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Original Communication
Cholesterol-lowering effects of plant sterol esters and non-esterified stanols in margarine, butter and low-fat foods

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Objectives: To determine the efficacy on plasma cholesterol-lowering of plant sterol esters or non-esterified stanols eaten within low-fat foods as well as margarine.
Design: Randomised, controlled, single-blind study with sterol esters and non-esterified plant stanols provided in breakfast cereal, bread and spreads. Study 1 comprised 12 weeks during which sterol esters (2.4 g) and stanol (2.4 g) -containing foods were eaten during 4 week test periods of cross-over design following a 4 week control food period. In Study 2, in a random order cross-over design, a 50% dairy fat spread with or without 2.4 g sterol esters daily was tested.
Subjects: Hypercholesterolaemic subjects; 22 in study 1 and 15 in study 2.
Main outcome measures: Plasma lipids, plasma sterols, plasma carotenoids and tocopherols.
Results: Study 1 — median LDL cholesterol was reduced by the sterol esters (-13.6%; $P < 0.001$ by ANOVA on ranks; $P < 0.05$ by pairwise comparison) and by stanols (-8.3%; $P = 0.003$, ANOVA and < 0.05 pairwise comparison). With sterol esters plasma plant sterol levels rose (35% for sitosterol, 51% for campesterol; $P < 0.001$); plasma lathosterol rose 20% ($P = 0.03$), indicating compensatory increased cholesterol synthesis. With stanols, plasma sitosterol fell 22% ($P = 0.004$), indicating less cholesterol absorption. None of the four carotenoids measured in plasma changed significantly. In study 2, median LDL cholesterol rose 6.5% with dairy spread and fell 12.2% with the sitosterol ester fortified spread ($P = 0.03$ ANOVA and $< 5\%$ pairwise comparison).
Conclusion: 1. Plant sterol esters and non-esterified stanols, two-thirds of which were incorporated into low-fat foods, contributed effectively to LDL cholesterol lowering, extending the range of potential foods. 2. The LDL cholesterol-raising effect of butter fat could be countered by including sterol esters. 3. Plasma carotenoids and tocopherols were not reduced in this study.
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Introduction

Plant stanol and sterol esters incorporated within spreads have been shown to augment the low density lipoprotein cholesterol lowering effect of other strategies. Alone, in dosages of around 2 g daily, stanol or unhydrogenated sterol esters lower LDL cholesterol by about 10% or more (Miettinen *et al*, 1995; Westrate & Meijer, 1998; Gylling & Miettinen, 1994). When added to a cholesterol-lowering diet, sitostanol ester-containing margarine reduces LDL cholesterol by at least a further 5% (Hallikainen & Uusitupa, 1999; Andersson *et al*, 1999). Each of these studies had provided sterols in the form of esters and within margarine.

Non-esterified or free sterols have also been shown to be effective by a 17% reduction in LDL cholesterol during treatment with a high dose of a soy sterol suspension, although lesser amounts (3 g tall oil sterols, mainly sitosterol) were also effective (Lees *et al*, 1977). The effect of non-esterified sitostanol is less certain with one study demonstrating 15% LDL cholesterol lowering in a limited number of hypercholesterolaemic subjects (Heinemann *et al*, 1986) treated with as little as 1.5 g non-esterified sitostanol dispersed in sunflower oil, whereas Denke

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(1995) observed no benefit from 3 g crystalline sitostanol taken within capsules.

Esterification of these compounds may improve efficacy because it allows greater 'solubilisation' within the margarine and optimal dispersion within fat-containing micelles during the intestinal metabolism of lipids. The exact mechanisms that lead to a substantial reduction in the absorption of cholesterol of about 50% (Von Bergmann *et al*, 1999) are not fully understood but include the displacement of cholesterol from the micelles by the presence of the stanols or sterols. This does not exclude similar efficacy from non-esterified phytosterols (Jones *et al*, 1999), nor the delivery of plant sterols and stanols within low-fat foods. Current community preferences lie in the direction of reduced fat intake that has reduced consumption of margarines. On the other hand preference for dairy fats remains significant and the addition of sterol or stanol esters to butter has been shown to be a useful cholesterol-lowering approach (Pelletier *et al*, 1995; Gylling & Miettinen, 1999).

The current studies address several issues but in particular the public health perspective of widening the availability of the sterol/stanol-fortified, cholesterol-lowering range of foods. The LDL cholesterol-lowering potential of sterol esters or of non-esterified stanols (which may have advantages in the incorporation of these compounds into some foods), eaten in low-fat foods such as bread and breakfast cereals, was examined in study 1. The value of adding sterol esters to a dairy spread containing 50% butter fat was determined in study 2, since it allowed a comparison with the effect in the same individuals who participated in study 1. We have also measured the concentrations of a range of plasma carotenoids, since a reduction in some of these has been postulated as a matter of concern in the use of sterol and stanol-enriched spreads (Plat & Mensink, 1998; Hallikainen *et al*, 1999).

Methods

Experimental design

Two sets of experiments were carried out, termed study 1 and study 2, each beginning with a 2 week familiarisation period followed by several 4 week test phases. In study 1, 2.4 g plant sterol ester or a similar amount of non-esterified plant stanols was provided within three different foods. During the run-in period and subsequent 4 week control period unsupplemented foods similar to the test foods were eaten. The two supplemented diets were then tested in random order. Each period was of 4 weeks' duration, giving a total study period of 14 weeks. During the familiarisation phase habitual diets were determined by a 3 day food frequency questionnaire which served to establish for each person a constant background pattern of eating. Since all subjects had been known to have moderately raised plasma cholesterol levels they were already on a low saturated fat, low cholesterol diet. The three test foods comprised a wheat-based breakfast cereal, wholemeal bread and a canola oil-based soft margarine

The sterol esters and the stanols were incorporated equally into the three foods in amounts that provided a total of 2.4 g plant sterols as an ester or 2.4 g non-esterified sitostanol/campestanol when eaten as cereal (40 g), sliced bread (70 g) and margarine (20 g). The foods were eaten partly in the morning (cereal with skimmed milk, bread and margarine) and partly in the evening (bread and margarine). All foods were colour-coded, but only the test subjects were blinded to the identity of the foods. However the laboratories carrying out the various assays were also ignorant of the nature of the test periods.

Vegetable oil sterol esters and vegetable oil stanols were obtained from the Food Ingredient Division of COGNIS Australia Pty Ltd (Ermington, NSW). Both the sterol esters and stanols were derived from predominantly soybean oil distillates. The sterol esters were made by esterification with fatty acids from soybean to an esterification degree of 94%. The sterol profile of the sterol esters was 50% sitosterol, 20% stigmasterol and 20% campesterol. The stanol profile was 70% sitostanol, 27% campestanol and approximately 1% free sterols. In the manufacture of cereal and bread, sterol esters were first dispersed in oil and produced a 97% fat-free product. The oil was mechanically dispersed among the cereal grain and flour with negligible loss. Plant stanols in crystalline form were also dispersed mechanically during the manufacture of the bread and cereals (the temperature being below the melting point of the stanols); losses were again measured and found to be minimal both before and following processing. The margarine contained 70% fat by weight and its fatty acid composition was predominantly that of canola oil.

Study 2 also tested a similar amount of sterol (2.4 g) as esters but within a dairy spread that contained 80% fat by weight. The control spread contained 40% butter fat, 32% canola oil and 8% hardened *trans*-free palm oil blend. The test dairy spread which also contained 40% butter fat included canola oil, soybean sterol ester and *trans*-free palm oil blend. Its fatty acid composition was 46% saturated fatty acids (11% lauric plus myristic, 21% palmitic, 9% stearic), 28% oleic, 17% linoleic and 3% α-linolenic acid. The saturated fatty acids in the control spread constituted 43% of the total so that monounsaturated and polyunsaturated fatty acids, at 47%, were slightly lower than in the test spread 49%. Each spread contained 2% *trans* fatty acids.

Although all the sterol ester was in 20 g spread, the subjects who participated in this trial, being members of the first study, were also asked to eat the same amounts of bread and breakfast cereal, partly in the morning and partly in the evening, as in the first study. The duration of the study was shorter. It was carried out several weeks after study 1 and comprised an initial retesting of plasma lipids after a period on the preceding habitual diet followed by two test periods each of 4 weeks' duration and administered in random order. The control period comprised the

unsupplemented dairy spread and the test period included the sterol ester supplemented spread.

Wash-out periods were not considered necessary, since sterols and stanols are minimally absorbed and 3–4 weeks is generally considered adequate to reach new steady-state values for plasma cholesterol, triglyceride and HDL cholesterol.

Subjects

Twenty-two men and women were recruited by advertisements that sought people with known hypercholesterolaemia but who had not been treated with lipid-lowering drugs for the preceding 6 months or longer. Inclusion criteria for plasma lipids were total cholesterol > 5.5 mmol/l and triglycerides < 3 mmol/l. Exclusion criteria included hormone replacement therapy, smoking, alcohol exceeding four standard drinks daily for men and two for women, medication likely to affect plasma lipids, bowel, liver and kidney disorders, thyroid dysfunction and diabetes mellitus.

Fifteen subjects volunteered to continue in study 2, several having been advised to seek more active cholesterol lowering medication and some being unavailable for a further 8 weeks.

Each subject completed initially and after each intervention a 3 day food frequency questionnaire, simplified to focus on foods containing fats, cholesterol and fibre, since the aim was to monitor the amounts and types of nutrients most likely to influence plasma lipids. The need to recall fewer individual foods has been found in our hands to improve the intra-individual correlations for fat, major fatty acids and cholesterol. Subjects were required to return all food packages for reweighing.

The studies were approved by the Human Ethics Committee of the Alfred Group of Hospitals, Melbourne and volunteers gave written consent following full disclosure and explanation of the studies.

Laboratory procedures

Blood was drawn with subjects having fasted > 12 h twice at the beginning and then twice at the end of each phase of the studies. Values from the two samplings were averaged. Plasma was stored at -80°C. Total cholesterol, triglyceride and HDL cholesterol (after precipitation of non-HDL lipoproteins) were measured by enzymatic kits on a Cobas-Bio automated analyser (Roche, Basel, Switzerland). LDL cholesterol was calculated using the Friedwald equation. Measurements were made on single samples of plasma after each intervention for the plant sterols, campesterol and sitosterol, and for lathosterol a precursor of cholesterol (an index of cholesterol synthesis). Plasma sterols were determined by gas-chromatography by the method of Wolthers *et al* (1991). Plasma sterol concentrations were derived from standard curves from the respective sterol peak areas referenced against the internal standard 5β-cholestan-3α-ol. This method does not distinguish sitostanol from avenasterol, the concentration of which is much higher than that of sitostanol, and the two sterols are reported as a single value since quantitation of plasma sitostanol was not a major outcome in this study.

Since carotenoids have been reported to be decreased following consumption of sterol and stanol esters, measurements were also made of several carotenoids (lycopene, α- and β-carotene, β-cryptoxanthin), and of tocopherols by high pressure liquid chromatography.

Plasma phytosterols were related to the cholesterol concentrations in the corresponding samples of plasma, all measurements being carried out by gas-chromatography.

Statistical analyses

Data were analysed by repeated measures ANOVA on ranks (Freedman), followed by pairwise multiple comparison procedures, due to the absence of normal distributions for plasma total and LDL cholesterol. Although unusual, this skewness was due to the inclusion of several subjects with high cholesterol levels. Results for these parameters are shown in Tables 1 and 2 as medians, although means are also given in the text. For normally distributed parameters including plasma triglyceride and HDL cholesterol, values are given as means and those data including that for plasma sterols and carotenoids have been analysed by paired *t*-test.

Results

Clinical characteristics

The average age of the four women and 18 men was 60 ± 9 y (range 34–70); their body mass index (BMI) was 24 ± 1.9 kg/m^2 (range 18.3–26.9) and they were normotensive (none $> 145/95$ mmHg). Their plasma lipid values on screening showed that only one subject was inadvertently entered with a cholesterol value below 5.5 mmol/l (the inclusion criterion): 5.2 mmol/l. Five subjects had triglyceride values between 2.0 and 2.6 mmol/l

Table 1 Plasma lipids following plant sterol ester or plant stanol enriched foods

Intervention (n = *22*)	*Total cholesterol (mmol/l) median (25–75%)*	*LDL cholesterol (mmol/l) median (25–75%)*	*HDL cholesterol (mmol/l) mean ± s.d.*	*Triglyceride (mmol/l) mean ± s.d.*
Control	7.00 (6.25–7.40)	4.77 (4.0–5.35)	1.69 ± 0.36	1.37 ± 0.48
Sterol esters	6.40 (5.85–7.15)*	4.12 (3.45–5.10)*	1.54 ± 0.33	1.43 ± 0.56
Stanols	6.75 (5.80–7.30)*	4.37 (3.60–4.95)*	1.56 ± 0.39	1.34 ± 0.56

*Significantly different from control by repeated measures analysis of variance on ranks ($P < 0.001$ for total cholesterol and $P = 0.003$ for LDL cholesterol; $P < 0.05$ for all by pairwise multiple comparison procedures).

Table 2 Plasma lipids following a plant sterol ester enriched dairy fat spread

Intervention (n = 15)	*Total cholesterol (mmol/l) median (25–75%)*	*LDL cholesterol (mmol/l) median (25–75%)*	*HDL cholesterol (mmol/l) mean ± s.d.*	*Triglyceride (mmol/l) mean ± s.d.*
Baseline	6.65 (5.70–6.98)*	4.60 (3.67–4.85)*	1.48 ± 0.19	1.11 ± 0.29†
Control dairy fat	7.05 (6.14–7.42)	4.90 ± (4.12–5.35)	1.52 ± 0.25	1.37 ± 0.59
Sterol ester in dairy fat	6.40 (6.05–7.15)*	4.30 (3.62–4.97)*	1.51 ± 0.18	1.36 ± 0.50

*Significantly different from control dairy fat by repeated measures analysis of variance on ranks ($P < 0.001$ for total cholesterol and $P = 0.03$ for LDL cholesterol; $P < 0.05$ for all by pairwise multiple comparison procedures).
†Significantly lower than both dairy fat values.

(inclusion criterion being < 3 mmol/l) and none of the subjects had an HDL cholesterol < 1.0 mmol/l. They were therefore primarily hypercholesterolaemic. The screening data are not shown but were similar to those at the end of the initial control dietary period, excluding systematic regression to the means during the control phase. None of the subjects gained or lost more than 1 kg during any of the studies.

Background diet

In study 1, the 3 day food frequency questionnaire suggested a small increase in fat consumption during the study that probably reflected the additional margarine (34 *vs* 32% energy from fat; $P = 0.02$). However the subjects maintained their relatively low saturated fat (11.5 ± 2% energy) and low cholesterol (164 ± 47 mg) intakes almost unchanged; fibre intake was 19 and 18 g, respectively, at the start and end of the study.

Similar information was not collected at the end of study 2 (sterol ester fortified butter fat spread) because the subjects had shown such excellent compliance in the first, longer part of the experiments. Since their intake of saturated fat would have increased by the amount in the spread, as described in the *Methods* section, and their cholesterol intake by about 25 mg daily, corresponding adjustments were made in other sources of saturated fat.

Lipid changes with plant sterol ester or non-esterified stanol-containing foods: study 1

Subjects first ate the three test foods that were devoid of supplement, termed control in Table 1. They then ate either sterol ester or plant stanol supplemented foods in random order. Plasma median total cholesterol concentrations fell significantly by 8.5% with sterol esters and by 3.5% with the non-esterified stanol ($P < 0.001$ by ANOVA and < 0.05 by pairwise comparison of each test intervention *vs* control diet). The response with sterol esters was possibly significantly greater than with stanol ($P = 0.05$). The effects of the test supplements were greater for LDL cholesterol: 13.6% reduction with sterol ester and 8.3% reduction with stanol ($P = 0.003$ by ANOVA and < 0.05 by pairwise comparison for each test intervention *vs* control diet). The changes with the two interventions were not significantly different for LDL cholesterol. Mean values ± s.d. for total cholesterol for the control sterol ester and stanol phases were 7.03 ± 1.26, 6.57 ± 1.16 and 6.73 ± 1.13 mmol/l. The corresponding values for LDL cholesterol were 4.82 ± 1.13, 4.37 ± 1.18 and 4.52 ± 1.12 mmol/l. However, since the values for total and LDL cholesterol were not normally distributed, statistical analyses were confined to the median values.

The minor changes in mean HDL cholesterol and in plasma mean triglyceride were not significant.

Lipid changes with sterol ester in 50% butter fat spread; study 2

Fifteen of the original 22 subjects agreed to a further study beginning several weeks after the conclusion of study 1. The median total cholesterol and LDL cholesterol concentrations were initially lower than at the commencement of study 1 because several of the most hypercholesterolaemic subjects had been advised to lower their cholesterol pharmacologically and therefore became unavailable. Compared with the new baseline, the control dairy fat spread raised both the median total and LDL cholesterol levels significantly: by 6.5% for LDL (Table 2). By comparison, 4 weeks on the sterol ester fortified dairy spread lowered median total cholesterol by 8.5% ($P < 0.001$ by ANOVA and < 0.05 by pairwise comparison) and LDL cholesterol by 12.2% ($P = 0.03$ by ANOVA and < 0.05 by pairwise comparison) and remained 7% below the baseline value ($P = 0.03$ by ANOVA and < 0.05 by pairwise comparison).

The mean values for total cholesterol at the end of the control and test spreads respectively were 6.86 ± 0.97 and 6.32 ± 0.85 mmol/l. Corresponding values for LDL cholesterol were 4.68 ± 0.78 and 4.31 ± 0.69 mmol/l.

Plasma mean triglyceride also rose significantly with dairy fat (+23%, $P = 0.017$) and remained at the higher level with the sterol ester-supplemented spread. HDL cholesterol concentrations did not change between dietary changes.

Since the subjects were hypercholesterolemic, 13 of the 15 were reviewed between 4 and 8 weeks after the conclusion of the trial and their lipids re-measured. Means for total and for LDL cholesterol were, respectively, 6.69 ± 1.1 and 4.71 ± 1.0 mmol/l.

Plasma lathosterol, campesterol and sitosterol concentrations; study 1

Table 3 shows the changes in plasma phytosterols, expressed as a ratio with cholesterol (means ± s.d.),

Table 3 Plasma plant sterol and lathosterol concentrations following plant sterol ester and non-esterified stanol enriched foods

Intervention (n = *22*)	*Sitostanol* (μ*M*/*mM cholesterol*)	*Campesterol* (μ*M*/*mM cholesterol*)	*Sitostansol+avenasterol* (μ*M*/*mM cholesterol*)	*Lathosterol* (μ*M*/*mM cholesterol*)
Control	0.74 ± 0.34	1.87 ± 0.93	0.50 ± 0.22	0.45 ± 0.10
Stanols	0.58 ± 0.23**	1.73 ± 0.78	0.43 ± 0.17*	0.48 ± 0.08
Sterol esters	0.99 ± 0.44**	2.82 ± 1.54**	0.43 ± 0.12	0.54 ± 0.21*

*Different from control, $P < 0.05$.
**Different from control, $P < 0.01$.

during sterol ester and non-esterified stanol supplementation. Plasma lathosterol, an index of cholesterol synthesis, rose 20% ($P = 0.03$) after the sterol ester interventions, but changed insignificantly with stanol intervention. Plasma plant sterols (being present in the sterol ester supplement), rose 51% ($P < 0.001$) for campesterol and 35% ($P < 0.01$) for sitosterol with the sterol ester supplement. However plasma sitosterol did fall significantly with stanol supplementation (by 22%; $P = 0.004$), indicating significant inhibition of cholesterol absorption. Plasma campesterol also tended to decrease. The combined plasma sitostanol plus avenasterol concentration fell significantly ($P = 0.04$) with stanol supplementation, presumably because avenasterol is a plant sterol and its fall has the same implication as that of sitosterol.

Plasma carotenoids and tocopherols

None of the carotenoids (lycopene, α- or β-carotene, β-cryptoxanthin) was significantly affected by sterol ester supplementation in study 1 (the stanol period was not assayed). The tocopherols, α- and γ-tocopherols both increased in plasma with the sterol ester supplement—α-tocopherol 35.2 and 40.1 μM/l; γ-tocopherol 11.1 and 17.7 μM/l, significantly different for γ-tocopherol (61%, $P = 0.044$). Equal amounts of tocopherols had been added to all the margarines in the amounts needed for antioxidant activity.

Discussion

This study has confirmed the substantial LDL cholesterol-lowering effect achievable with sterol esters in moderately hypercholesterolaemic people (Tables 1 and 2). The average 13.6% reduction in median values is in line with that reported by Westrate and Meijer (1998), who reported similar benefits with sitostanol esters and sitosterol esters in margarines. However in our study only one-third of the sterol was in a fat spread, the remaining two-thirds having been eaten as bread and breakfast cereal. This extends the range of foods within which such cholesterol-lowering compounds may be delivered. It suggests that lipid within the intestine derived from other foods and endogenously is sufficient for sterol esters to become incorporated into micelles.

Although the LDL cholesterol reduction was greater with the sterol esters than with non-esterified stanols, the difference was not statistically significant. It was of the order reported by Sjerksma *et al* (1999), who observed a 6% reduction in LDL cholesterol with as little as 0.8 g non-esterified soybean sterols eaten daily, but less than that reported for a mixture of non-esterified sterols and sitostanol (Jones *et al*, 1999). It appears likely that cholesterol absorption is similarly inhibited by sitostanol esters and sterol esters (Normen *et al*, 2000; Jones *et al*, 2000). The differentiation between esterified and non-esterified phytosterols may therefore be minor, if any, and shows that either can be utilised for the manufacture of this class of novel foods. Nevertheless, phytosterol esters have greater fat-like characteristics, a property that enhances their dispersion within the fat phase of food digests through which the inhibition of cholesterol absorption eventually takes place.

The trial with the 50% butterfat spread (Table 2) suggests that much of the cholesterol-raising effect of butterfat can be countered through the inclusion of sterol esters. The average 12.2% reduction in LDL cholesterol lowered its concentration below the baseline, pre-butterfat level. The relative reductions with sterol esters were therefore similar in the context of a low-fat diet (study 1) and a diet high in saturated fat (study 2). Gylling and Miettinen (1999) have reported a 12% reduction in LDL cholesterol when 2.43 g sitostanol (as ester) was added to whole butter and Pelletier *et al* (1995) had previously shown the value of phytosterols added to butter.

Concern over the use of these additives lies in two main areas. Sterol esters eaten in amounts necessary to be effective raise the concentration of these sterols in plasma (Jones *et al*, 2000). By contrast, consuming sitostanol esters lowers plasma plant sterol levels (Vanhannen *et al*, 1993). The subsequent metabolism of sitosterol and its excretion through the biliary tract is generally rapid (Salen *et al*, 1970). We have also observed significant increments in plasma sitosterol (+35%) and of campesterol (+51%; Table 3), possibly reflecting their relative absorption rates (Heinemann *et al*, 1993). As shown by others (Vanhannen *et al*, 1993), sitostanol esters will reduce the absorption of plant sterols as well as of cholesterol and this study has also shown a significant reduction in plasma sitosterol (−22%) during the consumption of non-esterified stanols. Whether the different effects on the absorption of plant sterols impact on health is unknown, but the concentration in plasma of sitosterol is only 0.1% that of cholesterol.

Our source of sterols had a sitosterol:campesterol ratio of 5:2. It has now been shown that the precise mix of these sterols as hydrogenated stanols is not a major determinant of their LDL cholesterol-lowering capacity (Gylling & Miettinen, 1999; Plat & Mensink, 2000). Further, the mix of sterols and sitostanol in tall oil, which differs importantly from that in soybean, leads to LDL cholesterol-lowering of the same order as that found with soybean derived sterols (Jones *et al*, 1999).

The other parameter of cholesterol metabolism, the plasma lathosterol concentration, showed, as expected, an increase in cholesterol synthesis in response to the sterol esters (Table 3). The 20% increase in plasma lathosterol with sterol ester consumption is in line with reported increments after sitostanol esters (Vanhannen *et al*, 1993). This cholesterol precursor reflects increased cholesterol synthesis in response to the diminished absorption of cholesterol. The failure of plasma lathosterol to change during the non-esterified stanol period is at first sight inconsistent with the significant decrease in plasma sitosterol which shows that there had been reduction in cholesterol absorption. Howell *et al* (1998) have shown more directly that the fractional synthetic rate of cholesterol is increased with greater consumption of non-esterified phytoesterols, although Jones *et al* (1999) failed to find such an increase despite a 15% reduction in LDL cholesterol when a mixture of non-esterified saturated (sitostanol) and unsaturated (sitosterol/campesterol) phytosterols was eaten. The compensation in cholesterol synthesis seen in most studies does not, however, appear to stimulate lipoprotein secretion, Gylling & Miettinen (1994) having reported a 20% fall in LDL apolipoprotein B production with 3 g daily of sitostanol ester in subjects with non-insulin-dependant diabetes mellitus.

The optimal dosage of stanols and probably also of sterols in their esterifed forms appears to be about 2 g daily above which little further benefit appears to accrue (Normen *et al*, 2000). However recently, Ostlund *et al*, (1999) have shown that when administered within lecithin micelles, as little as 0.3 g of non-esterified sitostanol reduced the absorption of cholesterol by 34%, compared with only 11% achieved with 1 g of sitostanol powder.

Interference with fat-soluble vitamins has been a theoretical concern over the use of these compounds. However, only some carotenoids have been reported to be significantly lowered, but when corrected for the lower concentrations of plasma lipids such reductions have often failed to be statistically significant (Gylling & Miettinen, 1999; Hallikainen *et al*, 1999). In the present analysis of plasma carotenoids, none was reduced in concentration with the sterol ester foods. This may relate to the generally high consumption of fruit and vegetables by Australians, especially those on cholesterol-lowering diets.

A limitation of study 1 is that the control foods were eaten initially by all subjects and only the two test diets were randomised. This raises the possibility of regression towards the mean for the lipid concentrations, a phenomenon that has affected cholesterol levels in some trials. However, the present subjects had been on a cholesterol-lowering diet for some time before entering the trial and furthermore the combined run-in and control periods, during which unsupplemented foods were eaten, lasted 6 weeks. Such a period is generally adequate for cholesterol concentrations to reach steady states.

This report is to our knowledge the first demonstration of the LDL cholesterol-lowering efficacy of either phytosterol esters or non-esterifed phytostanols delivered in bread and breakfast cereal. The magnitude of the response was in the upper range of LDL cholesterol-lowering reported by others with similar dosages, making it unlikely that the response was solely attributable to the 0.8 g sterols/stanols in the margarine. The similar reduction in LDL cholesterol when sterol esters were delivered in a 50% butterfat spread further extends the range of effective foods.

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