British Journal of Nutrition (2001), **86**, 233–239
DOI: 10.1079/BJN2001395

Effects of yoghurt enriched with plant sterols on serum lipids in patients with moderate hypercholesterolaemia

Roberto Volpe[1], Leena Niittynen[3], Riitta Korpela[3,4]*, Cesare Sirtori[2], Antonello Bucci[1], Nadia Fraone[2] and Franco Pazzucconi[1]

[1]*Institute of Systematic Medical Theraphy, University of Rome 'La Sapienza', Rome, Italy*
[2]*Institute of Pharmacologiacal Sciences, University of Milan, Milan, Italy*
[3]*Valio Ltd, Research Centre, Po Box 30, FIN-00039 Helsinki, Finland*
[4]*Foundation for Nutrition Research, Helsinki, Finland*

(Received 17 June 1999 – Revised 14 March 2001 – Accepted 19 March 2001)

The objective of the present study was to assess the effect of consumption of a yoghurt-based drink enriched with 1–2 g plant sterols/d on serum lipids, transaminases, vitamins and hormone status in patients with primary moderate hypercholesterolaemia. Thirty patients were randomly assigned to one of two treatment groups: a low-fat low-lactose yoghurt-based drink enriched with 1 g plant sterol extracted from soyabean/d *v.* a low-fat low-lactose yoghurt, for a period of 4 weeks. After a 2-week wash-out period, patients were crossed over for an additional 4-week period. Second, after a 4-week wash-out period, eleven patients were treated with 2 g plant sterols/d in a second open part of the study for a period of 8 weeks. The yoghurt enriched with plant sterols significantly reduced, in a dose-dependent manner, serum total cholesterol and LDL-cholesterol levels and LDL-cholesterol:HDL-cholesterol ($P<0{\cdot}001$), whereas no changes were observed in HDL-cholesterol and triacylglycerol levels, either in the first or the second part of the study. There were only slight, not statistically significant, differences in serum transaminase, vitamin and hormone levels. To conclude, a low-fat yoghurt-based drink moderately enriched with plant sterols may lower total cholesterol and LDL-cholesterol effectively in patients with primary moderate hypercholesterolaemia.

Plant sterols: Hypercholesterolaemia: Sex hormones: Fat-soluble vitamins

Hypercholesterolaemia is a major risk factor for the premature development of CHD (Stamler *et al.* 1986; Anderson *et al.* 1987). Dietary modification is the first step in all lipid-lowering regimens and is useful for lowering total cholesterol (TC) and LDL-cholesterol (LDL-C) in patients with mild hypercholesterolaemia (TC levels between 5·2 and 6·2 mmol/l: Consensus Development Conference, 1985). On the other hand, for patients with moderate hypercholesterolaemia (TC levels between 6·2 and 7·8 mmol/l) or severe hypercholesterolaemia (TC levels >7·8 mmol/l) dietary therapy is often not adequate on its own, and drug therapy is required for optimal reduction (Volpe *et al.* 1992). Various drugs traditionally used to reduce concentrations of TC and LDL-C both in primary prevention studies, e.g. resins (Lipid Research Clinics Program, 1984), fibrates (Frick *et al.* 1987) and statins (Shepherd *et al.* 1995), and secondary prevention studies, e.g. statins (Sacks *et al.* 1996; Scandinavian Simvastatin Survival Study Group, 1994; The Long-term Intervention with Pravastatin in Ischaemic Disease (LIPID) Study Group, 1998), have brought about significant reductions of the incidence of coronary events. However, drug treatment may be associated with adverse effects and may also be costly. Furthermore, in primary prevention evidence of the reduction of total mortality in long-term studies is still not available (Lipid Research Clinics Program, 1984; Frick *et al.* 1987; Shepherd *et al.* 1995). Moreover, the benefit of long-term treatment with statins in hypercholesterolaemic premenopausal women is not well documented, and those patients of childbearing age may be at risk of faetal malformations (Ghidini *et al.* 1992). Thus, non-pharmacological treatment is considered to be an important alternative for patients with moderate hypercholesterolaemia or for hypercholesterolaemic premenopausal women.

An interesting alternative among non-pharmacological treatments of hypercholesterolaemia is the use of plant

Abbreviations: HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol; TC, total cholesterol.

* **Corresponding author:** Dr R. Korpela, fax + 358 10381 3019, email riitta.korpela@valio.fi

sterols which are analogues of animal sterols. The major dietary sources of plant sterols are seeds and oils, but the sterol content of plants varies with their geographic location and climate. The most common plant sterols are β-sitosterol, campesterol and stigmasterol (Pollak, 1985).

The intake of plant sterols in a Western diet is 200–400 mg/d (Jones *et al.* 1997), and a vegetarian diet may provide twice that amount (Ling & Jones, 1995). Plant sterols are obtained from the diet by intestinal absorption. The absorption rate for sitosterol is about 5 %, but higher absorption rates of 10 % have been reported for campesterol (Salen *et al.* 1970; Heinemann *et al.* 1993). Plant sterols apparently inhibit the intestinal absorption of cholesterol, possibly by displacing cholesterol from micelles and thus reducing absorption (Ikeda *et al.* 1989). In earlier studies very large doses (up to 50 g/d) of plant sterols were used to reduce cholesterolaemia (Best *et al.* 1955). Later, smaller doses (3–18 g/d) were reported to reduce serum TC and LDL-C by 10–20 % (Grundy & Mok, 1976; Schlierf *et al.* 1978; Becker *et al.* 1992, 1993). The maximum effect was obtained with 3 g plant sterols/d (Grundy & Mok, 1976; Lees & Lees, 1976; Lees *et al.* 1977). In addition, Pelletier *et al.* (1995) reported that plant sterol levels as low as 1 g/d can reduce cholesterolaemia.

Plant sterols are poorly absorbed, and are believed to be free of adverse effects. High doses of plant sterols may reduce serum levels of fat-soluble vitamins (Gylling *et al.* 1996; Weststrate & Meijer, 1998), and very high doses of plant sterols may affect reproduction in animals (Malini & Vanithakumar, 1993; MacLatchy & Van der Kraak, 1995).

The aim of the present study was to examine the effect of a yoghurt-based drink moderately enriched with plant sterols (1 g/d) on serum cholesterol in patients with primary moderate hypercholesterolaemia, in a randomized double-blind cross-over trial. In addition, the effect of 2 g plant sterols/d on transaminases, vitamins and hormone status was investigated to obtain further information on the efficacy, tolerability and safety of the product.

Materials and methods

The effects of yoghurt-based drinks moderately enriched with plant sterols (1 g/d) on serum lipids were examined in outpatients with primary moderate hypercholestrolaemia attending the Lipid Clinics of Rome and Milan Universities, in a randomized double-blind cross-over trial. In addition, the effects of 2 g plant sterol/d on serum lipids and transaminases, vitamins and hormonal status in a subgroup of patients were examined. Nutritional instructions to patients to follow a lipid-lowering diet were enforced. All patients were trained individually, by a dietitian, to achieve or maintain an acceptable body weight and to consume a diet low in total fat (≤30 % energy) and in saturated fat (≤10 % energy), with a daily cholesterol intake of <300 mg. The recommendations adhered to the dietary goals established by the American Heart Association Step I diet (National Cholesterol Education Program Expert Panel, 1993). After this 8-week low-fat low-cholesterol diet, thirty patients started the yoghurt treatment (twenty-one males, nine females, age range 33–69 years, with TC values of between 6·2–7·8 mmol/l confirmed at weeks −2 and 0). None of the patients suffered from CHD, hypertension, diabetes or obesity. All subjects had normal fasting glucose levels and exhibited normal liver, renal and thyroid functions. None of the patients used drugs with documented lipid-modifying effects, such as diuretics, beta-blockers, corticosteroids, sex steroids or anti-fungal agents.

Patients were randomly assigned to one of two treatment groups for a period of 4 weeks: a low-fat (1 % (w/w) fat) low-lactose yoghurt-based drink enriched with a 1 g plant sterol extract from soyabean per portion (100 ml) *v.* a low-fat low-lactose yoghurt (100 ml). After a 2-week wash-out period, the patients were crossed over for an additional 4-week period. The nutrient content of the yoghurt (one dose) was: energy 384 kJ, carbohydrates 15 g, protein 3 g, fat 2 g, cholesterol 5 mg. The sterol-enriched yoghurt also contained a 1·08 g plant sterol mixture consisting of 85–95 % (w/w) plant sterols, of which 37–55 % was β-sitosterol, 20–30 % campesterol and 15–25 % stigmasterol.

The dietary intake was assessed at the beginning (weeks 0 and 6) and at the end (weeks 4 and 10) of each treatment period and at the end of the follow-up period (week 12) by means of a 3 d food record (two working days and one day of rest) and by body-weight control. Patients were asked to record detailed descriptions of all food and beverages consumed (ingredients, methods of preparation, cooking) and to give quantities using weights or household measurements from a standardized list. Nutrient intakes were calculated using a computer analysis programme of the Italian National Institute of Nutrition (Instituto Nazionale della Nutrizione, 1996), and total energy intake was calculated using the Atwater factors (Atwater & Bryant, 1989).

After a 4-week wash-out period, eleven of the thirty patients (seven males, four females, age range 34–69 years) were chosen at random to be treated with 2 g plant sterols/d for a period of 8 weeks in a second open part of the study.

The aim and modalities of the study were explained carefully to all patients, and signed informed consent was obtained from each patient.

Every 2 weeks the patients attended the clinic, where a blood sample (after at least a 12 h fast) was taken for serum lipid determination. At each visit arterial blood pressure was taken and patients were asked about their physical activities, the occurrence of adverse effects, transient diseases and use of drugs. Compliance with the treatment was checked by counting the returned yoghurts, and compliance with the diet was checked by food records and body-weight control.

Serum TC and triacylglycerols were measured by an automated enzyme method (Roschlau *et al.* 1974; Wada *et al.* 1979). HDL-cholesterol (HDL-C) was determined after precipitation of apolipoprotein B-containing lipoproteins with heparin–$MgCl_2$ (Warnich *et al.* 1979), and LDL-C was calculated according to Friedewald's equation (Friedewald *et al.* 1972). Blood samples for serum transaminases, hormones (gonadotropins, testosterone, oestradiol) and fat-soluble vitamins (vitamins A, D and E) were taken at the beginning and at the end of the second part of the study and stored frozen (−70°C). Measurements were made at United Laboratories Ltd, Helsinki.

Data are expressed as means and standard deviations. Changes were analysed by Student's '*t*' test. CI (95 %) were

Table 1. Serum lipids (mmol/l) for thirty patients with primary moderate hypercholesterolaemia at baseline and at the end of the 4-week treatment periods when they consumed a low-fat yoghurt-based drink with or without (placebo) 1 g plant sterol/d*
(Mean values and standard deviations and 95 % CI)

	Baseline		Sterol				Statistical significance of difference from baseline: *P*	Baseline		Placebo				Statistical significance of difference from baseline: *P*
	Mean	SD	Mean	SD	Δ%	95 % CI		Mean	SD	Mean	SD	Δ%	95 % CI	
Total cholesterol	6·83	0·4	6·37	0·5	−6·7	−27·5, −8·1	0·0005	6·87	0·5	6·71	0·7	−2·3	−18·3, +5·3	0·3
LDL-cholesterol	4·67	0·4	4·15	0·7	−11·1	−31·2, −8·6	0·0009	4·72	0·5	4·49	0·8	−4·9	−22·4, +4·2	0·2
HDL-cholesterol	1·37	0·3	1·47	0·4	+7·3	−3·7, +10·9	0·3	1·37	0·4	1·42	0·4	+3·6	−5·7, +9·7	0·6
LDL-cholesterol: HDL-cholesterol	3·64	1·0	3·09	1·1	−15·1	−11·0, −0·1	0·05	3·78	1·3	3·45	1·2	−8·7	−9·6, +3·0	0·3
Triacylglycerols	1·96	0·6	1·88	0·6	−4·1	−29·9, +17·2	0·6	2·03	0·7	1·98	0·7	−2·5	−33·3, 25·2	0·8

Δ%, Percentage difference between values at baseline and those after treatment.
*For details of subjects and procedures, see p. 234.

constructed based on arithmetic means or medians, as appropriate. The period, treatment and carry-over effects were also calculated. To reject a null hypothesis, $P<0{\cdot}05$ was considered statistically significant.

Results

Part 1 of the study

Serum lipids. The effects of the yoghurts on the serum lipids of the patients with primary moderate hypercholesterolaemia are shown in Table 1. When compared with baseline values the consumption of yoghurt enriched with plant sterols was shown to significantly reduce serum TC (−6·7 %, $P=0{\cdot}0005$) and LDL-C levels (−11·1 %, $P=0{\cdot}0009$) and LDL-C:HDL-C (−15·1 %, $P=0{\cdot}05$). No differences were observed in HDL-C and triacylglycerol levels. The period effect and the carry-over effect were not statistically significant.

Nutrient intake. Table 2 summarizes nutrient intakes based on the average intake over 3 d at the beginning and at the end of each active period and at the end of the follow-up period. Nutritional analysis shows that patients were able to keep their nutrient intake low in fat and low in cholesterol over the 12 weeks of the study. No statistically significant differences were found between periods, except for the cholesterol intake. However, the intake of cholesterol was always <300 mg/d. All patients ingested <30 % total energy as total fat and <10 % total energy as saturated fat. Total energy intake was <8·4 MJ/d and the mean BMI remained stable (Table 2) during the study, confirming that the patients complied with the dietary recommendations.

Part 2 of the study

After the 4-week wash-out period, eleven of the thirty patients took part in an open trial, during which the daily dose of the yoghurt enriched with plant sterols was doubled to 2 g plant sterols in order to obtain further information on the efficacy, safety and tolerability of the sterol enrichment.

The results of the effects of 2 g plant sterols/d on serum lipids (Table 3) show that the cholesterol-lowering effect of plant sterols is dose dependent; a greater reduction in TC, LDL-C and LDL-C:HDL-C values was observed after the daily dose of two yoghurts (2 g plant sterols). Percentage decreases in TC, LDL-C and LDL-C:HDL-C were respectively 11·2 % ($P<0{\cdot}001$), 15·6 % ($P<0{\cdot}001$) and 13·3 % ($P=0{\cdot}03$). No statistically significant effects were observed in HDL-C or triacylglycerol levels. With reference to the National Cholesterol Education Program Expert Panel guidelines (1993), nine of the eleven patients reached an LDL-C level of <4·2 mmol/l at week 4 and ten of the eleven patients reached this level at week 8, compared with eighteen of the thirty patients in part 1 of the study.

The effects of 2 g plant sterols on liver enzymes and vitamin and hormone levels are shown in Table 4. There were only slight, not statistically significant, differences in serum transaminases or vitamin A and E levels between those at the baseline and those at the end of the treatment period. However, a significant increase in the vitamin D level was seen after the treatment with plant sterols

Table 2. Nutrient intake from 3 d food records and BMI for thirty patients with primary moderate hypercholesterolaemia who consumed a low-fat yoghurt-based drink with or without (placebo) 1 g plant sterol/d during the 4-week treatment periods (weeks 0–4 and 6–10)†
(Mean values and standard deviations)

Week of study…	0				4				6				10				12			
Treatment group…	Sterol		Placebo		Sterol		Placebo		Sterol		Placebo		Sterol		Placebo		Sterol		Placebo	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Total energy (kJ)	7599	729	7750	577	7478	840	7825	502	7716	602	7700	577	7541	660	7758	585	7633	677	7674	627
Saturated fat (%)	6	1	5	2	5	2	5	2	7	1	5	2	6	1	6	2	6	2	6	1
Polyunsaturated fat (%)	8	2	8	3	9	2	8	3	8	3	9	3	10	2	9	3	7	3	8	3
Monounsaturated fat (%)	12	2	12	2	11	3	12	2	12	3	13	2	11	3	13	2	12	2	12	2
Cholesterol (mg)	207	53	228	44	191*	67	239*	51	220	48	229	53	198*	59	238	61	216	47	221	50
Carbohydrate (%): Complex	56	4	56	4	56	5	55	5	54	5	55	6	55	5	54	5	56	4	55	5
Sugar	2		2		1		2		1		1		1		1		1		1	
Protein (%)	16	3	17	3	18	3	18	2	18	3	17	3	17	4	17	3	18	3	18	3
Alcohol (wine: g)	16	2	13	3	15	3	14	3	15	3	13	4	16	2	14	3	15	2	13	3
Vitamin A (μg)	505	35	503	45	511	42	515	51	501	49	511	54	520	46	498	45	501	49	508	54
Vitamin D (μg)	4	1	5	1	5	0	6	1	4	1	6	0	5	0	5	0	4	1	5	1
Vitamin E (mg)	12	2	11	3	13	2	10	3	12	1	11	2	12	2	10	3	11	3	11	2
BMI (kg/m^2)	24·6	2	24·1	2	24·5	2	24·2	2	24·7	2	24·3	2	24·5	2	24·5	2	24·3	2	24·2	2

Mean values were significantly different from those for the placebo group: * $P<0·05$.
† For details of subjects and procedures, see p. 234.

($P=0·008$). There were no significant changes in serum hormone (follicle-stimulating hormone, sex hormone-binding globulin, oestradiol and testosterone) values before and after the treatment period.

Compliance

The taste of the yoghurt was well accepted by all the patients, who could not distinguish it from the low-fat yoghurt without plant sterols. Compliance was more than 95 % during all treatment periods, and both yoghurts were well tolerated. All subjects completed both parts of the study.

Discussion

Our study suggests that a yoghurt-based drink moderately enriched with plant sterols is effective in reducing cholesterol levels in primary moderate hypercholesterolaemia.

The treatment period lasted 4 weeks, which should be long enough for the reduction of serum cholesterol levels. In previous studies a reduction in cholesterol was noted after 2–4 weeks of plant sterol consumption (Pollak, 1953; Farquhar *et al.* 1956; Pelletier *et al.* 1995; Weststrate & Meijer, 1998). The wash-out period lasted 2 weeks, i.e. long enough for the cholesterol values to return to their original levels in most of the subjects (Pollak, 1953; Farquhar *et al.* 1956). As these patients were considered to be unresponsive to diet therapy on its own (the therapeutic targets of a reduction in TC to $<6·2$ mmol/l and LDL-C to $<4·2$ mmol/l had not been achieved only by dietary modifications), the reduction in TC and LDL-C can be attributed mainly to plant sterols.

In the placebo-controlled double-blind cross-over study, a yoghurt-based drink enriched with 1 g plant sterols/d caused a significant decrease in serum TC and LDL-C. Expressed as a percentage, the decrease relative to baseline was 7 for TC and 11 for LDL-C. In addition, the double dose (yoghurt enriched with 2 g plant sterols/d) caused a greater reduction in TC (11 %) and LDL-C (16 %) relative to baseline. It should be noted that results for TC and LDL-C obtained in the second part of the study are similar to, if not better than, those obtained in long-term studies with cholestyramine (Lipid Research Clinics Program, 1984) and gemfibrozil (Frick *et al.* 1987), and not much lower than the results obtained with the lowest statin doses (Isaacsohn *et al.* 1998), indicating that the yoghurt could be used to delay the use of lipid-lowering drug therapy or could be given in combination with the lipid-lowering drugs, at least in moderate hypercholesterolaemia.

In other studies plant sterols (3–12 g/d) have reduced TC and LDL-C levels to within the ranges of 6–17 and 7–19 % respectively (Lees *et al.* 1977; Schlierf *et al.* 1978; Becker *et al.* 1992, 1993; Weststrate & Meijer, 1998). In several studies the maximum effect was obtained with 3 g/d (Lees & Lees, 1976; Grundy & Mok, 1976; Lees *et al.* 1977). However, Pelletier *et al.* (1995) found that 0·74 g plant sterol/d reduced serum TC and LDL-C by 10 and 15 % respectively, and Schlierf *et al.* (1978) found a smaller reduction (6–7 %) with 12 g plant sterols/d. The wide variation in response shows that, in addition to the

Table 3. Serum lipids (mmol/l) for eleven patients with primary moderate hypercholesterolaemia at baseline (week 0), during (week 4) and at the end (week 8) of the treatment period when they consumed a low-fat yoghurt-based drink with 2 g sterols/d*

(Mean values and standard deviations)

Measurement weeks…	0		4		8	
	Mean	SD	Mean	SD	Mean	SD
Total cholesterol	6·70	0·31	6·29	0·51	5·95	0·48
HDL-cholesterol	1·51	0·47	1·59	0·44	1·46	0·40
LDL-cholesterol	4·42	0·53	3·88	0·78	3·73	0·67
LDL-cholesterol:HDL-cholesterol	3·23	1·13	2·68	1·11	2·80	0·98
Triacylglycerols	1·79	0·56	1·80	0·68	1·66	0·41

* For details of subjects and procedures, see p. 234.

phytosterol dose, certain study conditions may promote a more efficient cholesterol-lowering effect. It has been suggested that the composition of the plant sterol mixture (Lees *et al.* 1977; Becker *et al.* 1993; Weststrate & Meijer, 1998), diet (Denke, 1994), characteristics of the study subjects and the type of lipid disorder (Miettinen & Vanhanen, 1994; Gylling *et al.* 1997) may influence the efficacy of plant sterols.

The cholesterol-lowering effect of plant sterols has usually been linked to sitosterol, but the sterol mixtures used in most of the studies have also contained campesterol and stigmasterol. In addition, results from studies of the cholesterol-lowering effect of sterol mixtures containing different amounts of campesterol, stigmasterol and sitosterol are contradictory. Lees & Lees (1976) reported that a sterol mixture containing 93 % plant sterols was far more effective than a mixture containing only 60 % sitosterol. However, the plant sterol mixture found to be effective in our study contained about 50 % sitosterol and 50 % campesterol and stigmasterol; a similar mixture of plant sterols was used with good results by Pelletier *et al.* (1995) and Westrate & Meijer (1998). On the other hand, plant sterol mixtures containing sitostanol seem to be more efficient than mixtures containing sitosterol, and a decrease of 11–20 % has been reported after the ingestion of 1·5–3 g sitostanol or sitostanyl ester (Heinemann *et al.* 1986; Vanhanen *et al.* 1993, 1994; Gylling *et al.* 1995, 1997; Miettinen *et al.* 1995).

In our study the cholesterol response to plant sterol treatment was obtained in patients on a low-fat low-cholesterol diet. The significant difference in cholesterol intake could not have influenced the serum cholesterol levels, because it was found in the same group of patients in both the active period and the placebo period. It has been suggested previously that plant sterols are ineffective if the diet is low in cholesterol (Denke, 1994). Our results confirm the hypothesis (Lees *et al.* 1977; Gylling *et al.* 1997) that plant sterols can inhibit the absorption not only of dietary, but also of biliary, cholesterol in the gastrointestinal tract. In our subjects saturated fatty acid intake was very low (6 % energy intake). This factor may improve the effect of plant sterol treatment, because saturated fatty acids increase

Table 4. Serum transaminase, vitamin and hormone levels for eleven patients with primary moderate hypercholesterolaemia at baseline (week 0) and at the end (week 8) of the treatment period when they consumed a low-fat yoghurt-based drink with 2 g sterols/d*

	Baseline (week 0)			After (week 8) treatment period			Difference from baseline		
	Mean	SD	Min–max	Mean	SD	Min–max	Mean	95 % CI	*P*§
Vitamin A	2·20	0·5	1·5–3·1	2·23	0·4	1·4–2·8	+0·03	−0·20, +0·25	0·79
Vitamin E	41·4	8·9	30–59	40·0	6·3	27–51	−1·4	−7·3, +4·6	0·62
Vitamin D	53·9	19·1	34–94	63·7	18·8	42–96	+9·8	+3·1, +16·5	0·008
AST (U/l)	24	8·2	14–30	26	7·9	15–37	−3·7 %		0·69
ALT (U/l)	26	10·3	13–36	25	9·8	13–38	−4·9 %		0·59
FSH: Female+male	25·2	36·5	2·4–99·2	21·5	31·2	1·5–98·9	−3·7	−14, 7	0·56
Female†	59·2	44·8	2·4–99·2	49·9	39·1	3·1–98·9	−0·93	−53, +35	0·72
Male‡	5·8	2·8	2·6–10·6	5·3	2·8	1·5–9·6	−0·56	−1·06, −0·05	0·06‖
SHGB: Female+male	45·3	18·0	13–79	44·8	18·7	15–82	−0·45	−3·3, +2·4	0·72
Female†	62·8	14·3	45–79	61·5	19·6	36–82	−1·25	−9·9, +7·4	1·00
Male‡	35·3	10·7	13–45	35·3	9·9	15–45	0·00	−3·4, +3·4	0·79
Oestradiol: Female†			<0·02–0·26			<0·02–0·24			
Testosterone: Male‡	15·5	4·4	10·8–19·5	17·0	6·3	8·3–26·2	+1·5	−1·3, 4·3	0·24

AST, aspartate transaminase; ALT, alanine transaminase; FSH, follicle-stimulating hormone; SHBG, sex hormone-binding globulin.
* For details of subjects and procedures, see p. 234.
† *n* 4.
‡ *n* 7.
§ Conjugated I test and Wilcoxon's test.
‖ FSH was reduced in six of seven cases.

cholesterol synthesis in the liver (Glatz & Katan, 1993) and may thus impair the cholesterol-lowering effect of plant sterols.

It seems that the efficacy of plant sterols is highly variable from patient to patient (Lees *et al.* 1977). In our study TC and LDL-C levels decreased by 1·6–19·3 and 3·0–42·4 % respectively after ingestion of yoghurt providing 2 g plant sterols/d. However, it should be noted that variability in effectiveness is commonly seen with any lipid-lowering drug (Lees *et al.* 1977).

In our study plant sterols were well tolerated and no adverse effects were reported. Several other studies have also shown that the oral supplementation with plant sterols is almost free from side effects (Farquhar *et al.* 1956; Heinemann *et al.* 1986; Becker *et al.* 1993). However, in some studies a few patients have complained of mild gastrointestinal symptoms such as constipation (Lees & Lees, 1976) or a decrease in appetite (Becker *et al.* 1992).

Plant sterols may reduce the serum levels of fat-soluble vitamins (Gylling *et al.* 1996; Weststrate & Meijer, 1998). We found no reduction in vitamin A, E or D levels in the eleven patients who ingested 2 g plant sterols/d for 8 weeks. On the contrary, there was a significant increase in vitamin D levels (P=0·008). The vitamin D content of the yoghurt used was about the same as that of skimmed milk, so it is unlikely that the yoghurt supplied this serum vitamin D. The increase in serum vitamin D level may be due to the fact that the treatment period of the second part of the study began in spring when more vitamin D is synthesized in the skin.

It has been reported that very high doses of plant sterols can affect reproductive tissue in animals (Malini & Vanithakumari, 1993; MacLatchy & Van der Kraak, 1995). However, the effect of plant sterols on human hormone status has not been reported previously. We found no significant changes in serum, oestradiol, follicle-stimulating hormone or sex hormone-binding globulin levels in the women, or testosterone, follicle-stimulating hormone or sex hormone-binding globulin levels in the men before and after the treatment with 2 g plant sterols/d. It appears that at least low doses of plant sterols are safe in the treatment of hypercholesterolaemia in adults. However, the safety of treatment with plant sterols must be investigated in long-term studies.

Conclusion

The results obtained in these short-term studies suggest that a natural simple nutritional self-care treatment with a low-fat yoghurt-based drink moderately enriched with plant sterols may effectively lower TC and LDL-C levels in patients with primary moderate hypercholesterolaemia who are considered unresponsive to dietary treatment alone, and for whom, if the global risk of cardiovascular disease is low, the aim is to delay statin therapy. In addition, simultaneous use of plant sterols and statin could be encouraged, because the combination appears to be more effective than statin alone, especially in subjects with low cholesterol synthesis and high cholesterol absorption (Gylling *et al.* 1997). The two approaches are complementary, and could together represent a coordinated strategy for reducing the risk of cardiovascular disease.

Cholesterol-lowering treatment with plant sterols appears to be safe. However, the adverse effects of treatment with plant sterols on serum transaminase, vitamin and hormone values need to be confirmed in longer-term studies.

References

Anderson KM, Castelli WP & Levy D (1987) Cholesterol and mortality. 30 years of follow-up from the Framingham Study. *Journal of the American Medical Association* **257**, 2176–2180.

Atwater WO & Bryant AP (1989) The availability and fuel value of food materials. *Report of the Storrs Agricultural Experimental Station* no. 1900, pp. 73–110. Storrs, CT: University of Connecticut.

Becker M, Staab D & von Bergmann K (1992) Treatment of severe familial hypercholesterolemia in children: effect of sitosterol and bezafibrate. *Pediatrics* **89**, 138–142.

Becker M, Staab D & Von Bergmann K (1993) Treatment of severe familiar hypercholesterolemia in childhood with sitosterol and sitostanol. *Journal of Pediatrics* **122**, 292–296.

Best MM, Duncan CH, van Loon EJ & Wathen JD (1955) The effect of sitosterol on serum lipids. *American Journal of Medicine* **15**, 61–70.

Consensus Development Conferences (1985) Lowering blood cholesterol to prevent heart disease. *Journal of the American Medical Association* **263**, 2080–2086.

Denke MA (1994) Lack of efficacy of low-dose sitostanol therapy as an adjunct cholesterol-lowering diet in men with moderate hypercholesterolemia. *American Journal of Clinical Nutrition* **61**, 392–396.

Farquhar JW, Smith RE & Dempsey ME (1956) The effect of beta sitosterol on the serum lipids of young men with arteriosclerotic heart disease. *Circulation* **14**, 77–82.

Frick MH, Elo O, Haapa K, Heinonen OP, Heinsalmi P, Helo P, Huttunen JK, Kaitaniemi P, Koskinen P, Manninen V *et al.* (1987) Helsinki Heart Study: primary prevention trial with gemfibrozil in middle-aged men with dyslipidemia. *New England Journal of Medicine* **317**, 1237–1245.

Friedewald WT, Levy RI & Fredrickson DS (1972) Estimation of the concentration of low density lipoprotein cholesterol in plasma without the use of ultracentrifuge. *Clinical Chemistry* **18**, 499–502.

Ghidini A, Sicherer S & Willner J (1992) Congenital abnormalities (VATER) in baby born to mother using lovastatin. *Lancet* **339**, 1416–1417.

Glatz JF & Katan MB (1993) Dietary saturated fatty acids increase cholesterol synthesis and fecal steroid excretions in healthy men and women. *European Journal of Clinical Investigation* **23**, 648–655.

Grundy SM & Mok HY (1976) Effects of low dose phytosterols on cholesterol absorption in man. In *Lipoprotein Metabolism*, pp. 112–118 [H Greten, editor]. Berlin, Heidelberg and New York: Springer.

Gylling H, Puska P, Vartiainen P & Miettinen TA (1996) Serum retinol α-tocopherol, carotenes and lipid peroxide production during serum cholesterol lowering by sitostanol ester margarine in a mildly hypercholesterolemic population. *Circulation* **94**, Suppl. 1, 578.

Gylling H, Radhakrishnan R & Miettinen TA (1997) Reduction of serum cholesterol in postmenopausal women with previous myocardial infarction and cholesterol malabsorption induced by dietary sitostanol ester margarine. *Circulation* **96**, 4226–4231.

Gylling H, Siimes MA & Miettinen TA (1995) Sitostanol ester margarine in dietary treatment of children with familial hypercholesterolemia. *Journal of Lipid Research* **36**, 1807–1812.

Heinemann T, Axtmann G & von Bergmann K (1993) Comparison of intestinal absorption of cholesterol with different plant sterols in man. *European Journal of Clinical Investigation* **23**, 827–831.

Heinemann T, Leiss O & von Bergmann K (1986) Effect of low-dose sitostanol on serum cholesterol in patients with hypercholesterolemia. *Atherosclerosis* **61**, 219–223.

Ikeda I, Tanabe Y & Sugano M (1989) Effects of sitosterol and sitostanol on micellar solubility of cholesterol. *Journal of Nutritional Science and Vitaminology* **35**, 361–369.

Isaacsohn J, Stein E, Weinstein R, Orchard T, Huh C, Whalen E & Ripen S (1998) Cerivastatin, a novel potent HMG-CoA reductase inhibitor: comparative efficacy versus fluvastatin. In *XIIIth International Symposium on Drugs Affecting Lipid Metabolism (DALM)*, pp. 62. Florence, Italy. Milan, Italy: Lorenzini Foundation.

Instituto Nazionale della Nutrizione (1996) *LARN Livelli di Assunzione Raccomandati di Enrgia e Nutrienti per la Popolazione Italiana* (*The enzymatic determination of serum total cholesterol*). Roma: Instituto Nationale della Nutrizione.

Jones PHJ, MacDougall DE, Ntanios F & Vanstone CA (1997) Dietary phytosterols as cholesterol lowering agents in humans. *Canadian Journal of Physiology and Pharmacology* **75**, 217–227.

Lees RS & Lees AM (1976) Effects of sitosterol therapy on plasma lipid and lipoprotein concentrations. In *Lipoprotein Metabolism*, pp. 119–124 [H Greten, editor]. Berlin, Heidelberg and New York: Springer.

Lees AM, Mok HYI, Lees RS, McLuskey A & Grundy SM (1977) Plant sterols as cholesterol-lowering agents: clinical trials in patients with hypercholesterolemia and studies of sterol balance. *Atherosclerosis* **28**, 325–338.

Ling WH & Jones PJH (1995) Minireview. Dietary phytosterols: a review of metabolism, benefits and side effects. *Life Sciences* **57**, 195–206.

Lipid Research Clinics Program (1984) The Lipid Research Clinics Coronary Primary Prevention Trial results: I and II. *Journal of the American Medical Association* **251**, 351–374.

MacLatchy DL & Van der Kraak GJ (1995) The phytoestrogen beta-sitosterol alters the reproductive endocrine status of coldfish. *Toxicology and Applied Pharmacology* **134**, 305–312.

Malini T & Vanithakumari G (1993) Effect of β-sitosterol on uterine biochemistry: A comparative study with estradiol and progesterone. *Biochemistry and Molecular Biology International* **31**, 659–668.

Miettinen TA, Puska P, Gylling H, Vanhanen H & Vartiainen E (1995) Reduction of serum cholesterol with sitostanol-ester margarine in a mildly hypercholesterolemic population. *New England Journal of Medicine* **333**, 1308–1312.

Miettinen TA & Vanhanen H (1994) Dietary sitostanol related to absorption, synthesis and serum level of cholesterol in different apolipoprotein E phenotypes. *Atherosclerosis* **105**, 217–226.

National Cholesterol Education Program Expert Panel (1993) Expert Panel on detection evaluation and treatment of high blood cholesterol in adults: summary of the second report of the National Cholesterol Education Program (NCEP) Expert Panel on detection evaluation and treatment of high blood cholesterol in adults (Adult treatment panel 2). *Journal of the American Medical Association* **34**, 1535–1544.

Pelletier X, Belbraouet S, Mirabel D, Mordret F, Perrin JL, Pages X & Derby G (1995) A diet moderate enriched in phytosterols lowers plasma cholesterol concentrations in normocholesterolemic humans. *Annals of Nutrition and Metabolism* **39**, 291–295.

Pollak OJ (1953) Reduction of blood cholesterol in man. *Circulation* **7**, 702–706.

Pollak OJ (1985) Effect of plant sterols on serum lipids and atherosclerosis. *Pharmacology and Therapeutics* **31**, 177–208.

Roschlau P, Bernt E & Gruber W (1974) Enzymatische Bestimmung des Gesamtcholesterins in Serum. *Zeitschrift für Klinische Chemie und Klinische Biochemie* **12**, 403–407.

Sacks FM, Pfeffer MA, Moye LA, Rouleau JL, Rutherford JD, Cole TG, Brown L, Warnica JW, Arnold JM, Wun CC, Davis BR & Braunwald E (1996) The effect of Pravastatin on coronary events after myocardial infarction in patients with average cholesterol levels. *New England Journal of Medicine* **335**, 1001–1009.

Salen G, Ahrens EH & Grundy SM (1970) Metabolism of β-sitosterol in man. *Journal of Clinical Investigation* **49**, 952–967.

Scandinavian Simvastatin Survival Study Group (1994) Randomized trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). *Lancet* **344**, 1383–1389.

Schlierf G, Oster P, Heuck CC, Raetzer H & Schellenberg B (1978) Sitosterolemia in juvenile type II hyperlipoproteinemia. *Atherosclerosis* **30**, 245–248.

Shepherd J, Cobbe SM, Ford I, Isles CG, Lorimer AR, MacFarlane PW, McKillop JH & Packard CJ (1995) Prevention of coronary heart disease with Pravastatin in men with hypercholesterolemia. *New England Journal of Medicine* **333**, 1301–1307.

Stamler J, Wentworth D & Neaton JD for MRFIT Research Group (1986) Is relationship between serum cholesterol and risk of premature death from coronary heart disease continuous or graded? Findings in 356222 primary screenees of the Multiple Risk Factor Intervention Trial (MRFIT). *Journal of the American Medical Association* **256**, 2823–2828.

The Long-term Intervention with Pravastatin in Ischaemic Disease (LIPID) Study Group (1998) Prevention of cardiovascular events and death with pravastatin in patients with coronary heart disease and a broad range of initial cholesterol levels. *New England Journal of Medicine* **339**, 1349–1357.

Vanhanen HT, Blomquist S, Ehnholm C, Hyvönen M, Jauhiainen M, Torstila I & Miettinen TA (1993) Serum cholesterol, cholesterol precursors, and plant sterols in hypercholesterolemic subjects with different apoE phenotypes during dietary sitostanol ester treatment. *Journal of Lipid Research* **34**, 1535–1544.

Vanhanen HT, Kajander J, Lehtovirta H & Miettinen TA (1994) Serum levels, absorption efficiency, faecal elimination and synthesis of cholesterol during increasing doses of dietary sitostanol esters in hypercholesterolaemic subjects. *Clinical Science* **87**, 61–67.

Volpe R, Angelico F & Arca M (1992) Effects of simple dietary advice on serum lipids in severe and moderate primary hypercholesterolemia. In *Treatment of Severe Dyslipoproteinemia in the Prevention of Coronary Heart Disease*, pp. 226–230 [A Gotto, M Mancini, WO Richter and P Schwandt, editors]. Basel: Karger.

Wada T, Omuro K & Shirai K (1979) Assay of triglycerides on use of alfa-glycerophosphate oxidase. *Eisei-Kensa* **29**, 367.

Warnich GR, Cheung MC & Albers JJ (1979) Comparison of current methods for high density lipoprotein cholesterol quantitation. *Clinical Chemistry* **25**, 596–604.

Weststrate JA & Meijer QW (1998) Plant sterol-enriched margarines and reduction of plasma total- and LDL-cholesterol concentrations in normocholesterolaemic and mildly hypercholesterolaemic subjects. *European Journal of Clinical Nutrition* **52**, 334–343.