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ORIGINAL COMMUNICATION

Effects of soy supplementation on blood lipids and arterial function in hypercholesterolaemic subjects

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Background: Studies on soy supplementation suggest a cardioprotective potential.
Objective: To examine the effects on LDL cholesterol and arterial function as a result of dietary enrichment with soy supplementation.
Design: A Randomized, double blind, parallel intervention trial.
Setting: Department of Endocrinology and Metabolism C, Aarhus University Hospital, and Department of Human Nutrition, The Royal Veterinary and Agricultural University, Denmark.
Subjects: In all, 100 hypercholesterolaemic but otherwise healthy subjects were included in the study of which 89 completed it.
Interventions: Subjects were randomly assigned to 24 weeks of daily intake of either a soy supplement, Abalon® (30 g soy protein, 9 g cotyledon fibre and 100 mg isoflavones) or placebo (30 g of casein). The soy supplement and placebo were provided in two sachets daily that were stirred in water. Fasting plasma lipids, TNF-α, homocysteine, insulin sensitivity, homeostasis model assessment (HOMA-IR), serum insulin, serum glucose, blood pressure as well as Glucagon-like peptide 1 (GLP-1), glucose-dependent insulinotropic peptide (GIP) and plasma lipids to a fat-rich meal were recorded before and after the intervention. In a sub study in 32 subjects, arterial dilatory capacity, compliance, and distensibility were recorded before and after the intervention.
Results: In the main study, no difference in fasting plasma lipid levels or insulin sensitivity was found between soy-based supplement and placebo. A significant postprandial increase in GIP to the meal test was observed in the soy group ($P<0.05$). In a substudy, no difference between the groups in changes in flow-mediated vasodilatation ($P=0.84$) was detected, while the soy supplementation caused a reduction in LDL and total cholesterol.
Conclusions: No significant effects on blood lipids were observed in the main study to a soy supplementation in hypercholesterolaemic subjects after 24 weeks. In the substudy, the soy supplementation, however, reduced LDL and total cholesterol but did not influence markers of arterial function.
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Introduction

Cardiovascular disease is a major cause of death in Western populations and represents a serious public health problem. Some premature manifestations of coronary heart disease (CHD) are due to mutations in major genes involved in lipoprotein metabolism. However, elevated lipoprotein concentrations in most patients with CHD primarily reflect the adverse effect of the modern sedentary lifestyle, excess body weight, and diets high in total and saturated fat on the genetic background. Also, endothelial dysfunction as demonstrated by flow-mediated vasodilatation (FMD) of the brachial artery is observed in early atherogenesis (Ross, 1986; Celermajer *et al*, 1994; National Heart, Lung and Blood Institute, 2002) and is considered an important contributing factor in manifestations of CHD (Neunteufl *et al*, 1997).

A large proportion of the population still consumes a diet providing more than 35% of the total amount of calories (E%) from fat (around 15 E% from saturated fat). As noted in the National Cholesterol Education Program ATP III report individuals with hyperlipidaemia should be encouraged to eat a diet lower in saturated fat and cholesterol (National Heart, Lung and Blood Institute, 2002). It has been convincingly demonstrated that dietary and pharmacological reductions in the total cholesterol and LDL cholesterol plasma concentrations do indeed lead to a reduction in CHD with a reduction in mortality and morbidity both as primary and secondary prophylaxis (Scandinavian Simvastatin Survival Study (4S), 1994; De Lorgeril *et al*, 1996; West of Scotland Coronary Prevention Study (WOSCOPS), 1998). A recent study demonstrates that a combination of dietary approaches to lower cholesterol including soy products could reduce LDL cholesterol by 30%, which was comparable with the effect of lovastatin (Jenkins *et al*, 2002).

The major components of soybean flour (ie, soy protein, soy cotyledon fibre, and isoflavones) appear to independently lower serum cholesterol. Isoflavones are phytoestrogens that display oestrogen-like activity due to their ability to bind to the oestrogen receptor (Kuiper *et al*, 1998). Recently, it was demonstrated in a meta-analysis of eight randomized controlled trials that isoflavones have LDL cholesterol-lowering effects independent of soy protein (Zhuo *et al*, 2004). In another meta-analysis of 38 studies investigating the effects of soy protein intake on serum lipids, Anderson *et al* (1995) found that a daily consumption of an average of 47 g soy protein rather than animal protein significantly reduced total cholesterol, LDL cholesterol and triacylglycerol (TG) by 9, 13 and 11%, respectively. In comparison, Weggemans and Trautwein (2003) found in their meta-analysis that a daily consumption of 36 g soy protein in combination with 53 mg of isoflavones only lowered LDL cholesterol by 4% in average.

The 6 weeks treatment with Abalon®, a soy-based dietary supplement has previously been shown to reduce effectively LDL cholesterol, LDL-to-HDL ratio, TG and homocysteine in type II diabetic subjects (Hermansen *et al*, 2001).

The present investigation aimed at comparing the long-term effects (24 weeks) of supplementation of the soy-based dietary supplement, Abalon®, with a control (casein), on LDL cholesterol and other cardiovascular risk factors (including endothelial function) in subjects with hypercholesterolaemia.

Study design, subjects and methods

Study design

The study was conducted as a prospective double-blind, randomized, placebo-controlled, parallel-group, two-centre trial evaluating the effects of Abalon® against placebo (ratio 1:1) over a treatment period of 24 weeks. Random allocation to receive soy supplement or corresponding placebo was based on a computer-generated randomization list. Subjects were stratified with respect to LDL cholesterol at screening. After screening, the participants completed a 2-week run-in period, followed by randomization and the 24-week intervention period. The soy supplement daily provided a total of 30 g soy protein, 9 g cotyledon fibre and 100 mg isoflavones (2 sachets/day) according to the supplier, while the matching placebo sachets contained 30 g of casein (2 sachets/day). The subjects were instructed to stir the content of the sachets in water and consume twice daily, one sachet in the morning and one in the evening. The subjects were seen at intervals of 4 weeks during the whole trial for trial nutrient accountability and dispensing. The trial nutrients were dispensed to the participants with the instruction to bring all nutrients including empty sachets/boxes on each visit. Three days dietary records were obtained at baseline and twice during the intervention period (in the middle and at the end of the intervention). The subjects were instructed to weigh and record their food on two workdays and one weekend day in each period. A dietitian validated the 3×3-day dietary records and subsequently calculated the macronutrient intake in the period before and throughout the trial in order to avoid trial-related weight changes. The trial material should substitute an isocaloric amount of the subject's habitual diet. The participants were instructed to maintain their weight (weight change from first to last visit of no more than ± 2 kg) and dietary habits throughout the study.

Substudy. In a substudy, the vascular function was evaluated in 32 subjects (17 men and 15 women) of whom 29 completed the study. A total of 19 subjects received the active substance and 13 received placebo (16 subjects in the active group and 13 in the placebo group completed the study). All women were postmenopausal and all participants in the substudy were recruited from the main study.

Subjects

Out of the 100 subjects (58 women and 42 men) included in the study, 89 completed (43 in the active group and 46 receiving placebo). In all, 99 of the 100 subjects, were

Caucasians. The inclusion criteria were as follows: women and men between 40 and 80 y with total cholesterol above 6 mmol/l and/or LDL cholesterol above 3.5 mmol/l. Exclusion criteria were fasting TG above 4.5 mmol/l, major cardiovascular events within 3 months prior to inclusion, diabetes mellitus, severe hypertension (>180/110 mmHg), BMI > 40 kg/m^2, or taking supplements or therapy for lowering lipids. Subjects were asked to continue with unchanged physical activity during the study. Characteristics of included subjects are outlined in Table 1. All subjects gave written informed consent. The ethics committees of Aarhus and Copenhagen counties approved the study.

The nutrient content was calculated using Dankost 2000 (Danish Catering Service, Herlev, Denmark). The nutrient composition is given in Table 2. Blood samples were drawn after an overnight fast (12 h) at the first day of the study, after 12 weeks and at the end of the intervention. Serum was separated after 30 min by centrifugation at room temperature. Plasma was stored in ice for 30 min and separated at 4°C in a refrigerated centrifuge. Samples were stored at −20°C until analysis.

Methods

Meal test. A high-fat test meal was ingested at 08.00 after an overnight fast at the first visit and at the end of the intervention to evaluate the effect of Abalon®. Blood samples for glucose, insulin, HOMA are used as a surrogate measure of insulin resistance. HOMA-IR is calculated as insulin (μU/ml) × glucose (mmol/l)/22.5 (Matthews *et al*, 1985). Glucagon-like peptide-1 (GLP-1), glucose-dependent insulinotropic peptide (GIP), total cholesterol, HDL cholesterol and TG were drawn from the start and 8 h thereafter. The test meal consisted of white bread and vegetable soup with butter and provided 4.157 MJ. Fat, carbohydrate and protein provided 78 per cent of the total energy content (E%), 20 E% and 2 E%, respectively. Blood samples were drawn immediately before the start of the meal and at 2, 4, 6 and 8 h after for the analysis.

Flow-mediated vasodilatation. Flow-mediated vasodilatation (FMD) was measured using an Acuson 128 × P/10 system with extended frequency imaging option and a 7 MHz linear transducer resulting in 10 MHz imaging capability.

Table 1 Subject characteristics of the100 hypercholesterolaemic subjects (42 men and 58 women) at the start and after 24 weeks of treatment with a soy supplementation

	Placebo		*Active*	
	Start	*24 weeks*	*Start*	*24 weeks*
Male/female	19/32		23/26	
Age (y)	58.0±4.6		60.6±3.4	
Weight (kg)	75.3±3.4	75.4±3.7	77.1±3.6	76.6±13.6
BMI (kg/m^2)	25.6±3.4	25.8±3.4	26.4±3.6	26.5±3.7
Systolic BP (mmHg)	133±16.1	131±16.2	133±17.7	133±16.5
Diastolic BP (mmHg)	81.4±9.1	79.6±9.1	80.2±9.2	79.2±10.2
Pulse rate (beats/min)	67.5±9.2	65.9±8.3	66.1±8.1	64.4±8.9
HOMA-IR[a]	1.26±0.67	1.2±0.57	1.29±0.56	1.1±0.54
S-glucose (mmol/l)	5.3±0.5	5.3±0.6	5.5±0.6	5.4±0.6
S-insulin (pmol/l)	52.3±24.1	50.1±21.6	52.4±20.1	44.9±19.1
Smokers	6		8	
Former smokers	22		17	
Never smokers	23		24	

Data are means±s.d.
[a]HOMA-IR is calculated as [insulin (μU/ml) × glucose (mmol/l)/22.5].

Table 2 Composition of the diet to the 100 hypercholesterolaemic (42 men and 58 women) individuals at the start and after 24 weeks of treatment with a soy supplementation

	Placebo		*Active*	
	Start	*24 weeks*	*Start*	*24 weeks*
Total energy (MJ/day)	9.7±2.3	9.4±2.1	8.7±2.0	8.8±1.8
Carbohydrate (% of energy)	50.6±6	49.4±7	47.8±7	47.4±6
Fat (% of energy)	26.1±6	25.0±6	27.0±7	26.8±5
Protein (% of energy)	17.8±4	20.2±3	19.5±4	20.8±3
Alcohol (% of energy)	5.5±4	5.0±5	6.1±6	6.1±5
Cholesterol (mg/day)	200 (142–267)	200 (133–300)	200 (167–300)	200 (167–292)

Data are means±s.d. or median (25–75 percentiles).

All subjects were examined at baseline and at the end of the intervention. The subjects were instructed to fast and avoid smoking for 2 h prior to the examination. All examinations were performed by the same operator and were videotaped for later evaluation. The vasodilatory responses of the brachial artery to increased flow and to nitroglycerine (NGT) were examined as described by Clausen *et al* (2001). The brachial artery was scanned in a longitudinal section above the elbow. Depth and gain settings were set to optimize images of the lumen/arterial wall interface, and all settings were kept unchanged throughout the study. When a clear picture was obtained ensuring that the centre of the artery was identified, the skin was marked and the transducer was held in place throughout the examination. Arterial diameter was measured (I) at baseline, (II) during ischaemia after inflation of a cuff to 300 mmHg, placed around the forearm distal to the segment of the artery being scanned for $4\frac{1}{2}$ min, (III) 50–60 s after cuff release, (IV) at least 10 min after release to allow vessel recovery, and (V) 3–4 min after sublingual administration of 400 μg nitroglycerine (Nitrolingual spray, G. Pohl-Boskamp GmbH & Co). Vessel diameter was measured with ultrasonic calibres at a fixed point defined at the time of examination. Each observer measured the diameter in four cardiac cycles concurrently with the R wave on a simultaneously recorded electrocardiogram. A mean was calculated from all eight measurements. Vasodilatation was expressed as percentage of baseline diameter.

Analytic assays. Serum total cholesterol (Siedel *et al*, 1983), HDL cholesterol (Sugiuchi *et al*, 1995), TG and glucose concentrations were measured using enzymatic methods (Trinder, 1969). Serum LDL cholesterol was measured enzymatically using a kit insert from Roche (Roche diagnostics, Mannheim, Germany). Apolipoprotein (Apo) B100 and Apo A1 concentrations were measured by immunoturbidometry using a kit insert from Roche (Roche diagnostics, Mannheim, Germany). Plasma homocysteine was measured using a chemiluminescense immunometric assay (Babson *et al*, 1991). Serum tumour necrosis factor alpha (TNF-α) was measured with an enzyme-enhanced chemiluminescence immunoassay (Babson *et al*, 1991). Serum insulin concentrations were measured by an enzyme-linked immunosorbent assay method (CV: 1.7%) (Andersen *et al*, 1993). GIP was measured by radioimmunoassay with the use of antiserum R65, mono-iodinated human GIP, and human GIP for standards after extraction of the peptide from plasma according to a previously described method (Krarup *et al*, 1983). The sensitivity and detection limits of the assay are 1 pmol/l. The assay is highly specific for GIP and does not crossreact with the 8-kDa immunoreactive component of unknown nature crossreacting in most GIP assays. Plasma concentrations of GLP-1 were measured as previously described (Hvidberg *et al*, 1994) against standards of synthetic GLP-17–36 amide (proglucagon 78–106 amide) (Orskov *et al*, 1991) with the use of antiserum code no. 89390, which can be used at a final dilution of 1:250 000 and endows the assay with a detection limit of 1 pmol/l and has an intra-assay CV < 5% at 20 pmol/l. This antiserum is highly specific for the COOH-terminus of proglucagon 78–107 amide and reacts neither with glycine-extended GLP-1 (proglucagon 78–108) nor with proglucagon 78–106. Thus, it mainly reacts with GLP-1 of intestinal origin (Orskov *et al*, 1992). Before the analysis, plasma was extracted with ethanol (70%, v:v) (Orskov *et al*, 1991).

Statistical analysis. Normally distributed data are given as mean ± s.d. and not normally distributed variables as medians (25–75 percentiles). The Mann–Whitney test was used to compare height and age for the groups at randomization, and Fisher's exact test was used to compare the distribution of sex in the two groups. Analyses of differences (within and between treatments) were performed by analysis of variance (ANOVA). Changes in dietary data and LDL cholesterol were tested with repeated measurement ANOVA.

In the substudy, treatment-induced changes within groups were tested with paired student's *t*-test. ANCOVA was used to determine if initial concentrations had any effect on changes in total and LDL cholesterol. $P < 0.05$ was considered significant. Statistical analyses were performed using SAS software (Carry, NC, USA).

Results

There were three withdrawals due to adverse events; six withdrew consent and two subjects left for other reasons. In both treatment groups, a total of 57% experienced at least one adverse event. The majority of these adverse events were mild and were assessed to have a likely relation to the treatment in 25% of the events. A total of 20 subjects did not fulfil the criteria of stable weight during the study (weight change from first to last visit of more than ± 2 kg). The perprotocol (PP) population consists of 69 subjects. Compliance was defined as treatment in 85–115% of the planned period of 24 weeks, that is, between 20.4 and 27.6 weeks. Length of treatment was calculated from number of sachets dispensed and number of sachets returned. A total of 79 subjects were compliant (89%). There were no significant differences in clinical characteristics between the two groups at baseline (Table 1). No differences were seen at the end of treatment in weight, body mass index (BMI), blood pressure, pulse rate, HOMA-IR, serum-insulin or serum-glucose. Dietary data for the groups are presented in Table 2. No significant differences in the dietary nutrient composition between the groups were seen at baseline or at the end of the intervention. However, a significant decrease in fat intake was seen in the placebo group when compared to the active group ($P = 0.006$, adjusted for baseline values).

Fasting blood lipids and other cardiovascular risk factors
Lipid profiles and other cardiovascular risk markers before and after 24 weeks of dietary supplementation are summarized in Table 3. There were no significant differences before the intervention in blood lipids, Apo B100, Apo A1, TNF-α or homocysteine. None of the measured blood lipids (total cholesterol, LDL cholesterol, HDL cholesterol and TG) was affected by the treatment with the soy-based supplement when analysed as *intention to treat* (ITT). Both groups tended to decrease in LDL from baseline LDL—the mean decrease in the active group being 0.4 mmol/l (−7.2±11.7%) and in the placebo group 0.2 mmol/l (−3.4±16.4%), respectively—however, not attaining statistical significance. The change in LDL cholesterol neither differed among treatment groups ($P=0.22$) nor changed when gender, compliance and baseline weight were taken into account. A significant decrease in LDL cholesterol was seen in the group receiving soy supplement (−7.6±10.7%) compared to placebo (−0.6±16.2%, $P=0.04$), when analysed on the PP population. However, the treatment effect in the PP population disappeared when accounting for differences in fat intake, compliance, lipid concentration at the end of the intervention. Repeated measurements ANOVA showed no effect of interaction between treatment and visit on absolute LDL cholesterol concentrations, neither for the ITT nor the PP population ($P=0.48$ and $P=0.23$, respectively).

Meal test
The responses to the meal tests are summarized in Table 4. The Abalon® group experienced a significant increase in GIP measured as incremental area under the curve (iAUC). A mean change from baseline of 10.1 pmol/l (7.7±20.3%) was seen in the active group and a change from baseline of −4.9 pmol/l (−5.2±16.3%) was seen in the placebo group. Abalon® compared to control caused no significant effect on iAUC of glucose, insulin, GLP-1, total cholesterol, HDL cholesterol and TG to the test meal.

Table 3 Effects of a soy-based dietary supplement (Abalon) on cardiovascular risk markers in 100 hypercholesterolaemic subjects (42 men and 58 women)

	Placebo		*Active*		*Active* vs *placebo*	*Mean treatment difference (%)*	
	Start	*24 weeks*	*Start*	*24 weeks*	P[a]	*Mean (95% CI)*	P[b]
T-cholesterol (mmol/l)	6.9±1.1	6.9±1.2	6.9±0.9	6.6±0.8	NS	−4.3 (−0.23 to 8.74)	NS
LDL	4.6±1.1	4.4±1.0	4.6±0.8	4.2±0.7	NS	−3.8 (−2.34 to 9.85)	NS
LDL (PP)	4.6±1.3	4.5±1.1	4.5±0.7	4.2±0.7	NS	−7.0 (−0.43 to −13.73)	0.04
HDL	1.5±0.4	1.4±0.3	1.4±0.4	1.5±0.4	NS	−2.0 (−3.92 to 7.81)	NS
LDL/HDL ratio	3.3±1.1	3.2±1.0	3.4±1.1	3.1±1.1	NS	−0.5 (−6.44 to 7.61)	NS
Triacylglycerol (mmol/l)	1.9±1.1	1.9±1.2	1.8±0.7	1.5±0.7	NS	−8.0 (−19.3 to 35.09)	NS
Apo B100 (g/l)	1.3±0.2	1.3±0.2	1.3±0.2	1.2±0.2	NS	−0.2 (−5.07 to 5.40)	NS
Apo A1 (g/l)	1.5±0.3	1.5±0.2	1.4±0.2	1.5±0.3	NS	0.3 (−4.52 to 3.79)	NS
TNF-α (ng/l)	4.7±1.6	4.9±2.4	4.7±1.8	4.9±2.6	NS	4.1 (−12.4 to 4.19)	NS
Homocysteine (μmol/l)	10.4±2.5	10.1±2.5	11.9±3.5	11.0±3.0	NS	−4.0 (−3.15 to 11.24)	NS

ANOVA. Data are means±s.d., unless otherwise indicated.
[a]Significance of differences in active and placebo treatments after 24 weeks.
[b]Significance of mean treatment differences.
NS: nonsignificant, CI: confidence interval.

Table 4 Average effects during an 8-h period of a standard meal test on blood responses (iAUC/min) after 6 months on a soy-based dietary supplement in 100 hypercholesterolaemic subjects (42 men and 58 women) (ITT)

	Placebo		*Active*		*Active* vs *placebo*		
(IAUC/min)	*Start*	*24 weeks*	*Start*	*24 weeks*	P[a]	*95% CI for treatment difference*	P[b]
Total cholesterol (mmol/l)	0.23±0.12	0.26±0.14	0.21±0.13	0.21±0.14	NS	−0.04 to 0.12	NS
HDL cholesterol (mmol/l)	0.07±0.05	0.06±0.04	0.06±0.05	0.07±0.05	NS	−0.04 to 0.01	NS
Triacylglycerol (mmol/l)	0.84±0.56	0.72±0.44	0.84±0.52	0.63±0.33	NS	−0.18 to 0.19	NS
Glucose (mmol/l)	0.61±0.32	0.61±0.36	0.63±0.26	0.68±0.36	NS	−0.23 to 0.10	NS
Insulin (pmol/l)	35±21	35±26	32±24	43±39	NS	−26 to 1	NS
GLP-1 (pmol/l)	17±8	19±15	16±8	20±15	NS	−7 to 4	NS
GIP (pmol/l)	50±27	43±19	46±20	56±32	NS	−23 to 7	<0.05

ANOVA. Data are means±s.d., unless otherwise indicated.
[a]Significance of differences in active and placebo treatments after 24 weeks.
[b]Significance of mean treatment differences.
iAUC: incremental area under the curve, CI: confidence interval.

Substudy

The results of the substudy are summarized in Table 5. There were no differences between the groups with respect to changes in FMD ($P=0.84$) or pulse pressure ($P=0.49$). The group treated with soy experienced a significantly larger decrease in total cholesterol (-0.56 ± 0.63 mmol/l (-7.9%), $P=0.003$) and LDL cholesterol (-0.54 ± 0.56 mmol/l (-11.5%), $P=0.002$). The changes in total- and LDL cholesterol concentrations differed significantly between the treatment and the control group. The treatment difference in total and LDL cholesterol did not change when baseline values were included as a covariate ($P=0.002$ and $P=0.015$, respectively). There were no differences between the groups in changes of HDL cholesterol ($P=0.19$), TG ($P=0.25$) or homocysteine ($P=0.49$).

Discussion

The purpose of the present study was to examine the long-term effect of supplementation with soy-based dietary supplement (Abalon®) on LDL cholesterol and other cardiovascular risk factors (including endothelial function) in adults with hypercholesterolaemia. In the main study no significant treatment effects of 24 weeks of soy supplementation were seen on LDL cholesterol or other cardiovascular risk factors. The soy-based dietary supplement significantly increased the GIP responses to a standard test meal. In the substudy, no effect on markers of endothelial function was detected. More than half the participants in both groups experienced at least one episode of mild side effects. In the soy group, the content of fibre may in part explain some of the gastrointestinal side effects. It should be underlined that we had not independently analysed the Abalon® soy supplement for its constituents. Another limitation of the study was that we did not measure the isoflavone contents from urinary and/or blood samples during the intervention period. This would have provided a more precise impression of the compliance and bioavaiability of the supplement.

Blood lipids

In 1999, the United States Food and Drug Administration (FDA) approved the health claim for cholesterol-lowering benefits of soy protein referring to consumption of 25 g/day of soy protein (Food and Drug Administration, 1999).

The lack of effect on blood lipids in the main study is not consistent with the meta-analysis of Anderson *et al* (1995) including 38 controlled human trials (primarily in subjects with hyperlipidaemia). An average daily intake of 47 g soy protein resulted in significant reductions in total [9.3% decrease, 95% CI (0.35–0.85 mmol/l)] and LDL cholesterol [10.5% decrease, 95% CI (0.30–0.82 mmol/l)], with the cholesterol-lowering effect being positively related to baseline cholesterol concentrations. However, durations of the studies included in the meta-analysis (Anderson *et al*, 1995) were all of only 4 weeks or less, and consequently the analysis only applies to short-term effects of soy protein supplementation. The present study is the first to report on the long-term effects of a soy-based dietary supplement on plasma lipids. The results of the present study are also in contrast with a recent study examining the effect of the same product (Abalon®) on cardiovascular risk factors in type II diabetics (Hermansen *et al*, 2001). In the latter study, a significant decrease in LDL cholesterol (10% reduction), LDL to HDL cholesterol ratio (12% reduction), TG (22% reduction) and homocysteine (14% reduction) was detected. In the present study, there were no significant changes in plasma homocysteine, LDL/ HDL ratio, LDL or TG. One explanation for the lack of effect on LDL cholesterol in the present study compared to the results of Anderson *et al* (1995) and Hermansen *et al* (2001) might be the lower amount of soy protein ingested (30 *vs* 47 g/day and 30 *vs* 50 g/day, respectively). This explanation is corroborated by

Table 5 Effects of 3 months treatment with a soy-based dietary supplement (Abalon) on endothelial function and lipids in a substudy consisting of 32 subjects

	Placebo		*Active*		*Active* vs *placebo*	*Mean treatment difference (%)*	
	Start	*24 weeks*	*Start*	*24 weeks*	P[a]	*(%)*	P[b]
Cholesterol (mmol/l)							
Total	6.2±0.6	6.7±1.0	7.1±1.1*	6.5±1.1	NS	−16	<0.001
LDL	4.0±0.4	4.1±0.6	4.7±1.1**	4.1±1.0	NS	−14	<0.005
HDL	1.3±0.4	1.4±0.3	1.5±0.5	1.5±0.4	NS	−7	NS
FMD (% of baseline)	103.1±3.1	104.0±4.9	103.6±4.9	104.5±4.2	NS	−22	NS
NID (% of baseline)	109.3±5.6	108.6±6.9	112.7±5.1	113.9±5.4	0.04	4	NS
Pulse pressure (mm Hg)	47±8	45±19	48±9	52±10	NS	11	NS

Student's *t*-test.
Data are means±s.d., unless otherwise indicated.
[a]Significance of differences in active and placebo treatments after 24 weeks.
[b]Significance of mean treatment differences.
*,**Significantly higher than placebo.
FMD: Flow mediated dilatation, NID: Nitroglycerine-induced vasodilatation.

the findings in the two meta-analyses of Weggemans and Trautwein (2003) and Anderson *et al* (1995) showing that 36 *vs* 47 g/day caused a 4 *vs* 13% reduction in LDL cholesterol (Hermansen *et al*, 2001;Weggemans and Trautwein, 2003). The type II diabetic patients (Hermansen *et al*, 2001) may be more sensitive to the soy treatment than the nondiabetic subjects in the present study. However, it is not known, whether this is the case since no direct comparisons of effect of soy supplementation in type II diabetic and nondiabetic subjects have been performed. It is noteworthy that the soy supplementation elicited a reduction in LDL and total cholesterol in the substudy (Table 5). We have no obvious explanation for the puzzling diversity in results between the main and the substudy regarding influence on the lipids. Since the study was run in parallel with concomitantly similar numbers of active and placebo-treated subjects, it seems less likely that seasonal variations in lipids could explain the negative results in the main study. Interestingly, Meyer *et al* (2004) in a recent retrospective analysis found indications that improvement of plasma lipids by soy products may be limited to subjects having a high level of equol, a metabolite of the isoflavone daidzein. Whether this in part could explain the diversity of our results between the main and the substudy is unknown, since the equol content in urine or plasma samples were not determined.

Meal test

No differences were observed in the postprandial lipid, insulin, glucose or GLP-1 responses while a standard test meal caused an increased GIP response after long-term soy protein treatment. The difference in GIP responses is puzzling. However, it is known that different types of fatty acids can elicit different incretin responses (Thomsen *et al*, 1999). Most recently, it has also been demonstrated that two different milk proteins, casein and whey, exert different effects on plasma amino acid profiles and gastrointestinal hormone response including GIP (Hall *et al*, 2003). It may be that the different amino acid compositions of the soy and milk protein (casein) in part may explain the difference in GIP responses to the test meal. The functional importance of the increased GIP response after long-term soy protein supplementation is unknown. The increased GIP response may influence the insulin release and the subsequent insulin-mediated metabolic effects.

Substudy

The trial supplement did not improve the endothelial function assessed by FMD. The results from this study are fairly consistent with other clinical trials evaluating the effects of soy protein or isoflavones on endothelial function. Our results corroborate a recent placebo-controlled study with 179 men and postmenopausal women, also showing no effect of three months of soy protein supplementation (50 g/day, 118 mg isoflavones) on FMD (Teede *et al*, 2001). Also three placebo-controlled interventions, evaluating the effect of a soy-derived isoflavone supplementation (80 mg/day) on FMD in men and postmenopausal women, showed no effects (Simons *et al*, 2000; Hale *et al*, 2002; Teede *et al*, 2003). In contrast, 28 healthy postmenopausal women consuming 25 g of soy protein with isoflavones daily showed enhanced vasodilatation without experiencing any change in lipid values (Steinberg *et al*, 2003). The technique for measuring FMD has its limitations, being significantly operator dependent, and is under the influence of external factors such as time of day (Gaenzer *et al*, 2000), physical activity (Huonker *et al*, 1996), and food ingestion (Brown and Hu, 2001; Cuevas *et al*, 2000). In the present study, a single experienced operator performed FMD under controlled conditions, with the study powered to detect a 4% change in FMD. Nitroglycerine-induced vasodilatation (NID) was significantly higher in the treatment group after the intervention when compared to the control group. However, a nearly significant difference was seen before the intervention. NID did not change significantly in the treatment group during the intervention when compared to placebo.

In conclusion, supplementation with Abalon® had no significant effect on blood lipids in hypercholesterolaemic men and women in the main study. In the substudy, the soy supplementation, however, reduced the LDL and total cholesterol but did not influence markers of arterial function.

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