscous fiber, 23 g/1000 kcal for almonds, and 1 g/1000 kcal for plant sterols. The full intake of each of the 4 components was expressed as 25%, adding up to the total dietary portfolio intake of 100%.

period between weeks 52 and 62. This period represented the dietary portfolio without plant sterols. Data from weeks 32 to 52 and 62 to 80 were used as the full dietary portfolio that included plant sterols. Data are given as absolute changes and as percentage changes from baseline. The effect of plant sterol removal from the diet was assessed as the mean percentage reduction in measurements while on the portfolio diet without plant sterols (weeks 58 and 62) minus the mean percentage reduction while on the portfolio diet that included plant sterols (weeks 32-52 and weeks 72 and 80). Values were derived from the 42 subjects of whom both complete and incomplete data were available and from the subgroup of 18 of whom complete data for all time points were available. Among the 24 subjects with incomplete data, there were 17 subjects who had not taken plant sterols for 1 or more months during the full diet period (weeks 32-52 and 62-80). Data at the end of these periods were included with the off-sterol data for weeks 52 to 62. Significance was calculated using Student *t* test (2-sided) for paired data.

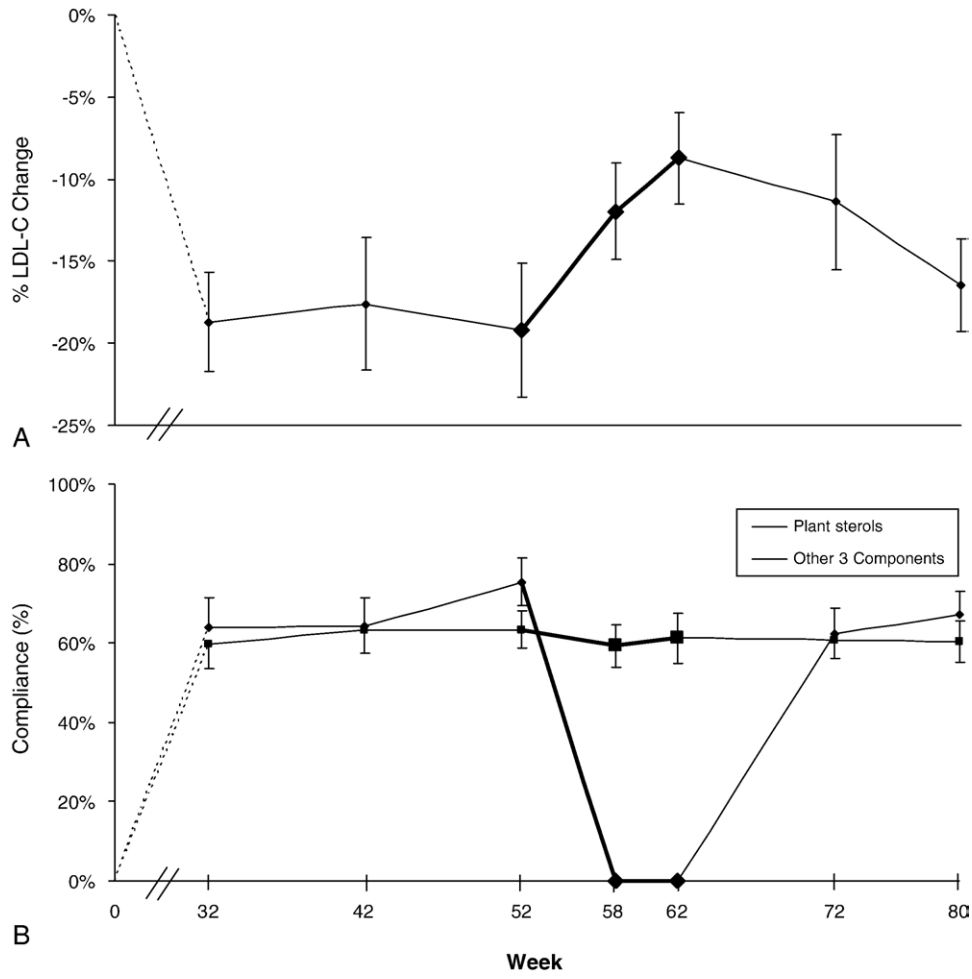

Fig. 2. Mean LDL-C change from baseline (A) and mean dietary portfolio compliance of each component (B) during the 32- to 80-week study period for the group of 18 subjects. Plant sterol margarine was consumed by subjects as part of a full dietary portfolio until week 52, at which time they were asked to not consume plant sterol margarine for the following 10-week period (darkened segment). At week 62, subjects were advised to resume adding plant sterol margarine to their diets.

Linear associations between mean LDL-C reduction and mean compliance measures were tested using Pearson correlation. Compliance was assessed for the 4 portfolio components (soy protein, plant sterols, viscous fibers, and almonds), where the prescribed amount for each component represented 25% of the total (100%). All data were analyzed using SAS version 8.2 (SAS, Cary, NC) [19].

3. Results

For the 42 subjects, compliance for plant sterol–enriched margarine was 65.8% ± 4.3%, whereas for the non–plant sterol components (ie, viscous fiber, soy protein, and almonds), the combined compliance was 52.0% ± 3.7%. No significant change in the compliance of the non–plant sterol components was seen when plant sterol margarine was removed from the diet (combined compliance after plant sterol elimination was 49.9% ± 4.0%, $P = .281$). In the 18 subjects who adhered most completely to the study protocol, the sterol and non–plant sterol compliance was 67.0% ± 5.9% and 61.9% ± 4.8%, respectively (Table 4). Mean body weight was slightly but significantly higher on plant sterol–enriched margarine than when off the margarine (73.9 ± 1.9 kg vs 73.4 ± 1.9 kg, n = 42, $P = .049$).

3.1. Blood lipids

In the whole group of 42 subjects, the mean LDL-C reduction while taking the full dietary portfolio with plant sterols during weeks 32 to 80 was 15.4% ± 1.6% ($P < .001$). After elimination of the plant sterol–enriched margarine, the LDL-C reduction was reduced to 9.0% ± 1.5% ($P < .001$) (Fig. 2). For the 18 subjects with complete data points, the comparable LDL-C reduction when taking the full dietary portfolio including the plant sterol–enriched margarine was 16.7% ± 3.1% ($P < .001$); and after elimination, the LDL-C reduction diminished to 10.3% ± 2.6% ($P < .001$), resulting in an LDL-C reduction attributable to plant sterols of 6.3% ± 2.0% (n = 18, $P = .005$) (Fig. 3).

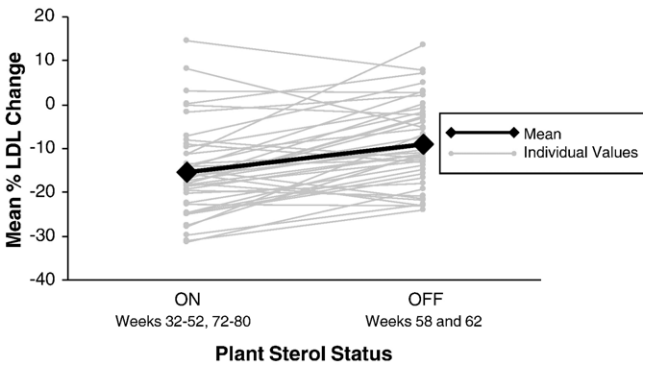

Fig. 3. Comparison of individual lipid changes from baseline while on plant sterols vs off plant sterols for 42 subjects. Lipid changes for each subject are shown in gray (●), whereas mean group lipid change is shown in black (◆) ($P < .001$).

Elimination of plant sterols had no effect on triglyceride or HDL-C, where the reduction of 5.3% ± 4.1% and increase of 3.5% ± 1.8%, respectively, while on the complete dietary portfolio were maintained (Table 2).

In the 3 participants who took no plant sterol margarine by intention during the entire duration of the study, a mean 32- to 80-week LDL-C reduction of 15.8% ± 7.9% was achieved, with a mean compliance of 77.4% ± 14.7%.

3.2. Intake of portfolio components and lipid changes

The elimination of plant sterols and the resulting reduction in the cholesterol-lowering capacity of the dietary portfolio were reflected in a significant negative correlation between the difference in total dietary portfolio intake and difference in total cholesterol as well as LDL-C in the on– minus off–plant sterol phases ($r = -0.36$, n = 40, $P = .024$ and $r = -0.37$, n = 40, $P = .021$, respectively). In addition, the change in intake of the nonsterol cholesterol-lowering components (ie, soy protein, almonds, and viscous fiber), although small, also related significantly to the change in total cholesterol and LDL-C ($r = -0.38$, n = 40, $P = .017$ and $r = -0.35$, n = 40, $P = .028$, respectively), although the reductions with plant sterol intake per se were not significant ($r = -0.08$, n = 40, $P = .647$ and $r = -0.13$, n = 40, $P = .435$, respectively). No other associations between change in total dietary portfolio intake and blood lipid changes were significant.

4. Discussion

Plant sterol withdrawal from a cholesterol-lowering diet resulted in a reduction in the overall cholesterol-lowering effect of the diet of similar magnitude to that ascribed to plant sterols when administered as the sole cholesterol-lowering component. However, in compliant subjects, even when not consuming plant sterol–enriched margarine, substantial reductions in serum cholesterol can still be achieved through good adherence to the other components of the dietary portfolio.

Meta-analyses and many studies in subjects who are healthy, overweight, diabetic, or hyperlipidemic, both in the short and long term, have indicated the cholesterol-lowering potential of plant sterols [14,20-28]. They have also been found to provide additional improvements in blood lipids when taken in combination with statins [29]. It is also apparent that the dose-response curve of plant sterol intake demonstrates a physiologically significant lowering of LDL-C of 6% to 8% with as little as 1 g of plant sterols per day [14]. A near-maximum effect is seen at 2 to 2.5 g/d, with a 9% to 14% LDL-C reduction [14]. The effect of plant sterols was seen in subjects taking plant sterols for 1 year and confirms the data from previous long-term trials where the plant sterol–induced LDL-C reductions were maintained over 1 year of observation [20,23].

There has been concern that plant sterols may not be as effective when the saturated fat and cholesterol contents of

the diet are low [25]. It is possible that had our diet been higher in saturated fat and cholesterol, larger reductions might have been observed. However, the saturated fat intakes were less than 7% of calories in our study both before the start and during the diet. Nevertheless, other studies have also demonstrated plant sterols to be effective in the context of a low-fat Step 1 diet [30,31].

Viscous fiber, soy protein, plant sterols, and almonds are all well recognized for their cholesterol-lowering properties [5-9,14,20-24,28,32-40]. Their mechanisms of action differ and are complementary such that their cholesterol-lowering effects are likely to be additive. Viscous fibers increase bile acid losses in the feces [41,42]; plant sterols block endogenous and exogenous cholesterol absorption and increase fecal losses [21,22,43]. Vegetable proteins in general and soy in particular appear to reduce hepatic cholesterol synthesis, with specific proteins (7S globulin) [44] inhibiting apolipoprotein B synthesis. The potential bioactive components of almonds, which lower serum cholesterol, may be several [37-40], relating to their plant protein, monounsaturated fat, and plant sterol contents [14,21,38,45].

Genetic differences, especially regarding adenosine triphosphate–binding cassette half transporter G5/8 polymorphism, may make a difference in the plant sterol cholesterol-lowering response of individuals [46]. However, in the present study, compliance is likely to be a major factor determining the effect of the diet. Many of the participants in this study had also taken part in earlier dietary portfolio studies in which great differences between subjects were observed in the ad libitum study, but the cholesterol-lowering effect was uniformly large when all foods were provided to the subjects under metabolically controlled conditions [10-12].

Some concerns have been raised that plant sterols as opposed to stanols, their less absorbed hydrogenated form, may increase the risk of CHD [47] because in the rare condition of homozygous sitosterolemia or phytosterolemia early arteriosclerosis is seen. However, there is no evidence that those heterozygous for sitosterolemia are affected in this way; and their sterol absorption is not notably increased [48]. Furthermore, there is evidence that cardiovascular disease determined by coronary artery calcification was not more marked in those with modestly elevated serum plant sterol levels [49]. The lack of effect of modest elevation of plant sterols on CHD risk has also been confirmed in the European Prospective Investigation into Cancer and Nutrition and Longitudinal Aging Study Amsterdam (LASA) studies [50,51]. Likewise, concern has been raised that replacement by plant sterols of cholesterol in membranes may weaken the membranes [52]. Although this may occur in animal models with very low serum cholesterol levels, as evidenced by increased red cell fragility, there is no evidence in humans that plant sterol consumption makes a physiologically significant difference to red cell fragility [53].

There was a lack of relation between compliance with plant sterols and LDL-C reduction (Table 5). It is possible that the dose of plant sterols taken by many participants was already on the relatively flat part of the dose-response curve and that with the relatively small number of subjects, there was insufficient contrast to see a significant association with sterol consumption and LDL-C reduction.

This explanation is supported by a meta-analysis of the reduction on LDL-C related to the dose of plant sterol intake. The data indicated that the dose-response curve was relatively flat from 0.75 to 1.5 g/d [14]. With 2 g/d of plant sterols as the goal for a 2000-kcal/d diet and 65.8% compliance, most of the subjects will be on the flat part of the dose-response curve, reducing the likelihood of seeing a dose response to plant sterols with our data. Using the meta-analysis data, an approximately 0.25-mmol/L reduction in LDL-C or 6% would be expected from the 1.3 g/d of plant sterols as consumed in our study [14]. This reduction corresponds to the 6.3% reduction that we observed.

If it is assumed that the participants' compliance with the portfolio components was 100%, including the plant sterols at the prescribed dose of 2 g/d rather than the approximately 1.3 g/d consumed, then the plant sterol effect in the 18 subjects with complete data points would be equivalent to a 9.7% reduction in LDL-C rather than the 6.3% observed. The overall LDL-C reduction was 16.7% for a total dietary compliance of 63%. Again, assuming 100% compliance, the LDL-C reduction would be 26.4% for the total diet effect. Although the association with plant sterol compliance and LDL-C reduction was not significant in our study, the compliance with the nonsterol components was significantly related to LDL-C reduction.

There are a number of weaknesses with our study. It is possible that removal of plant sterol margarine from the overall diet negatively influenced compliance with other components of the diet, in which case, the resulting diminished cholesterol reduction may have been due, in part, to elimination of nonsterol cholesterol-lowering components in addition to the plant sterol margarine. However, in this respect, no change was recorded in the percentage compliance with the other portfolio components during the time when the plant sterol margarine was eliminated. Interpretation of the data cannot be made with

Table 5
Correlation between mean LDL-C reduction and mean dietary adherence to the dietary portfolio while on plant sterols and while off plant sterols during weeks 32 to 80 of study period (n = 40)

	On plant sterols		Off plant sterols	
	r	*P*	*r*	*P*
Total dietary portfolio	−0.39	.013	−0.34	.032
Plant sterol	−0.14	.392	NA	NA
Soy protein	−0.40	.011	−0.39	.014
Viscous fiber	−0.27	.098	−0.24	.133
Almonds	−0.29	.067	−0.21	.191

NA indicates not applicable.

the same degree of confidence that would be possible with a placebo-controlled, double-blind crossover trial. The 3 subjects who never took plant sterols still had a mean LDL-C reduction of 15.8%, which was similar to the rest of the subjects when taking the full dietary portfolio with the plant sterol margarine. Nevertheless, the compliance to the nonsterol components was good at more than 77% in the 3 subjects who had never taken plant sterols. This figure is compared with a more modest compliance, in the low 60% range, for the per protocol group. With good compliance, it appears that good reductions in LDL-C are achievable even in the absence of plant sterol intake. In addition, a lack of a significant correlation was seen between plant sterol intake and LDL-C reduction, although there was a significant overall association with compliance to the portfolio components.

However, the study also has some strengths. This study is the first to attempt to assess the effect of withdrawing plant sterols from a very effective long-term cholesterol-lowering combination diet. The diet has been applied under the conditions of everyday life, and the results can be expected to reflect the results of plant sterol withdrawal from the dietary portfolio in this situation. Finally, the resulting loss of effectiveness of the dietary portfolio in lowering serum cholesterol after sterol withdrawal is as would be predicted from the results of studies where plant sterols were given as the sole lipid-lowering dietary agent.

We conclude that in the context of a low–saturated fat diet and in combination with other cholesterol-lowering dietary components, plant sterols appear to exert a very significant effect on LDL-C reduction of the order of 10% for 2 g/d of plant sterols. This figure is similar to studies where plant sterols have been given as the only cholesterol-lowering agent [14]. Furthermore, the effect appears to be maintained in the long term. Increased plant sterol intakes are likely to have been a part of the ancestral human diet at about 1 g/d [54] and are part of a more plant-based diet as currently recommended for CHD risk reduction, including green leafy vegetables, raw or dry roasted nuts, and nonhydrogenated vegetable oils. Their reintroduction into the Western diet to prevent CHD may be seen as similar to the desire to reintroduce fiber into the diet to reduce the risk of a number of chronic diseases [55-57]. Plant sterols therefore appear a good fit with other cholesterol-lowering components in a dietary portfolio to reduce CHD risk.

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