

Plant sterol-enriched spread enhances the cholesterol-lowering potential of a fat-reduced diet

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Objective: To determine the effect of a plant sterol-enriched spread on plasma cholesterol concentrations when replacing butter or a standard polyunsaturated spread in a diet containing 30% of energy fat.

Design: Parallel butter phase followed by double-blind, randomized, cross-over polyunsaturated spread phases.

Setting: General community.

Subjects: Volunteer sample of 50 free-living men and women with mean age (s.d.) 46.7 y (10.5), moderately elevated plasma total cholesterol 5.95 mmol/l (0.78), and body mass index 26.0 (3.9) kg/m².

Intervention: Participants ate a moderately low-fat diet (30% of energy) for the 11-week intervention. During the first 3 weeks the diet included 20 g per day of butter. Participants were then randomized to replace the butter with 25 g of polyunsaturated spread with or without 2 g of plant sterols for 4 weeks, crossing over in the last 4 weeks to the alternate spread.

Main outcome measures: Plasma cholesterol and fatty acids.

Results: Replacing butter with a standard polyunsaturated fat spread reduced mean plasma total cholesterol concentrations by 4.6% (from 6.09 (0.82) to 5.81 (0.77) mmol/l, $P < 0.01$) and low-density lipoprotein cholesterol by 5.5% (from 3.98 (0.76) to 3.76 (0.74) mmol/l, $P < 0.05$). Replacing butter with a polyunsaturated spread containing plant sterols reduced plasma total cholesterol by 8.9% (from 6.09 (0.82) to 5.55 (0.76) mmol/l, $P < 0.01$) and low density lipoprotein cholesterol by 12.3% (from 3.98 (0.76) to 3.49 (0.72) mmol/l, $P < 0.01$). Plasma high density lipoprotein cholesterol concentration was the same on the three diets.

Conclusion: In people with moderately raised plasma cholesterol concentrations consuming reduced-fat diets the reduction in plasma total and low-density lipoprotein cholesterol concentrations achieved by replacing butter with a polyunsaturated spread is enhanced by addition of plant sterols.

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Introduction

Strategies to lower plasma cholesterol concentrations are of great public health importance because raised concentrations are a major risk factor for coronary heart disease and

clinical interventions have shown that decreasing total and low density lipoprotein cholesterol significantly reduces coronary heart disease mortality. Individuals with very high concentrations of plasma cholesterol are commonly prescribed cholesterol-lowering medication, whereas those with mild-to-moderate elevations in concentration are advised, in the first instance, to make dietary changes that will help reduce plasma cholesterol (Anonymous, 1993; Ansell *et al*, 1999). Reducing saturated fat intake, particularly from dairy fat, is a key element of any dietary strategy to lower plasma cholesterol concentration (Grundey & Denke, 1990; Anonymous, 1993; Hegsted *et al*, 1993; Kris-Etherton & Yu, 1997), though the relative advantages of replacing it with polyunsaturated fat, monounsaturated fat or carbohydrate are still debated (Connor & Connor, 1997; Katan *et al*, 1997).

The advent of commercially available table spreads with plant sterols has the potential to offer the person with

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moderately raised plasma cholesterol concentrations an additional cholesterol-lowering strategy. Plant sterols reduce cholesterol absorption (Mattson *et al*, 1982) and the results of several randomized controlled trials show that ingesting 1–3 g/day of plant sterols lowers total and low-density lipoprotein cholesterol concentrations in participants with normal or slightly raised cholesterol concentrations (Moghadasian & Frohlich, 1999). The fat content of the diets used in these studies has been, in the vast majority of cases, considerably higher (>35% of energy) than would be recommended for individuals on cholesterol-lowering diets. Furthermore, in the few studies where low-fat diets (\leq 30% of energy) have been used the cholesterol-lowering effect of plants sterols has been variable (5.2–10.6%; Denke, 1995; Hallikainen & Uusitupa, 1999; Hallikainen *et al*, 2000; Maki *et al*, 2001; Nigon, 2001), with no significant change reported in one study (Denke, 1995). In the two studies (Hallikainen & Uusitupa, 1999; Hallikainen *et al*, 2000) that showed the largest decrease in serum cholesterol concentrations, participants adhered to diets that were highly prescribed rather than self-selected, as they would be outside a research setting. Studies with less controlled diets, modelling the advice people with mildly increased blood cholesterol concentrations would receive, will give more accurate estimates of the cholesterol-lowering effect of plant sterols in a community setting.

In New Zealand, the average serum total cholesterol concentration in adults 15 y or older is 5.8 mmol/l (Skeaff *et al*, 2001), thus a substantial proportion of the population has raised cholesterol concentrations and would benefit from a lipid-lowering diet. Because 50% of New Zealand adult consumers use butter, ingesting on average 18 g/day (Anonymous, 1999), replacing butter with a polyunsaturated table spread is a simple food substitution that would help to reduce serum cholesterol concentrations.

The purpose of the present study was to examine in participants with moderately raised plasma cholesterol concentrations who were following freely chosen low-fat diets, the effect of replacing butter with a vegetable oil-based table spread with or without plant sterols. Study diets were not prescribed; rather participants were given general advice—as they would be in a community setting—on how to reduce total and saturated fat intake by selecting wisely from foods they were accustomed to eating. An important feature of this study was that compliance with the fat composition of the diet was monitored by measuring the fatty acid composition of plasma triacylglycerol and cholesterol ester.

Methods

Participants were recruited through notices in the local (Dunedin, New Zealand) newspapers. Eligible participants were between the ages of 18 and 70 y, were not taking any cholesterol-lowering medication, were not being treated for any serious illness, and had a plasma cholesterol

concentration between 5.0 and 7.5 mmol/l and triacylglycerol concentration below 3.0 mmol/l at screening. Eighty-seven individuals were screened, 58 were enrolled and 53 completed the trial. The study was approved by the Otago University Human Ethics Committee and all volunteers gave informed written consent before taking part.

Participants were asked to follow a self-selected low-fat diet (30% of energy from total fat) throughout the study to which they were to add butter, a standard polyunsaturated spread or an identical polyunsaturated spread with plant sterols. Before the study began, participants met in small groups with a research dietitian and discussed ways to reduce their fat intake by choosing sensibly from foods the participants were accustomed to eating. They were given a booklet containing general advice and a number of common low fat recipes as well as the New Zealand National Heart Foundation food guide. For the first 3 week phase of the study, all participants were asked to incorporate 20 g/day of butter into their low-fat diet. The second and third phases of the study followed a randomized double-blind cross-over design. Half of the participants were asked to replace the butter with 25 g/day of plant sterol-containing spread (2.0 g plant sterols) for 4 weeks while the other half were asked to consume an equivalent amount of a spread of identical composition without plant sterols. The two groups then crossed over without washout to the alternate spread for the final 4 weeks of the study. It was emphasized to participants that the same low-fat diet was to be followed in all three dietary phases and that substitution of the spread was the only change required between phases. Participants were asked to use only the plant sterol spread supplied to them. Unintentional intake of added plant sterols from other food sources was not possible because no other manufactured food in New Zealand is enriched with plant sterols. Participants were given plastic spoons to measure out the correct amount of butter and polyunsaturated spreads required each day. Participants were asked to use soybean oil if they needed any additional fat during the study because the plant sterol spread, although appropriate for baking, was not suitable for high temperature cooking. Participants were given NZ\$5 per clinic visit for their time and to help defray transport and parking costs.

The two polyunsaturated spreads used in this study were virtually identical in composition (Table 1) except that the plant sterol spread had 8 g/100 g phytosterol esters derived from vegetable oils. The plant sterols were predominantly beta-sitosterol (80% of the total), campesterol and stigmasterol. The two commercially available polyunsaturated spreads (Flora and Flora Pro-activ) were manufactured by Unilever Australia, packaged in unmarked tubs, and supplied to the researchers in boxes marked A or B. After all blood samples had been analysed the manufacturers revealed which letter corresponded with the plant sterol spread.

Body weight was measured in each phase of the study. Participants were also encouraged to keep their lifestyle, such as level of physical activity, constant throughout the study.

Table 1 Energy and nutrient composition of spreads

	Butter ^a	Standard spread ^b	Plant sterol spread ^b
Energy (kJ/100 g)	3100	2605	2388
Total fat (g/100 g)	82	70	64
SAFA (%TF)	73	24	22
MUFA (%TF)	24	32	32
PUFA (%TF)	3	43	47
Cholesterol (mg/100 g)	201	0	0
Plant sterols (mg/100 g)	0	400	8000

Saturated fatty acids, SAFA; monounsaturated fatty acids, MUFA; polyunsaturated fatty acids, PUFA; percentage of total fat, %TF.

^aNew Zealand Food Composition Database.

^bAnalysis supplied by manufacturer.

Participants met with researchers at the beginning and the end of the run-in phase and fortnightly in the second and third dietary phases of the study. Compliance was encouraged through this regular contact. Compliance was measured using 4 day diet records and fatty acid composition of plasma triacylglycerol and cholesterol ester.

Four-day diet records were filled out by each participant in each of the three dietary phases of the study. Participants were given electronic scales to use and a colour picture booklet to help estimate food portion sizes. Participants were advised how to use these tools when they met with the dietician. Energy and nutrient composition of the diets were calculated based on the 1999 food composition data from the New Zealand Institute For Crop and Food Research Ltd.

Blood was taken on eight occasions; at screening, at baseline and then twice, on non-consecutive days, at the end of each of the three dietary phases. A registered nurse collected venipuncture fasting blood samples into tubes containing K₂EDTA. Blood was centrifuged at 3000 g for 15 min, then plasma was removed and frozen at -20°C for lipoprotein analysis and -70°C for fatty acid analysis. All blood analyses were conducted within the Human Nutrition Department at the University of Otago. Plasma total cholesterol was measured using an enzymatic kit (Cholesterol CHOD-PAP) by Boehringer on a Cobas Mira Plus autoanalyser. High-density lipoprotein cholesterol was measured in the supernatant following precipitation of apoB-containing lipoproteins with phosphotungstate-magnesium (Assmann *et al*, 1983). Triacylglycerol concentration was measured enzymatically on the autoanalyser using a Roche kit (Unimate 5 TRIG). All seven samples from the same person were analysed in the same batch and all samples were completed in four batches. The within-batch coefficient of variation for these methods was 4.0% for total cholesterol, 3.4% for HDL cholesterol and 6.9% for triacylglycerols. These assays were validated according to the Royal Australasian College of Pathologists Quality Assurance Program. Low-density lipoprotein cholesterol was calculated from the total and high-density lipoprotein cholesterol and

triacylglycerol measurements using the Friedewald equation (Friedewald *et al*, 1972).

The fatty acid composition of plasma triacylglycerols and cholesterol esters was determined at baseline and in one of the two samples collected at the end of each dietary phase. Lipids were extracted from 200 µl of plasma using the Bligh and Dyer (1959) method and fatty acid composition determined as described by Holub and Skeaff (1987). The precision of fatty acid measurement was determined by repeated analysis of pooled plasma; $n = 9$ for triacylglycerol and $n = 16$ for cholesterol ester. The coefficients of variation for selected triacylglycerol fatty acids were: C18:2n-6, 2.1%; C14:0, 1.5%; C15:0, 3.7%; C16:0, 0.8%. The coefficients of variation for cholesterol ester fatty acids were: C18:2n-6, 1.3%; C14:0, 5.0%; C15:0, 5.8%; C16:0, 2.3%.

The criteria we used to assess dietary compliance was the average change in linoleic acid composition of plasma cholesterol esters between the butter phase and each of the two polyunsaturated spread phases.

Three participants who had triacylglycerol concentrations above 3.0 mmol/l in both of the blood tests within one phase were excluded from the analysis because the high values were incompatible with calculation of low-density lipoprotein cholesterol by the Friedewald equation; their exclusion had no statistical or interpretive bearing on the final results. Complete results are presented for 50 participants. We calculated that a sample size of 48 was needed to detect, using a crossover design, a 5% difference in total cholesterol concentration between treatments with a power of 0.95 and a confidence of 0.05. This assumed an average blood cholesterol concentration of 6.0 mmol/l and within-person variation of 0.4 mmol/l in the study population. The average coefficient of variation between the two plasma total cholesterol measurements at the end of each dietary phase was less than 4%; furthermore, there was no significant difference between the mean of the first and the mean of the second plasma lipid measurements. An average of the two measurements was taken to represent the individual result. There was no order effect of polyunsaturated spread consumption on blood cholesterol concentration; therefore, the results were analysed ignoring the order of plant sterol administration. Linear regression was used to test for significant differences in cholesterol concentrations, energy and nutrient intake and blood fatty acids between the three dietary phases. All results were analysed using SPSS version 10 for the Mac.

Results

The baseline characteristics of participants are presented in Table 2. Weight was unchanged during the three dietary phases of the study.

Participants reported consuming an average of 21 g/day of butter during the butter phase, 27 g/day of standard spread and 26 g/day of plant sterol spread in the respective polyunsaturated spread phases (Table 3). The latter amount of

Table 2 Participant characteristics at baseline

Number	50
Female/male	31/19
Age (y)	46.7 (10.5)
BMI (kg/m ²)	26.0 (3.9)
Total cholesterol (mmol/l)	5.95 (0.78)
LDL cholesterol (mmol/l)	3.91 (0.72)
HDL cholesterol (mmol/l)	1.30 (0.40)
Triacylglycerol (mmol/l)	1.62 (0.54)

Body mass index, BMI; low-density lipoprotein, LDL; high-density lipoprotein, HDL.

Results are presented as mean (s.d.).

spread consumption equated to a daily intake of 2.1 g of plant sterols. The average amount of fat consumed by the participants from the butter or spreads did not differ significantly between the three phases.

Total fat content of the diets during the three dietary phases was very similar; however, saturated fat intake was 3% of total energy lower and polyunsaturated fat intake 3% of total energy higher during the polyunsaturated spread phases compared with the butter phase. These significant changes in saturated and polyunsaturated fat intake were entirely accounted for by the replacement of butter with polyunsaturated spread. There were no significant differences between any of the dietary phases in energy, carbohydrate or protein intake.

The linoleic acid (C18:2n-6) content of plasma triacylglycerol and cholesterol ester during the polyunsaturated phases was significantly higher as compared with the butter phase, whereas pentadecanoic acid (C15:0), heptadecanoic acid (C17:0) and myristic acid (C14:0) content were significantly lower. With the exception of a slightly lower alpha-linolenic acid (C18:3n-3) the fatty acid composition of plasma triacylglycerol and cholesterol ester during the

Table 3 Energy and nutrient composition of diets

	Butter (n = 48)	Standard spread (n = 48)	Plant sterol spread (n = 48)
Energy (kJ)	8630 (2235)	8504 (2199)	8397 (1963)
Protein (%TE)	17 (4)	16 (3)	16 (4)
Carbohydrates (%TE)	47 (6)	48 (6)	48 (6)
Fat (%TE)	31 (6)	31 (6)	31 (5)
SAFA (%TE)	14 (3)	11 (3) [†]	11 (3) [†]
MUFA (%TE)	9 (3)	10 (2) [*]	10 (2)
PUFA (%TE)	4 (2)	7 (2) [†]	7 (1) [†]
Spread consumption (g)	21 (8)	27 (8)	26 (7)
Fat intake from spread (g)	18 (5)	19 (6)	17 (5)
Soy bean oil consumption (g)	2 (3)	2 (2)	2 (3)
Cholesterol (mg)	249 (100)	219 (93)	232 (100)
Dietary fibre (g)	23 (6)	22 (7)	20 (7) [*]
Alcohol (g)	10 (15)	7 (14)	11 (16)

Results are presented as mean (s.d.).

Percentage of total energy, %TE.

**P* < 0.05, [†]*P* < 0.01, significantly different compared to the butter phase.

Table 4 Fatty acid composition of plasma triacylglycerol

	Butter (n = 50)	Standard spread (n = 50)	Plant sterol spread (n = 50)
C14:0	3.21 (1.12)	2.85 (0.96) [*]	2.89 (0.84) [*]
C15:0	0.51 (0.13)	0.43 (0.09) [†]	0.43 (0.11) [†]
C16:0	29.02 (4.26)	26.84 (4.06) [†]	27.60 (3.61)
C16:1	5.74 (1.37)	5.10 (1.56) [*]	5.01 (1.53) [†]
C17:0	0.42 (0.09)	0.37 (0.07) [†]	0.38 (0.09) [*]
C18:0	3.92 (0.83)	4.13 (3.50)	3.99 (1.01)
C18:1	39.22 (4.43)	38.09 (4.25)	38.61 (3.55)
C18:2n-6	12.04 (3.13)	16.10 (4.60) [†]	15.35 (3.75) [†]
C18:3n-3	1.10 (0.37)	1.33 (0.44) [†]	1.13 (0.33) [§]
C20:4n-6	1.00 (0.32)	1.15 (0.46) [†]	1.14 (0.40) [*]
C20:5n-3	0.38 (0.48)	0.33 (0.23)	0.28 (0.12)
C22:6n-3	0.88 (1.03)	0.72 (0.43)	0.66 (0.31)

Results are presented as mean (s.d.).

**P* < 0.05, [†]*P* < 0.01, significantly different compared to the butter phase.

[§]*P* < 0.01, significantly different compared to the standard spread phase.

polyunsaturated phases with or without plant sterols were virtually identical.

Mean total plasma cholesterol concentration was significantly lower, by 4.6%, at the end of the standard polyunsaturated spread phase than at the end of the butter phase (Table 6). Including the plant sterol-enriched spread in the diet significantly reduced plasma total cholesterol by 8.9% compared with the diet containing butter. Total cholesterol concentration was significantly lower, by 4.5%, while using the plant sterol spread compared to using the standard spread.

Low-density lipoprotein cholesterol concentration was 5.5% lower on the standard spread and 12.3% lower on the plant sterol spread as compared with butter. In comparison with the standard polyunsaturated fat spread phase, low-density lipoprotein cholesterol was 7.2% lower when

Table 5 Fatty acid composition of plasma cholesterol esters

	Butter (n = 50)	Standard spread (n = 50)	Plant sterol spread (n = 50)
C14:0	1.11 (0.25)	0.90 (0.22) [†]	0.92 (0.22) [†]
C15:0	0.30 (0.05)	0.23 (0.04) [†]	0.24 (0.05) [†]
C16:0	12.52 (0.98)	11.75 (0.99) [†]	11.85 (0.87) [†]
C16:1	4.80 (1.78)	3.53 (1.35) [†]	3.96 (1.47) [†]
C17:0	0.10 (0.04)	0.08 (0.04) [†]	0.07 (0.04) [†]
C18:0	1.32 (0.26)	1.29 (0.27)	1.19 (0.26) ^{†‡}
C18:1	21.30 (2.52)	19.14 (2.39) [†]	19.00 (2.02) [†]
C18:2n-6	49.02 (5.16)	53.78 (4.66) [†]	53.44 (4.42) [†]
C18:3n-3	0.73 (0.16)	0.63 (0.14) [†]	0.57 (0.13) ^{†‡}
C20:4n-6	5.67 (1.76)	6.00 (1.46)	5.87 (1.86)
C20:5n-3	1.08 (0.53)	0.93 (0.57)	0.87 (0.50) [*]
C22:6n-3	0.53 (0.17)	0.51 (0.16)	0.51 (0.17)

Results are presented as mean (s.d.).

**P* < 0.05, [†]*P* < 0.01, significantly different compared to the butter phase.

[‡]*P* < 0.05, [§]*P* < 0.01, significantly different compared to the standard spread phase.

Table 6 Plasma lipid concentrations at the end of each dietary phase

	Butter (n = 50)	Standard spread (n = 50)	Plant sterol spread (n = 50)
Total cholesterol (mmol/l)	6.09 (0.82)	5.81 (0.77) [†]	5.55 (0.76) ^{†‡}
LDL-cholesterol (mmol/l)	3.98 (0.76)	3.76 (0.74) [*]	3.49 (0.72) [§]
HDL-cholesterol (mmol/l)	1.35 (0.41)	1.37 (0.41)	1.38 (0.41)
Triacylglycerols (mmol/l)	1.64 (0.46)	1.47 (0.38) [†]	1.48 (0.46) [*]

Results are presented as mean (s.d.).

^{*} $P < 0.05$, [†] $P < 0.01$, significantly different compared to the butter phase.

[‡] $P < 0.05$, [§] $P < 0.01$, significantly different compared to the standard spread phase.

participants included the plant sterol-enriched spread in their diet. Participants were ranked according to dietary compliance. Low-density lipoprotein cholesterol concentration in participants who were ranked in the highest tertile of compliance was 11.2% lower during the plant sterol than standard spread phases. In the remaining participants the decrease with added plant sterols was significantly less, 4.6% ($P < 0.05$).

There were no significant differences in the mean high-density lipoprotein cholesterol concentrations at the end of the three dietary phases. Mean triacylglycerol concentration was 10.4% lower in the standard spread phase and 9.8% lower in the plant sterol spread phase than in the butter phase.

Discussion

This study confirms the potential of a plant sterol-enriched polyunsaturated spread to favourably influence lipoprotein-mediated risk of cardiovascular disease. This effect was apparent even in the context of a diet in which intake of total and saturated fatty acids is below that usually consumed in a typical Western diet, a dietary modification that on its own would be expected to appreciably reduce levels of total and low-density lipoprotein cholesterol. The experimental design permitted a comparison of the cholesterolaemic effects of three different spreads in the context of this low-fat diet—butter, and a widely used polyunsaturated spread with and without plant sterols added.

Compared with the diet including butter, low-density lipoprotein cholesterol was reduced by 5.5% on the polyunsaturated spread diet and by 12.3% when participants used the plant sterol spread. The only significant differences in dietary intakes between the butter phase and standard polyunsaturated phase were the relative amounts of saturated and polyunsaturated fats. These differences in diet composition were entirely accounted for by the removal of butter from the diet and its replacement with polyunsaturated spread. Therefore, the 4.6 and 5.5% decrease in total and low density lipoprotein cholesterol, respectively, was due to this substitution; the reduction in total cholesterol being remarkably similar to that predicted using the equa-

tions of Keys *et al* (1957) and Hegsted *et al* (1993; 5.2% and 5.0%, respectively). The diets consumed during the two polyunsaturated phases were virtually identical, as indicated by the reported dietary intakes. Although the fatty acid composition of plasma triacylglycerol and cholesterol esters are not specific markers for spread consumption, the very similar fatty acid results during the two polyunsaturated spread phases objectively confirms that the fat composition of the two diets was the same. Therefore, the additional 7.2% reduction in low-density lipoprotein cholesterol between the two diets containing the polyunsaturated spreads can be attributed entirely to the plant sterols. Triacylglycerol concentration decreased by about 10% in both the polyunsaturated spread phases compared with concentrations in the butter phase. High-density lipoprotein cholesterol concentrations were similar on the three diets.

Only five of the many studies that have examined the effects of plant sterols or stanols on lipids and lipoproteins have been carried out in the context of diets relatively low in total and saturated fatty acids (Denke, 1995; Hallikainen & Uusitupa, 1999; Hallikainen *et al*, 2000; Maki *et al*, 2001; Nigon, 2001). The first of these reported no effect on total and low-density lipoprotein cholesterol, the author concluding that the hydrogenated plant sterol, sitostanol, probably had little effect when used in a diet low in cholesterol (Denke, 1995). However, it is conceivable that the negative results might have been due to the low solubility of unesterified sitostanol. The three other studies showed appreciable reduction of total and low-density lipoprotein cholesterol in association with the use of spreads enriched with plant sterols or stanols. The order of magnitude of the changes in the two Finnish studies (Hallikainen & Uusitupa, 1999; Hallikainen *et al*, 2000) appeared slightly greater than that observed in the present study; the findings in the American (Maki *et al*, 2001) and French studies (Nigon, 2001) being virtually identical to our own data.

Our study and the American study differ from the Finnish studies in at least one important respect which has considerable practical application. In both of the Finnish studies it appears that participants, although free-living, received detailed dietary instructions to an extent which may not be possible in community-based intervention programmes. On the other hand our volunteers were given practical and simple dietary instructions regarding a fat-reduced diet into which they were asked to incorporate the three different spreads. Despite this relatively modest level of intervention, several measures of dietary compliance suggested an impressive level of adherence to the dietary advice. Four-day records suggested a dietary intake which achieved to a considerable extent the targets which had been set. Perhaps even more impressive were the changes in fatty acid composition of the two plasma lipid fractions we measured. It has been established that serum pentadecanoic acid (C15:0) is a good biological marker of dairy fat intake (Wolk *et al*, 2001); therefore, the lower pentadecanoic acid content of plasma

triacylglycerol and cholesterol ester is objective confirmation of the decrease in butter intake during the polyunsaturated dietary phases. In a similar way, the increase in linoleic acid (C18:2n-6) content of the plasma lipid fractions during the polyunsaturated diet phases indicates a higher intake of the polyunsaturated spreads (Zock *et al*, 1997). Despite good group compliance to the diets, it is reasonable to expect that some participants were more compliant than others. It is notable that in participants classified in the highest tertile of compliance—assessed by the change in the linoleic acid composition of plasma cholesterol ester between the butter and spread phases—consumption of the plant sterol-enriched spread reduced low-density lipoprotein cholesterol by 11.2% in comparison with the standard spread. The magnitude of the reduction was consistent with that in the two Finnish studies (mean 11.4% decrease) and suggests that compliance may explain, to some extent, the variable cholesterol-lowering effect of plant sterols reported in studies of free-living individuals.

The relatively short duration of the experimental periods is acknowledged. However, there is now ample evidence that lipid and lipoprotein responses to dietary change in fat intake are rapid (Durrington *et al*, 1977; Mensink & Katan, 1987). Maximum changes after initiating use of plant sterols are observed after 2 weeks (Nguyen *et al*, 1999) and there is an appreciable body of evidence which confirms that diet-induced changes are sustained in the long term provided compliance is maintained (Miettinen *et al*, 1995; Agewall *et al*, 2001). The results of a few studies have suggested a slight reduction in the plasma concentration of some carotenoids with use of plant sterol-enriched spreads, but this has not been a consistent finding (Hallikainen *et al*, 1999; Hendriks *et al*, 1999; Sierksma *et al*, 1999; Plat *et al*, 2000), particularly when lipid-adjusted concentrations are compared. Indeed, it seems that, provided the background diet is appropriate and includes a variety of carotenoid-rich foods, any alteration in carotenoids is likely to be minor and of little or no clinical significance (Noakes *et al*, 2002).

The demonstration here that a polyunsaturated spread with the addition of plant sterols can produce, in free-living individuals following a moderately low-fat diet, a further reduction in low-density lipoprotein cholesterol suggests that such spreads may be a useful additional component of dietary strategies for the management of hypercholesterolaemia. It is particularly noteworthy that for some individuals with moderately raised total and low-density lipoprotein cholesterol this additional lipid-modifying effect may obviate the need for long-term lipid-lowering drug therapy.

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