Original Article

Cholesterol lowering benefits of soy and linseed enriched foods

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Foods such as breads and breakfast cereals enriched with a combination of soy protein (soy grits and/or soy flour) and whole linseed are gaining popularity. Regular consumption of either whole grains or soy protein can lower risk factors for coronary heart disease. Furthermore, linseed is a rich source of the omega-3 fatty acid, α-linolenic acid (LNA), with purported cardiovascular benefits. The aim of this study was to determine the effect of daily consumption of soy and linseed containing foods and Canola (as an added source of LNA) on plasma lipid concentrations in 20 mildly hypercholesterolaemic postmenopausal women. Fasted blood samples were taken initially and after 3 and 8 weeks to assay plasma lipids and both plasma and erythrocyte membrane fatty acids. Urinary isoflavones were also measured. Data from 18 subjects were used for analysis. Plasma total, low-density lipoprotein (LDL) and non-high-density lipoprotein (HDL) cholesterol concentrations fell significantly (10, 12.5 and 12%, respectively) within 3 weeks. Although attenuated, there were still significant reductions in total and non-HDL cholesterol (5 and 6.5%, respectively) after 8 weeks of intervention. These reductions were associated with increases in urinary isoflavone excretion. This pilot study indicates that regular inclusion of foods containing soy and linseed in the diet may improve plasma lipids in subjects with hypercholesterolaemia.

Key words: Australia, cholesterol, isoflavones, linseed, polyunsaturated fatty acids, postmenopausal women, soy, triglycerides.

Introduction

Foods containing a combination of soy and linseed ingredients are becoming increasingly popular in Australia. We have experienced a relatively recent proliferation of breads, cereals, snack foods and other novel soy and linseed containing foods offering numerous health benefits. There is considerable evidence, now embodied in independent health claims for soy protein and whole grains in the USA, that consumption of these ingredients can reduce the risk of coronary heart disease.[1,2] However, there has been no evaluation of the potential cardiovascular health benefits of foods containing a combination of these ingredients.

Epidemiological research shows that in many countries where the incidence of cardiovascular disease is low, consumption of soy-based foods is high.[3] Furthermore, there is substantial evidence that daily consumption of foods containing soy protein can reduce total and low-density lipoprotein (LDL) cholesterol and may increase high-density lipoprotein (HDL) cholesterol in hypercholesterolaemic individuals. This evidence has been summarised in a meta-analysis of 38 clinical trials.[4] It has also been included in numerous subsequent studies which have formed the basis of a health claim for soy protein recently approved by the U.S. Food and Drug Administration which states that a daily intake of 25 g of soy protein, in combination with a diet low in saturated fat and cholesterol may reduce the risk of heart disease.[1]

Soy protein is a rich source of the isoflavones, genistein and daidzein, which have recently been implicated as contributors to its hypocholesterolaemic effect.[5,6] It was recently demonstrated in an isoflavone dose–response study that a daily intake of 25 g of soy protein containing 37–62 mg of isoflavones could significantly lower total and LDL cholesterol.[6] However, the dose of isoflavones required to lower cholesterol was inversely related to the initial blood cholesterol level, such that individuals with high cholesterol experienced a reduction with only 37 mg of isoflavones/day, while those with normal or moderately elevated cholesterol required 62 mg of isoflavones/day, even though both groups of people were consuming 25 g of soy protein/day.

The health claim for whole grains states that regular consumption of foods containing whole grains can also reduce the risk of coronary heart disease.[2] In the case of linseed, this benefit could be attributed to a number of nutrients, including lignans, fibre and α-linolenic acid (LNA). The nutrient LNA comprises more than one-half of the fatty acid content of linseed. It is the plant precursor of the very long-chain

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omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which are obtained from marine sources and are known to have beneficial cardiovascular effects.[7,8] Dietary intake of LNA can increase EPA and DHA in the circulation by enzymatic desaturation and elongation shortly after consumption. Although the increase is not as great as that achieved by direct consumption of EPA and DHA from fish or fish oil,[9] the limited conversion of LNA can nevertheless influence the formation of cytokines and eicosanoids (thromboxane, prostaglandins, and leukotrienes) favouring those with less vasoconstrictor, platelet-aggregatory or inflammatory properties.[10,11]

Another possible benefit of whole linseed supplementation in the diet is the reduction of LDL cholesterol in both healthy and hypercholesterolaemic individuals, which may be attributable to the soluble fibre components of linseed.[12,13] This effect of linseed, together with the potential of LNA to lower plasma triglyceride concentrations (a property of EPA and DHA which has yet to be demonstrated with LNA supplementation) might further enhance the hypolipidaemic benefits of soy protein when consumed in a dietary combination.

The aim of this study was to conduct a preliminary evaluation, in mildly hyperlipidaemic postmenopausal women, of the potential lipid-lowering benefits of regular daily consumption of soy and linseed containing foods together with additional dietary sources of LNA.

Subjects and methods

Subjects

Newspaper advertisements were used to recruit 22 postmenopausal women with unmedicated mild hyperlipidaemia (plasma cholesterol > 5.5 mmol/L; triglycerides > 1.5 mmol/L) to participate in a dietary intervention trial. Eligibility was assessed from responses to a health and lifestyle questionnaire. Postmenopausal status was determined by the cessation of menses for more than 6 months. Exclusion criteria included insulin dependent diabetes, frequent consumption (i.e. several times per week) of soy or soy and linseed containing foods, or the use of hormone replacement therapy or lipid lowering medication in the past 3 months. The study was approved by the Human Ethics Committee of the University of Wollongong and informed consent was obtained.

Two women chose to withdraw; thus a total of 20 completed the trial, six of whom had been clinically diagnosed more than 2 years earlier with diabetes mellitus type 2. These women were taking hypoglycaemic medication which did not change during the course of the study. Subject characteristics at baseline are shown in Table 1. One subject was excluded from data analysis because she was initially on a very low-fat diet and consumption of the intervention foods resulted in an unusually large increase of fat intake. Another subject was excluded after commencing lipid lowering medication during the intervention. Thus, data from 18 subjects was included in the final analysis. This number of subjects could be expected to give at least 80% power to detect a significant ($P < 0.05$) change in total plasma cholesterol (based on an anticipated change of 10% with a 10% standard deviation, as estimated from previous studies).[14]

Dietary supplements and study design

After completing a 3-week run-in period during which consumption of soy and/or linseed containing foods, aspirin and other non-steroidal anti-inflammatory drugs (NSAIDS) was discontinued, subjects visited the clinic on 2 consecutive days having fasted overnight. Weight, blood pressure and fasted blood samples were taken on each day, after which subjects began consumption of the specified test foods. The clinic assessments were repeated after both 3 and 8 weeks. An overnight urine collection was made at the start of the study, while subsequent 24 h urine samples were collected after 3 and 8 weeks. Subjects were advised to maintain their usual physical activities during the course of the study.

At the end of the run-in period, subjects were provided with nutritional/dietetic advice on how to adapt their diet to incorporate the following soy and linseed foods daily: two slices of soy and linseed bread, one soy and linseed English muffin and a soy and linseed muesli bar and oatcake. All test foods were manufactured and supplied by Goodman Fielder (Sydney, Australia). The bread and muffins were commercial products: 720 g loaves of Uncle Tobys 97% fat free Soy & Linseed bread and Buttercup Bakeries English muffins. The muesli bar and oatcake were custom made products containing soy protein/isoflavones and added linseed oil for the purpose of enhancing dietary LNA intake. To maximise the intake of LNA, subjects were further instructed to consume

Table 1. Baseline characteristics of subjects

	Enrolled (n = 20)	Included in evaluation (n = 18)
Age (year)	57 (46–69)	57 (46–69)
Weight (kg)	77 (58–89)	78 (58–89)
BMI (kg/m^2)	30 (24–40)	30 (24–40)
Blood pressure		
Systolic (mmHg)	136 (102–168)	133 (102–167)
Diastolic (mmHg)	84 (60–97)	83 (60–97)
Plasma lipids (mmol/L)		
Total cholesterol	6.4 (4.9–7.7)	6.4 (4.9–7.7)
LDL cholesterol*	4.3 (2.5–5.6)	4.3 (3.5–5.6)
HDL cholesterol†	1.3 (0.9–2.1)	1.2 (0.9–1.9)
Triglyceride	2.0 (0.8–3.6)	2.1 (1.0–3.6)

BMI, body mass index; LDL, low-density-lipoprotein; HDL, high-density lipoprotein. All values given in mean (range).
*Determined by the Friedewald calculation. †HDL was separated from plasma by dextran sulphate magnesium chloride precipitation.

20 g of Canola oil and/or margarine daily. All soy and linseed foods were interchangeable; for example, a muesli bar could be replaced with an oatcake or English muffin. Collectively, these foods supplied approximately 45 mg of isoflavones, 6 g of LNA and an estimated 32 mg of lignans daily (Table 2).

Subjects visited the clinic fortnightly for collection of test foods. Dietary compliance was assessed by three sets of three unscheduled 24 h dietary recalls made by telephone during the 3 weeks prior to study commencement and repeated in the first 3 and last 5 weeks of the intervention. Subjects were also given a diary to record their daily consumption of soy and linseed foods. Urinary isoflavone and plasma fatty acid determinations provided additional measures of compliance. Diets were analysed by using the FoodWorks Nutrient Analysis program, Version 1.04.001, 1997 (Xyris Software, Highgate Hill, Brisbane, Australia). A questionnaire was also administered to the subjects after 3 and 8 weeks of intervention to assess both tolerance and acceptability of the soy and linseed test foods.

Blood collection

At each clinic visit, fasted venous blood was collected into tubes containing ethylenediamine tetraacetic acid (EDTA). Samples underwent centrifugation at 1000 *g* for 10 min at 4°C and the plasma removed and frozen at – 80°C for subsequent analysis. Erythrocytes were washed with a Tris saline buffer solution (1 M Tris (Bis Tris), pH 7.4; 0.84 M NaCl, 8.5×10^{-4} M EDTA, 0.02 M NaN_3) and subjected to centrifugation again at 1000 *g*. This process was repeated and the supernatant removed both times. The washed erythrocytes were stored at – 80°C for future fatty acid analysis. On the second day of each of the three visits, an additional 9 mL of venous blood was collected into plain blood tubes, for the subsequent determination of maximally stimulated platelet thromboxane production.

Blood lipid analysis

The HDL was separated from fresh plasma by dextran sulphate magnesium chloride precipitation.[15] Plasma samples were stored at – 80°C for subsequent analysis. Plasma total cholesterol, HDL-cholesterol and triglycerides were quantified using an automated analyser (Cobas Mira Plus automated analyser; Roche Diagnostics, Sydney, Australia) using commercially available kits (Cholesterol CHOD-PAP, Cat 1489232 Roche Diagnostics; Unimate 5 Trig, art 0736791 Roche Diagnostics, Australia). Non-HDL concentrations were calculated by subtracting HDL cholesterol from total cholesterol. The LDL cholesterol levels were estimated from total and HDL cholesterol and triglyceride concentrations according to the Friedewald equation.[16]

Isoflavone analysis

Urine samples were collected in 2 L plastic bottles containing 1.2 mg sodium azide and 1 g ascorbic acid as preservatives. Isoflavone content was determined by an enzymatic hydrolysis method adapted from King and Bursill.[17] Briefly, a 1 mL aliquot of 0.17 M ammonium acetate with 1.67×10^6 units/L of β-glucosidase/sulphatase (Sigma, Sydney, Australia) was added to each 500 µL urine sample and incubated overnight at 37°C. Three ethyl acetate extractions (2×2.5 mL and 1×2 mL) were performed. The ethyl acetate fractions were combined and dried under nitrogen gas at 37°C and reconstituted in 250 µL of HPLC mobile phase (60: 50: 1 (v/v/v) high performance liquid chromatography (HPLC) grade methanol: 0.1 M NH_4OAC, pH 4.6: 25 mM EDTA). Samples were separated by HPLC (Shimadzu, Sydney, Australia) using a C_{18} column (5µM, 4.6 mm by 250 mm, SGE, Melbourne, Australia) with mobile phase flowing at a rate of 1 mL/min. Samples were analysed by electrochemical detection (ESA, Coulochem) at a potential of 520 mV, with the conditioning set at – 50 mV and the analytical cell at 470 mV. An injection volume of 20 mL of extracted urine was applied to the column and measured at a sensitivity of 10 µA. Peak areas of daidzein (Sigma, Australia) and genistein (Sigma, Australia) were used to determine concentrations from standard curves formulae.

The isoflavone contents of the supplementary foods were determined by grinding a 5 g sample of each food in a mortar and pestle with 40 mL of absolute ethanol, 10 mL of 32% HCL and 1 mL of flavone standard (Sigma, Australia) (0.1 mM final concentration) and refluxing at 100°C for 2 h. The supernatant removed following centrifugation was stored at – 20°C until subsequent HPLC analysis. A 20 µL aliquot was applied to the column and measured at a sensitivity of 10 µA.

Fatty acid analysis

Washed erythrocytes were resuspended in a Tris buffer (10 mM Bis Tris, 2 mM EDTA Na_2, pH 7.2) in 1.7 mL tubes and transferred to centrifuge tubes (16 × 76mm). Additional Tris buffer was added to make a final volume of 10 mL. They were then capped, gently inverted several times and allowed to sit for 40 min to lyse the erythrocytes. The lysed cells were centrifuged in a Beckman L-8 ultracentrifuge (Beckman,

Table 2. Phytoestrogen and LNA content of the soy and linseed containing food supplements

	Total isoflavones (mg)*	Lignan (SECO) (mg)†	LNA (g)‡
Soy and linseed English muffin	10	22.5	1.2
Soy and linseed bread (2 slices)	9	9.0	0.5
Soy and linseed muesli bar	10	–	1.5
Soy and linseed oatcake	16	–	1.5
Canola margarine (15g)	–	–	0.8
Canola oil (5g)	–	–	0.5
Total	45	31.5	6.0

*Total isoflavones equals the sum of genistein and daidzein in the foods. †Estimated lignan content of the foods derived from values observed from assays performed with whole linseeds in our laboratories, SECO, secoisolariciresinol. ‡LNA, alpha-linolenic acid.
– Indicates the dietary component is not present in the food.

CA, USA) at 50 000 *g* for 30 min at 4°C. The pelleted erythrocyte membrane was recovered and resuspended in 200 µL of distilled water. Aliquots of 150 µL were removed for direct transesterification.[18] Briefly, 2 mL of methanol:toluene: (4: 1) was added and each sample vortexed on high while 200 µL of acetyl chloride was added slowly using a positive displacement pipette. Samples were then capped and placed in a heat block for 60 min at 100°C. Following heating, the tubes were placed in an ice bath for 5 min for rapid cooling. Then 3 mL of potassium carbonate was added and samples were centrifuged at 1000 *g* for 8 min at 4°C. The upper toluene phase, containing fatty acid methyl esters, was removed, ready for gas chromatography.

Fatty acid methyl esters were analysed by flame-ionization gas chromatography (model GC-17 A, Shimadzu) using a 30 m × 0.25 mm internal diameter capillary column. Individual fatty acids were identified by comparison with known fatty acid standards. The temperature program consisted of an initial temperature of 185°C, ramp function of 5°C/minute for 15 min, maintaining 260°C for 5 min, resulting in a total run time of 20 min. Injector and detector temperature were 260°C. The carrier gas was ultra-high purity hydrogen and the column flow rate was 1.54 mL/min. Peak quantification was calculated by area for corrected normalisation.

Thromboxane analysis

Fasting blood samples collected in plain blood collection tubes were placed immediately into an agitating water bath at 37°C for 60 min for maximal stimulation of platelets. Samples underwent centrifugation at 1000 *g* for 10 min at 4°C and subsequently, serum was removed from the pelleted blood clot. Maximally stimulated plated thromboxane levels were then assayed from the serum samples using an EIA Thromboxane B_2 Enzyme immunoassay kit (Cat. 519031; Cayman Chemical, Ann Arbor, USA).

Statistical analysis

Within subjects, changes were determined between (i) baseline and 3 weeks, (ii) baseline and 8 weeks and (iii) 3 and 8 weeks. Paired *t*-tests were used to determine statistical significance ($P < 0.05$) when comparing differences between two time points. Where assessments were made on three occasions, a Bonferroni correction was used ($P < 0.017$). Significance of an overall effect was determined using a repeated measures called MANOVA. Correlations between initial values and changes with intervention in various parameters were assessed by linear regression.

Results

Anthropometric and dietary data

Subject compliance with the soy and linseed diet intervention appeared to be good. Self-reporting indicated that, with a few exceptions as a result of illness or special occasions, all test foods were consumed daily. No subject omitted soy and linseed foods from their diet for more than 2 days.

Dietary intakes were estimated by analysing 24 h food recalls obtained from unannounced telephone calls (Table 3). The percent of total energy (%E) obtained from carbohydrate, protein and total fat after 3 and 8 weeks did not change when compared with baseline values. The ratio of dietary polyunsaturated to saturated fat (P:S) however, did change with polyunsaturated fat intake increasing 85% ($P < 0.001$) and saturated fat consumption decreasing by 23% of the baseline value ($P < 0.001$) after 3 weeks. The favourable change in P:S ratio was maintained for the study duration. Fibre consumption was doubled after 3 weeks of soy and linseed supplementation. Again these higher intakes were maintained through until the end of 8 weeks. There was no significant change in weight or body mass index (BMI) at either 3 or 8 weeks of the intervention.

Plasma lipids

Plasma concentrations of total, LDL and non-HDL cholesterol (total – HDL cholesterol) were significantly reduced with daily consumption of soy and linseed containing foods ($P < 0.001$, repeated measures MANOVA). Reductions of 10, 12.5 and 12%, respectively, were seen within 3 weeks of commencing the dietary supplementation (Fig. 1). However, these reductions were attenuated after 8 weeks, with only total and non-HDL cholesterol concentrations remaining significantly lower than baseline values ($P < 0.017$). A non-significant trend towards increased HDL cholesterol and reduced triglycerides was observed after 3 weeks, with concentrations returning to baseline by the end of the intervention (Table 4). There was a significant correlation between baseline concentrations of triglycerides and the reductions observed after 3 weeks ($r = -0.458$, $P < 0.05$).

Table 3. Estimates of subjects' daily nutrient intakes at baseline, weeks 3 and 8 of the intervention[†]

	Baseline	Duration of diet Week 3	Week 8
Total energy (KJ)	7756.1 ± 738.1	8287.2 ± 391.5	7942 ±405.7
Dietary intake (% Energy)			
Carbohydrate	44 ± 2.1	40.9 ± 1.2	42.2 ± 1.7
Protein	18.8 ± 0.9	19.1 ± 0.6	18.2 ± 0.8
Alcohol	1.3 ± 0.8	0.8 ± 0.6	0.2 ± 0.1
Fat	34.7 ± 2.4	37.2 ± 1.3	37.5 ± 1.5
PUFA	6.3 ± 0.5	11.3 ± 0.4*	11.7 ± 0.4*
SFA	13.4 ± 0.8	10.3 ± 0.5*	10.0 ± 0.7*
MUFA	14.3 ± 1.5	15.5 ± 0.7	15.7 ± 0.9
Fibre (g)	24.5 ± 1.7	51.2 ± 2.5*	47.0 ± 2.3*

[†]Mean ± SEM. PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids. Asterisk denotes values that are significantly different from baseline ($P < 0.017$).

Urinary isoflavones

Consistent with a normal western diet, the mean urinary isoflavone excretion rate at baseline was negligible (Fig. 2). No individual excretion rate exceeded 0.94 mg/day. Thus, it is not surprising that 3 weeks of soy and linseed supplementation resulted in a 30-fold increase in the total isoflavone excretion rate. Urinary excretion of genistein and daidzein increased from 0.12 to 1.3 mg/day and from 0.11 to 5.3 mg/day, respectively, after 3 weeks. At the end of 8 weeks, the excretion rate of total isoflavones (2.04 mg/day) was still significantly higher than the baseline, but lower than the rate of excretion observed at 3 weeks (6.6 mg/day), with the observed rate of excretion representing only an approximate eightfold increase from baseline values.

Plasma fatty acids

The proportion of LNA relative to total plasma fatty acids more than doubled during the intervention (Table 5), with a resultant increase in plasma EPA (57% after 3 weeks and 69% after 8 weeks). There were also modest increases in DHA and linoleic acid in plasma after 8 weeks but there was no change in arachidonic acid.

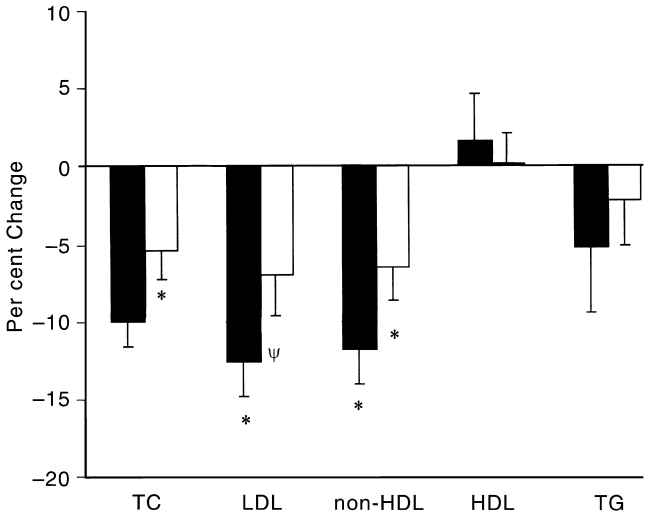

Figure 1. Percent changes in fasting blood lipids at 3 and 8 weeks compared with baseline values. Asterisks denote plasma lipid concentrations which are significantly different from baseline ($P < 0.017$) using a paired t-test with Bonferroni correction. ψ denotes significant difference between 3 and 8 weeks ($P < 0.017$). Repeated measures analysis confirmed significant reductions in total, LDL and non-HDL cholesterol ($P < 0.01$). TC, total cholesterol; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triglyceride; (■), week 3; (□), week 8.

Erythrocyte fatty acids and platelet thromboxane

Despite the significant increases observed in plasma levels of LNA, EPA and DHA, there was no evidence of increased incorporation of these fatty acids into erythrocyte membranes after 8 weeks of dietary LNA supplementation (Table 6). Similarly levels of arachidonic acid, the predominant omega-6 fatty acid precursor of eicosanoids, remained unaltered. Accordingly, maximally stimulated platelet thromboxane production, known to decrease as a consequence of increased intracellular levels of EPA and DHA, did not change significantly. Several subjects had still been taking NSAIDS at baseline. In the 15 subjects not taking NSAIDS, concentrations of thromboxane B_2 in serum from incubated blood taken initially and after 8 weeks of intervention, were 187 ± 28 and 153 ± 30 pg/mL, respectively, representing a within-individual reduction of 34 ± 23 pg/mL (not significant).

Discussion

The primary finding of this study was that daily consumption of soy and linseed containing foods and Canola by mildly hypercholesterolaemic women resulted in clinically significant improvements of plasma cholesterol after 3 weeks. The reductions of total, LDL and non-HDL cholesterol were not transient but were still evident, albeit reduced in magnitude, after 8 weeks of continuous dietary supplementation. Changes in the intakes of several nutrients which occurred as a consequence of the soy, linseed and Canola supplementation may have accounted for the observed changes in plasma lipid. Apart from the increased intake of soy protein and isoflavones, saturated fat intake decreased and there were increases in the intake of polyunsaturated fat (primarily LNA), fibre and lignans. All of these changes had the potential to contribute to the reduction of plasma cholesterol concentrations.

The influence of soy protein consumption on plasma cholesterol concentrations has been the subject of extensive investigation, culminating in the above-mentioned health claim for soy protein.[1] The meta-analysis by Anderson *et al.* found that in individuals with mildly elevated plasma cholesterol (5.2–6.6 mmol/L), soy protein consumption accounted for a 4.4% reduction in total plasma cholesterol independent of changes in saturated fat, total fat or dietary cholesterol intakes.[4] However, not all studies have confirmed this effect. Potter *et al.* found that daily consumption of 40 g of soy protein failed to reduce either total or LDL cholesterol in mildly hypercholesterolaemic postmenopausal women.[19]

Most of the studies included in the meta-analysis did not report the amount of dietary isoflavones contained within the soy protein supplements. Subsequent research, however,

Table 4. Plasma lipid concentrations (mmol/L) at baseline and during the intervention†

	Baseline	Duration of diet 3 weeks	8 weeks
Total	6.44 ± 0.19	5.80 ± 0.20*	6.11 ± 0.24*
LDL	4.30 ± 0.16	3.79 ± 0.18*	4.00 ± 0.19‡
Non-HDL	5.24 ± 0.17	4.65 ± 0.21*	4.92 ± 0.23*
HDL	1.20 ± 0.08	1.20 ± 0.07	1.19 ± 0.07
Triglycerides	2.06 ± 0.20	1.92 ± 0.18	1.99 ± 0.19

†Mean ± SEM. * denotes plasma lipid concentrations that are significantly different from baseline ($P < 0.017$). ‡ denotes plasma lipid concentrations that are significantly different from concentrations observed at week 3 ($P < 0.017$). Total, total cholesterol; LDL, low-density lipoprotein cholesterol; non-HDL, subtraction of HDL cholesterol from total cholesterol; HDL, high-density lipoprotein cholesterol.

suggests that the isoflavones are a necessary contributor to the hypocholesterolaemic effect.[6] However, it has not been established whether they can act independent of other components in soy protein to affect plasma cholesterol. Preliminary studies using extracts of red clover or soy in tablet form to provide a daily dose of 40–80 mg of isoflavones have failed to produce any change in plasma lipids.[20–22] However, these studies were conducted in subjects with normal cholesterol concentrations and should be replicated in hypercholesterolaemic individuals.

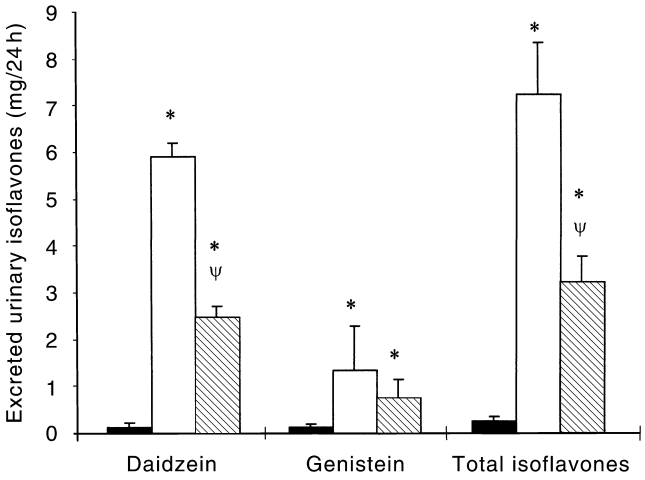

Figure 2. Amount of isoflavones recovered from urine samples collected at baseline and after 3 and 8 weeks of soy and linseed supplementation. Asterisks denote plasma lipid concentrations which are significantly different from baseline ($P < 0.017$). ψ denotes urinary concentrations which are significantly different from week 3 concentrations ($P < 0.002$). Data from 15 subjects was used to compare urinary excretion at weeks 3 and 8 with baseline levels. (■), baseline; (□), week 3; (▨), week 8.

Urinary isoflavone concentrations have been shown to be useful biomarkers of dietary soy consumption.[23] The significant increase in urinary isoflavones after 3 weeks demonstrates good dietary compliance by the subjects. Assuming full compliance with a dietary supplementation rate of 45 mg/day, urinary excretion rates observed after three weeks represent recoveries of 10 and 17% for genistein and daidzein, respectively, and a 14.7% recovery of total ingested isoflavones. These values correspond with recoveries reported by others.[24–26] By 8 weeks, the recovery of isoflavones in urine was substantially reduced. This could reflect a decrease in dietary compliance by the subjects; however, self-reporting of intakes in unannounced 24 h dietary recalls suggested that the consumption of isoflavones was unchanged. This is further supported by the sustained increase in plasma LNA concentration after 8 weeks. The richest sources of LNA, namely muesli bars and oatcakes, were also the richest source of isoflavones, particularly daidzein. If the decreased urinary excretion of isoflavones was as a result of reduced consumption of these foods, then plasma LNA concentrations may also have been expected to decline during the course of the study. An alternative explanation for the decrease in urinary isoflavone excretion after 8 weeks is that there is increased conversion of daidzein and genistein to their respective metabolites and thus a reduction in their bioavailability. There is evidence that the recovery of isoflavones in urine decreases progressively during chronic soy consumption.[27]

The pattern of change in urinary isoflavone excretion corresponds with the reduction of plasma cholesterol, suggesting a causal relationship. In an attempt to estimate the potential contribution of soy protein/isoflavone consumption to the observed change in cholesterol, we took an average of the cholesterol reduction attributed to soy protein consumption in the above-mentioned meta-analysis[4] (i.e., 4.4%) and the extent of cholesterol reduction which might be predicted

Table 5. Omega-6 and omega-3 fatty acids (expressed as percentage of total fatty acids) in plasma at baseline and after 3 and 8 weeks of the intervention†

	Baseline	Week 3	% change	Week 8	% change
LA	25.39 ± 0.99	26.03 ± 1.00	3.53 ± 3.28	27.92 ± 0.76* ‡	12.39 ± 4.28
AA	6.0 ± 0.45	5.67 ± 0.37	– 2.32 ± 3.85	5.97 ± 0.38	2.95 ± 3.48
LNA	0.47 ± 0.04	1.10 ± 0.10*	136.51 ± 15.19	1.17 ± 0.12*	164 ± 25.22
EPA	0.78 ± 0.08	1.20 ± 0.13*	57.33 ± 16.57	1.29 ± 0.13* ‡	68.97 ± 12.90
DHA	2.77 ± 0.17	2.89 ± 0.21	9.69 ± 9.87	3.18 ± 0.15* ‡	20.10 ± 8.32

†Mean ± SEM. LA, Linoleic acid; AA, Arachidonic acid; LNA, α-Linolenic acid; EPA, eicosapentaenoic acid, DHA, docosahexaenoic acid. * denotes a statistically significant change from baseline ($P < 0.017$). ‡Denotes percent of total fatty acids in plasma is significantly different from that observed at week 3 ($P < 0.017$).

Table 6. Omega-3 and omega-6 fatty acids (expressed as percentage of total fatty acids) in erythrocyte membranes at baseline and after 8 weeks of soy and linseed supplementation*

	Baseline	Week 8	% change
LA	7.81 ± 0.23	8.17 ± 0.23	4.78 ± 1.22
AA	15.01 ± 0.27	14.74 ± 0.26	– 1.67 ± 0.74
EPA	1.68 ± 0.06	1.63 ± 0.06	– 2. 70 ± 0. 95
DHA	10. 78 ± 0. 28	10.91 ± 0.26	1.48 ± 0. 97

*Mean ± SEM. LA, linoleic acid; AA, arachidonic acid; EPA eicosapentaenoic acid; DHA, docosahexaenoic acid.

from the above-mentioned dose–response study[6] following consumption of 37 mg of isoflavones/day (i.e., 6%). We thus predicted that the soy protein/isoflavone component of the diet may have reduced total cholesterol by 5.2% (i.e., 0.33 mM). This would account for all of the cholesterol reduction observed after 8 weeks. However, it would account for only half of the 10% decrease seen after 3 weeks, suggesting that other dietary factors may also have contributed to the decrease.

The increases in plasma LNA and EPA at 3 and 8 weeks do not correspond with the pattern of change observed for plasma lipids and are therefore unlikely to have contributed to the hypocholesterolaemic benefit. Any cardiovascular benefits of LNA supplementation are generally attributed to its conversion to the very long-chain omega-3 fatty acids.[28] However, despite a significant increase in plasma EPA and DHA, there was no evidence of increased incorporation of either into membrane storage sites. Antithrombotic and hypotriglyceridaemic effects are the hallmarks of omega-3 supplementation. Neither plasma triglycerides nor platelet thromboxane production were significantly changed in the present study. The increases in plasma LNA and EPA were similar to those reported by others using higher rates of dietary LNA supplementation (Table 7). However, they also found no changes in eicosanoids, plasma cholesterol or triglyceride concentrations.[29,31]

While these and other studies do not support a cholesterol lowering effect of LNA per se, the resultant change in dietary P:S ratio as a consequence of increased LNA consumption may have influenced plasma lipids. Reductions in plasma cholesterol have been observed in both hyperlipidaemic and healthy subjects fed whole or milled flaxseed supplements providing doses of 24 g and 14 g of LNA/day, respectively.[12,33] The authors hypothesised that the flaxseed gums and components contained within the whole flaxseed may contribute to cholesterol reduction by increasing bile acid excretion with increased laxation, or through lignan modulation of cholesterol metabolising enzymes. These mechanisms may have contributed to the reduction of cholesterol in the present study. However, it is more likely that the change in dietary P:S ratio from the increased LNA intake was a contributing factor.

It has been well established that saturated fat increases total and LDL plasma cholesterol and that polyunsaturated fats decrease it.[34,35] The observed reductions in total, LDL and non-HDL cholesterol after 3 weeks are consistent with the change in the dietary P:S ratio. However, the P:S ratio tended to increase slightly between 3 and 8 weeks, while the reductions in cholesterol were markedly attenuated. If this dietary factor were having a great influence on plasma lipids, it would be expected that plasma cholesterol concentrations would not change between 3 and 8 weeks.

Dietary fibre increased significantly with the soy and linseed foods, mostly in the form of soluble fibre from oats, predominate in the muesli bar and oatcake. A recent meta-analysis of 67 controlled trials concluded that 1 g of soluble fibre/day produced a change in total cholesterol of –0.045 mmol/L, irrespective of initial lipid concentrations.[36] Thus the increased soluble fibre intake resulting from the consumption of oats in the muesli bars and oatcakes could account for a 0.08 mmol/L decrease in total cholesterol. This represents about one-eighth of the observed decrease in total cholesterol after 3 weeks. Hence, the increase in dietary fibre is likely to be a minor contributor to the reduction in plasma cholesterol.

This study has demonstrated the feasibility of supplementing the diet with soy and linseed containing foods to achieve clinically useful reductions of total, LDL and non-HDL cholesterol. However, the specific dietary factors and mechanisms responsible for the favourable lipid change are unclear. The cholesterol reductions were most likely because of the increased consumption of soy protein/isoflavones and a concurrent increase in the P:S ratio of the diet. This favourable outcome warrants the conduct of longer duration, placebo-controlled, clinical trials to examine the health benefits of the soy and linseed dietary combination and to elucidate the underlying mechanisms. It highlights the potential to design foods with appropriate combinations of active nutrients to optimise dietary prevention and treatment of cardiovascular risk factors in populations with an elevated risk of developing cardiovascular disease.

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Table 7. Comparison of study population, duration and changes in plasma concentrations of LNA and EPA in clinical trials involving dietary LNA supplementation

Study	Subjects	Duration	LNA dose (g/day)	% change in plasma LNA	% change in plasma EPA
Sanders and Roshani[31]	Healthy (n = 6)	2 weeks	9.4	150	100
Kestin *et al.*[29]	Mildly hypercholesterolemic (n = 38)	6 weeks	9.2	265	100
Mantzioris *et al.*[32]	Healthy (n = 15)	4 weeks	13.7	1000	135
Li *et al.*[30]	Healthy, vegetarian (n = 10)	6 weeks	3.7	60	20
This study	Mildly hypercholesterolemic (n = 18)	8 weeks	6	164	69

EPA, eicosapentaenoic acid; LNA, α-Linolenic acid.

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