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ORIGINAL COMMUNICATION

Soy in hypercholesterolaemia: a double-blind, placebo-controlled trial

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Objective: To study whether Abacor®, a product based on isolated soy protein with high and standardised levels of isoflavones and cotyledon soy fibres, was more effective in lowering total and LDL cholesterol than placebo.
Design: Randomised, placebo-controlled, double-blind, parallel group, single centre study.
Setting: Primary care in Joensuu, North Karelia, Finland.
Subjects: Subjects were screened from the patient database of the health centre; 30 were randomised to the Abacor® group and 30 subjects to placebo. Eight subjects were withdrawn, six from the active group, two from the placebo group.
Intervention: The preparations were given as two daily liquid supplements in addition to the subjects' regular diets for 6 weeks.
Results: Abacor® showed a statistically significant lipid-lowering effect as compared to placebo, although an unexpected reduction was seen in the placebo group. The estimated difference between active treatment and placebo was 0.25 mmol/l (95% CI 0.01, 0.50; $P=0.049$) for total cholesterol, corresponding to reductions of 8.3 and 5.1%, respectively. The difference in reduction of LDL-cholesterol was 0.27 mmol/l (95% CI 0.06, 0.49; $P=0.014$) and corresponded to a reduction of 13.2% in the active treatment group, and 8.0% in the placebo group. Abacor® showed a rapid onset of effect, as compared with placebo. During a wash-out period of 4 weeks after treatment, the subjects returned to pre-treatment cholesterol levels.
Conclusion: Added to a regular diet, Abacor® significantly reduced LDL-cholesterol and total cholesterol. These beneficial effects occurred within 6 weeks of treatment.
Sponsorship: Commercial organisation.
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Introduction

Prospective, epidemiological studies have established that lipids and lipoproteins play a role as risk factors for atherosclerotic cardiovascular disease. It is generally recognised that the higher the total cholesterol, the higher the mortality of cardiovascular disease. The risk seems to be associated with the LDL-cholesterol fraction, while HDL-cholesterol seems to be protective. The important role of serum cholesterol and the impact of dietary changes is further underlined by population studies in Finland (Pietinen *et al*, 1996; Puska *et al*, 1995; Vartiainen *et al*, 1997).

At present, the most widely used lipid-lowering drugs are HMG-CoA-reductase-inhibitors (statins), bile acid resins, fibrates, nicotinic acid derivatives and fish oil concentrates with a high content of ω-3-fatty acids. Recently published studies generally conclude that statins are safe and that most adverse drug reactions are infrequent and non-serious. However, there are reports on increased hepatic enzymes, myopathies and psychiatric symptoms (Buajordet *et al*, 1997), and the risk of interaction with other drugs should not be underestimated. Developing other therapies with a different mode of action and with a milder tolerability profile would thus be beneficial.

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Contributors: **PP provided clinical input to study protocol and analysis; VK contributed practical organization of the trial at the North Karelia Project field office in Joensuu, Finland; LHH provided product composition and background product information; ES was responsible for design and statistical analysis; TL monitored the study according to ICH guidelines for GCP; KS held overall responsibility for project management and quality control.**
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Dietary changes are the most important non-pharmacological measures to reduce serum lipid levels. Food products with attempted beneficial effects, so-called 'functional foods', are being developed. Such products should be documented according to scientific principles and regulatory guidelines, as if they were drugs. A recent commentary (Katan, 1999) concluded that better regulation of health claims for foods is needed. The current legislation focuses on safety rather than efficacy, and most documentation of nutraceuticals has so far been uncontrolled or anecdotal.

It has been postulated that soy isoflavones may be one factor preventing Asian populations from developing certain diseases such as breast, colorectal and prostate cancer as well as coronary heart disease (Adlercreutz, 1998), and the preventive role of soy protein and soy fibre in cardiovascular disease has been in focus. A meta-analysis of 38 clinical trials (Anderson *et al*, 1995) concluded that the consumption of soy protein significantly decreases concentrations of total cholesterol, LDL-cholesterol and triglycerides in the circulation. Recently, the US Food and Drug Administration has concluded that soy protein, included in a diet low in saturated fat and cholesterol, may reduce the risk of coronary heart disease by lowering blood cholesterol levels (US FDA, 1999).

The mechanism of action is not known, but various proposals have been put forward (Anthony *et al*, 1998; Kirk *et al*, 1998; Potter, 1998; Sirtori *et al*, 1998). These mechanisms have included the effects of soy protein and/or soy isoflavones: (i) in the lowering of cholesterol levels by increasing LDL receptor activity; (ii) as antioxidants; (iii) as anti-proliferative and anti-migratory agents on smooth muscle cells; (iv) on thrombus formation; (v) in maintenance of normal vascular reactivity; and (vi) in improvement of endothelial function.

The aim of the present study was to explore the lipid-lowering effects of Abacor® (isolated soy protein with high and standardised levels of isoflavones and cotyledon soy fibre) *vs* a placebo control product.

Methods

Eligibility

Subjects were screened from the patient database of the Joensuu Health Centre area in North Karelia, Finland. Eligible subjects had to meet the following selection criteria: total serum cholesterol concentration 7.0–9.9 mmol/l; serum triglycerides < 4.5 mmol/l; age 18–70 y for men and 45–70 y for postmenopausal (defined as no vaginal bleeding within the last 6 months prior to study) women; written informed consent; no significant signs of cardiovascular, renal, hepatic, endocrine or gastrointestinal disease; no familiar hypercholesterolaemia; no type 1 or 2 diabetics treated with insulin; no past or concomitant use of statins, ω-3-fatty acids or other lipid lowering drugs during the last 8 weeks before randomisation; no hormone replacement therapy within the past 6 months; no drug or alcohol dependency; no eating disorder; no plans to lose weight during the study; and no clinically significant lactose intolerance.

Design, randomisation and blinding

This was a randomised, placebo-controlled, double-blind, parallel-group, single-centre study. Thirty patients per treatment group were included. This ensured that the power to detect a 10% difference in total cholesterol between the two study groups would be at least 90% with a 5% significance level (two-sided). The randomisation scheme was generated by a computerised procedure. Neither the investigators nor the patients knew the randomisation code, block size or the results of the blood lipid concentrations until after the statistical analysis. Furthermore, the statistical analyses were conducted before breaking the randomisation code.

Intervention and procedures

In the screening visit, conducted 3–21 days before randomisation, all subjects received standardised dietary advice in order to reduce the variability of their baseline lipid values. It was attempted to keep the calorie intake as constant as possible by advising the subjects to reduce their morning and evening meal by approximately the same amount of calories as they received through the study nutrients. There was, however, no detailed diary recording of calorie intake in any of the study phases. After randomisation, all subjects continued the intake of study nutrients for 6 weeks with weekly visits to the study nurse for weight control and blood sampling for lipid measurements. Total cholesterol, HDL-cholesterol and triglycerides were measured every week, whereas homocysteine, lipoprotein (a) and apolipoprotein B were measured at baseline and after 6 weeks of treatment. Blood samples were taken 5 min after supine rest in fasting state at the start of all visits. All lipids were analysed at the Department of Biochemistry of the National Public Health Institute, Helsinki, Finland. The concentrations of LDL-cholesterol were calculated according to Friedewald *et al* (1972). After discontinuation of the study nutrient, there was a wash-out period for 4 weeks in which the subjects were allowed to return to their regular pre-study diet.

Each Abacor® sachet contained isolated soy protein 26.0 g, standardised as 3.7 mg isoflavones per gram soy protein, cotyledon soy fibre 7.75 g (manufactured by Protein Technology International Inc., St. Louis, USA), lechinated fat reduced cocoa 5.77 g and soy lecithin 2.2 g (Contract Foods Ltd, Birmingham, UK). The isoflavones in Abacor® are natural parts of the soy protein and are not isolated and then added. Cotyledons are dehulled and defatted cell wall structures of the soy bean. The matching placebo was based on calcium caseinate, whole milk powder and cellulose with equal protein and fibre content (New Zealand Milk Products Ltd, Surrey, UK; Allchem Int. Ltd, Berkshire, UK and Contract Food Ltd, Birmingham, UK). In terms of energy intake, the

active treatment had 210 kcal (891 kJ) and the placebo had 218 kcal (922 kJ) per serving.

The study nutrient powder was mixed with cold tap water. The study subjects were advised to take one study nutrient sachet in the morning and one in the evening as a replacement of about half of the normal breakfast and evening meal. Compliance with the treatment schedule was checked by counting all returned sachets.

Statistical analysis

The statistical analyses were conducted before breaking the randomisation code. The change in serum lipids over time was analysed by a model for unbalanced repeated measurements (BMDP5V, Dixon, 1992). Parameters were estimated by the method of restricted maximum likelihood, and a compound symmetry covariance structure was assumed. The model included time and treatment group as main effects and in addition the interaction between treatment and time. The last measurement before the start of treatment was included as a covariate in the model. Total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides were analysed separately. Analysis of covariance was used to estimate the effect of treatment on homocysteine, lipoprotein (a) and apolipoprotein B. Active treatment and placebo have been compared for a total of seven efficacy variables. The *P*-values presented are not adjusted for multiple significance tests.

Ethics and administration

The study protocol and informed consent form were approved by the Ethics Committee of the Joensuu Health Centre. An independent contract research organisation (Smerud Medical Research AS) was responsible for monitoring the study according to the Good Clinical Practice principles.

Results

From the group of 60 subjects randomised, six subjects in the Abacor® group, and two subjects in the placebo group withdrew within 2 weeks because of gastro-intestinal symptoms. Fifty-two subjects thus received treatment for at least 2 weeks after randomisation (Figure 1). Their baseline characteristics are presented in Table 1, and Table 2 shows the mean effects and differences between the two treatments. The reported results are based on the modified intention-to-treat population ($n = 52$ subjects). Similar results were also seen with the intention-to-treat population ($n = 60$) and the per protocol population ($n = 38$).

Total cholesterol

Total cholesterol was significantly reduced with time in both groups ($P < 0.001$). The mean reduction from baseline in the active treatment group was 0.64 mmol/l and in the placebo group 0.39 mmol/l, corresponding to 8.3 and 5.1%, respectively (Table 2). The estimated difference between active treatment and placebo was 0.25 mmol/l ($P = 0.049$). As can be seen from Figure 2, the lipid-lowering effects seem to occur earlier in the active group. Four weeks after stopping the study nutrient, both groups had returned to their baseline values.

LDL cholesterol

Both treatment groups showed a reduction in LDL-cholesterol over time ($P < 0.001$). Here, the mean reduction from

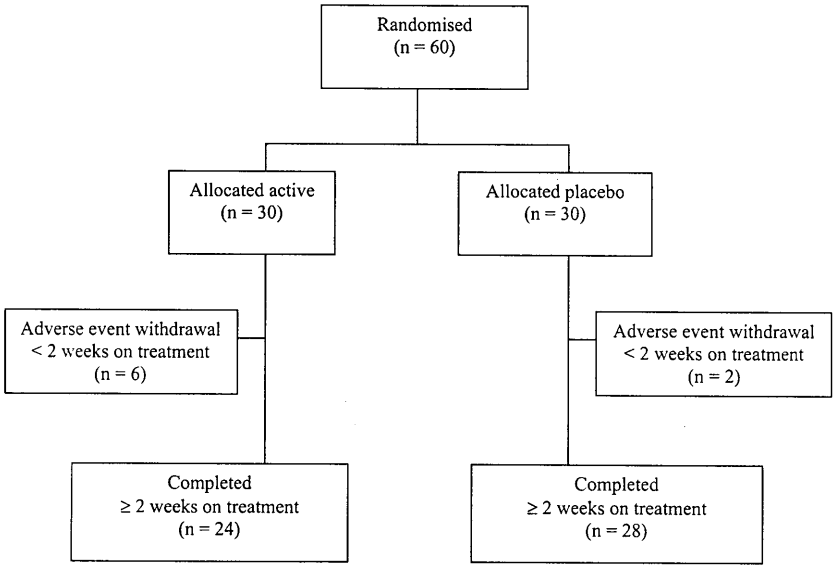

Figure 1 Trial profile.

Table 1 Baseline characteristics

		Active	*Placebo*
No. patients		24	28
Males		16	15
Females		8	13
Age	Median (range)	52 (35–70)	59 (38–70)
Weight			
Males	Mean ± s.d. (kg)	84 ± 11	85 ± 10
Females	Mean ± s.d. (kg)	67 ± 7	71 ± 8
Baseline measurement			
Total cholesterol	Mean ± s.d. (mmol/l)	7.50 ± 0.57	7.68 ± 0.54
LDL-cholesterol	Mean ± s.d. (mmol/l)	5.13 ± 0.48	5.15 ± 0.74
HDL-cholesterol	Mean ± s.d. (mmol/l)	1.58 ± 0.44	1.56 ± 0.43
Triglycerides	Mean ± s.d. (mmol/l)	1.76 ± 0.78	1.81 ± 1.30
Homocysteine	Mean ± s.d. (μmmol/l)	10.52 ± 3.68	9.65 ± 2.27
Apolipoprotein B	Mean ± s.d. (g/l)	1.32 ± 0.22	1.42 ± 0.28
Lipoprotein (a)	Mean ± s.d. (g/l)	272 ± 341	341 ± 495

Table 2 Mean effects and differences between treatments

Variable	*Mean reduction, active treatment*[a]	*Mean reduction, placebo*[a]	*Estimated difference, active − placebo*	*95% confidence interval for mean difference*	*P-value*
Total cholesterol (mmol/l)	0.64	0.39	0.25	(0.01, 0.50)	0.049
LDL-cholesterol (mmol/l)	0.68	0.41	0.27	(0.06, 0.49)	0.014
HDL-cholesterol (mmol/l)	− 0.09	− 0.08	− 0.01	(− 0.10, 0.09)	0.904
Triglycerides (mmol/l)	0.13	0.19	− 0.06	(− 0.35, 0.22)	0.663
Homocysteine (μmol/l)	0.32	− 1.42	1.74	(0.87, 2.60)	< 0.001
Lipoprotein (a) (g/l)[b]	− 57.2	− 13.6	− 43.6	(− 111.0, 23.9)	0.212
Apolipoprotein B (g/l)	0.10	0.10	0.00	(− 0.09, 0.09)	0.997

[a]Negative values correspond to an increase from baseline.
[b]Missing values for four patients in the placebo group.

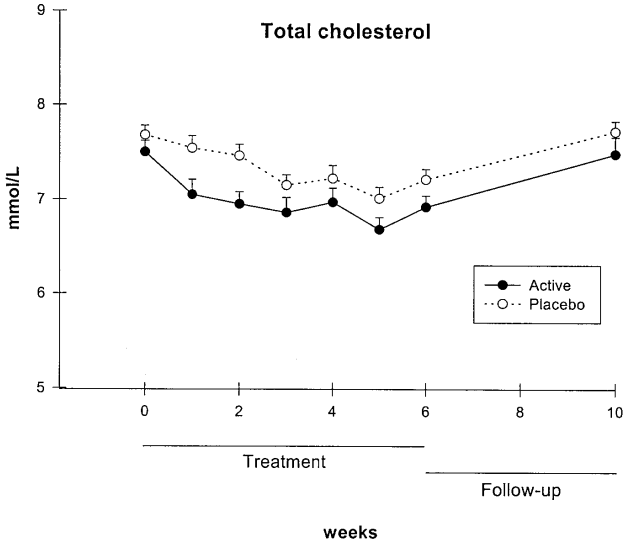

Figure 2 Total cholesterol. Error bars = s.e.

baseline was 0.68 mmol/l in the Abacor® group and 0.41 mmol/l in the placebo group (Table 2). The difference between treatment groups (0.27 mmol/l) was statistically significant ($P = 0.014$). The reduction of LDL-cholesterol corresponded to 13.2% in the active treatment group, and 8.0% in the placebo group. As for total cholesterol, the onset of effect was rapid (Figure 3).

Other efficacy variables

For homocysteine, Abacor® caused a 0.32 μmol/l reduction from baseline (corresponding to 3.2%), whereas the placebo group showed an increase of 1.42 μmol/l (14.1%) during treatment (Table 2). The difference between treatment groups was statistically significant ($P < 0.001$). No significant differences between treatment groups were found for HDL-cholesterol, triglycerides, lipoprotein (a), or apolipoprotein B. Effect estimates are shown in Table 2.

Safety assessments

Both Abacor® and placebo products were well tolerated. There were no serious adverse events reported in this trial. A slight increase in serum uric acid values was seen with Abacor®, but this was not considered to be of any clinical relevance. There were no significant weight changes during the study.

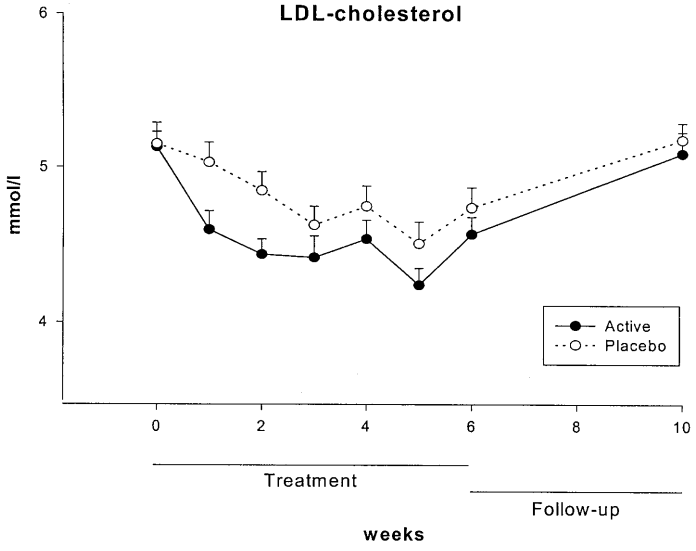

Figure 3 LDL-cholesterol. Error bars = s.e.

Discussion

In this 6 week diet intervention trial in subjects with mild-to-moderate hypercholesterolaemia, we have shown that Abacor®, a product based on isolated soy protein with high and standardised levels of isoflavones and cotyledon soy fibre, had significant lipid-lowering effects as compared with placebo. The estimated reduction from baseline was 0.64 mmol/l for total cholesterol, and 0.68 mmol/l for LDL-cholesterol. Also the placebo group experienced significant lowering of lipoproteins. Total cholesterol was reduced by 0.39 mmol/l, and LDL-cholesterol with 0.41 mmol/l, significantly less than Abacor®.

The relatively large change in lipid concentrations observed in the placebo group suggests that these patients may not have received an inactive treatment. Nilausen and Meinertz (1999) reported that casein lowers lipoprotein concentrations, whereas the study by Crouse *et al* (1999) did not show any effects. In order to ensure equal content of protein and fibre of our two treatments, we chose to add cellulose fibre to casein as the placebo, as we believed that this combination would not affect lipidaemia, as neither of the components did. Our results indicate that prospective, controlled studies are needed in order to investigate whether casein and cellulose fibre in combination exert any lipid-lowering effects.

Likewise, our results should preferably be compared with other studies using soy protein, isoflavones and cotyledon fibre in order to elucidate which component(s) are responsible for the effect. Other investigators (Lo, 1990; Bakhit *et al*, 1993; Potter *et al*, 1993) have published conflicting results regarding the lipid-lowering activity of soy cotyledon fibre. Our study was primarily designed in order to confirm or reject the hypothesis that the triple combination was effective, rather than trying to identify mechanism of action. However, our results encourage us to conduct further explorative studies aiming to determine the magnitude of effects for each of the soy components.

Our findings on the lipid-lowering efficacy of Abacor® confirm results from other studies of soy (Potter *et al*, 1998; Wong *et al*, 1998; Meinertz *et al*, 1989), but the onset of effect was already seen after 1 week with Abacor® in this study. The effects of soy are less pronounced than for long-term use of statins, but in the same order of magnitude as fibrates. It will therefore be of therapeutic interest to assess long-term effects of soy protein isolates with high contents of isoflavones and cotyledon fibres like Abacor®. Supported by clinical data from this double-blind study, the introduction of Abacor® in the diet might play a role for those patients who have little or no effect with other diet/lifestyle interventions, and for those patients who do not qualify for treatment with statins. Further well-designed and controlled studies are needed to verify this potential role of Abacor®.

In conclusion, the present study supports a lipid-lowering effect of isolated soy proteins with high and standardised contents of isoflavones and cotyledon soy fibres. Dependent on evidence from confirmatory and long-term trials, potential recommendations could be either to use soy protein with high and standardised levels of isoflavones and cotyledon fibres as a first intervention step in hypercholesterolaemia when dietary changes have not helped, or as addon to statins. The rapid onset of effect may prove beneficial to patients who need motivation in the early phases of intervention.

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References

Adlercreutz H (1998): Epidemiology of phytoestrogens. *Baillières Clin. Endocrinol. Metab.* **12**, 605–623.

Anderson JW, Johnstone BM & Cook-Newell ME (1995): Meta-analysis of the effects of soy protein intake on serum lipids. *New Engl. J. Med.* **333**, 276–282.

Anthony MS, Clarkson TB & Koudy Williams J (1998): Effects of soy isoflavones on atherosclerosis: potential mechanisms. *Am. J. Clin. Nutr.* **68**(Suppl), 1390S–1393S.

Bakhit RM, Klein BP, Essex-Sorli D *et al* (1993): Endocrinological responses to soy protein and fiber in mildly hypercholesterolemic men (25 g study). *F.A.S.E.B. J.* **7**, A802.

Buajordet I, Madsen S & Olsen H (1997): Adverse drug reactions caused by statins, with emphasis on psychiatric reactions. (In Norwegian.) *Tidsskr Nor Lægeforen* **117**, 3210–3213.

Crouse JR III, Morgan T, Terry JG, Ellis J, Vitolins M & Burke GL (1999): A randomised trial comparing the effect of casein with that of soy protein containing varying amounts of isoflavones on plasma concentrations of lipids and lipoproteins. *Arch. Intern. Med.* **159**, 2070–2076.

Dixon WJ (ed.) (1992): *BMDP Statistical Software*. Los Angeles, CA: UCLA Press.

Freidewald WT, Levy RI & Fredrickson DS (1972): Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin. Chem.* **18**, 499–502.

Katan MJ (1999): Functional foods. *Lancet* **354**, 794.

Kirk EA, Sutherland P, Wang S *et al* (1998): Dietary isoflavones reduce plasma cholesterol and atherosclerosis in C57BL/6 mice but not LDL receptor-deficient mice. *J. Nutr.* **128**, 954–959.

Lo GS (1990): Physiological effects of physico-chemical properties of soy cotyledon fiber. In *New Developments in Dietary Fiber*, eds. I Gurda and CJ Brine, pp 49–66. New York: Plenum Press.

Meinertz H, Nilausen K & Faergeman O (1989): Soy protein and casein in cholesterol-enriched diets: effects on plasma lipoproteins in normolipidemic subjects. *Am. J. Clin. Nutr.* **50**, 586–593.

Nilausen K & Meinertz H (1999): Lipoprotein (a) and dietary proteins: casein lowers lipoprotein (a) concentrations as compared with soy protein. *Am. J. Clin. Nutr.* **69**, 419–425.

Pietinen P, Vartianinen E, Seppänen R *et al* (1996): Changes in diet in Finland from 1972 to 1992: impact on coronary heart disease risk. *Prev. Med.* **25**, 243–250.

Potter SM, Bakhit RM, Essex-Sorlie D *et al* (1993): Depression of plasma cholesterol in men by consumption of baked products containing soy protein. *Am. J. Clin. Nutr.* **58**, 501–506.

Potter SM (1998): Soy proteins and cardiovascular disease: the impact of bioactive components in soy. *Nutr. Rev.* **56**, 231–235.

Potter SM, Baum JA, Teng H *et al* (1998): Soy protein and isoflavones: their effect on blood lipids and bone density in postmenopausal women. *Am. J. Clin. Nutr.* **68**(Suppl); 1375S–1379S.

Puska P, Tuomilehto J, Nissinen A *et al* (eds) (1995): *The North Karelia Project. 20 Y Results and Experiences*. Helsinki: University Press.

Sirtori CR, Lovati MR, Manzoni C, Gianazza E, Bondioli A, Staels B & Auwerx J (1998): Reduction of serum cholesterol by soy proteins: clinical experience and potential molecular mechanisms. *Nutr. Metab. Cardiovasc. Dis.* **8**, 334–340.

US FDA (1999): *Food labeling: health claims; soy protein and coronary heart disease*. 21 CFR part 101 (docket no. 98P-0683), 57699–57731.

Vartiainen E, Puska P, Pekkanen J *et al* (1994): Changes in risk factors explain changes in mortality from ischaemic heart disease in Finland. *Br. Med. J.* **309**, 23–27.

Wong WW, O'Brian Smith E, Stuff JE *et al* (1998): Cholesterol-lowering effect of soy protein in normocholesterolemic and hypercholesterolemic men. *Am. J. Clin. Nutr.* **68**(Suppl), 1385S–1389S.