Nutrition Journal

BioMed Central

Research

Open Access

Effects of isoflavones (soy phyto-estrogens) on serum lipids: a meta-analysis of randomized controlled trials

John Yeung*[1] and Tak-fu Yu[2]

Address: [1]Medical Centre, Kowloon Motor Bus Company, Hong Kong and [2]Medical Centre, G/F, Greenrich Mansion, 100 Castle Peak, Hong Kong

Email: John Yeung* - john@epidemiology.org.hk; Tak-fu Yu - yutakfu1@yahoo.com.hk

* Corresponding author

Published: 19 November 2003

Nutrition Journal 2003, **2**:15

Received: 19 September 2003

Accepted: 19 November 2003

This article is available from: http://www.nutritionj.com/content/2/1/15

Abstract

Objectives: To determine the effects of isoflavones (soy phyto-estrogens) on serum total cholesterol (TC), low density lipoprotein cholesterol (LDL), high density lipoprotein cholesterol (HDL) and triglyceride (TG).

Methods: We searched electronic databases and included randomized trials with isoflavones interventions in the forms of tablets, isolated soy protein or soy diets. Review Manager 4.2 was used to calculate the pooled risk differences with fixed effects model.

Results: Seventeen studies (21 comparisons) with 853 subjects were included in this meta-analysis. Isoflavones tablets had insignificant effects on serum TC, 0.01 mmol/L (95% CI: -0.17 to 0.18, heterogeneity p = 1.0); LDL, 0.00 mmol/L (95% CI: -0.14 to 0.15, heterogeneity p = 0.9); HDL, 0.01 mmol/L (95% CI: -0.05 to 0.06, heterogeneity p = 1.0); and triglyceride, 0.03 mmol/L (95% CI: -0.06 to 0.12, heterogeneity p = 0.9). Isoflavones interventions in the forms of isolated soy protein (ISP), soy diets or soy protein capsule were heterogeneous to combine.

Conclusions: Isoflavones tablets, isolated or mixtures with up to 150 mg per day, seemed to have no overall statistical and clinical benefits on serum lipids. Isoflavones interventions in the forms of soy proteins may need further investigations to resolve whether synergistic effects are necessary with other soy components.

Introduction

In recent years, phytoestrogens have attracted a great deal of interests in the medical and scientific literature. It also appears in the lay press for its effects on cardiovascular and menopausal health, and even cancer prevention. These compounds are present in large quantities in soybeans, clover and some legumes. Because of resemblances to human estrogen and the observations that Asian populations who consume more isoflavones compared with women in western countries have less menopausal symptoms, isoflavones are postulated as natural products that may be beneficial to postmenopausal women in cardiovascular health. Commercial products containing different quantities and mixtures of isoflavones are now widely available. A meta-analysis of soy protein in 1995 found significant cholesterols lowering effects when compared with animal proteins [1]. The authors suggest that isoflavones may be the principal physiologically active components responsible for the lipid lowering effects. However, it is still controversial [5]. Many reviews and editorials

[2,3] have discussed the relevance of phytoestrogens on cardiovascular health and hypocholesterolemic effects but there was no meta-analysis up to our searching.

Methods

Searching strategy

We searched the databases from ACP Journal Club 1991 to Oct 2002, Cochrane Controlled Trials Register 3rd Quarter 2002, Cochrane Database of Systematic Reviews 4th Quarter 2002, Database of Abstracts of Reviews of Effectiveness 4th Quarter 2002, British Nursing Index (BNI) 1994 to Oct 2002, CANCERLIT 1975 to Oct 2002, CINAHL 1982 to Oct Week 4 2002, CSA – Life Sciences Collection 1982 to Oct 2002, EMBASE 1980 to 2002 Week 45, International Pharmaceutical Abstracts 1970 to Oct 2002, PREMEDLINE Oct 27, 2002, MEDLINE 1996 to Oct Week 4 2002. We searched the keywords with Ovid software version rel6.2.0: 'soy', 'soy protein', 'soybean', 'tofu', 'phytoestrogen', 'isoflavone', 'genistein', 'daidzein', 'formononectin' and 'biochanin A' by the method described by Dickersin 1994 [4]. We did not restrict any languages during the searching. Hand searching was made by retrieving relevant articles from the obtained studies and unpublished data were obtained through contacting experts. We identified on-going trials by searching ClinicalTrials.gov, the UK National Research Register and Meta-register of controlled trials on the internet.

Selection of eligible trials

We included both single and double blind randomized controlled trials with baseline and after treatment values for synthesizing risk (mean) differences. The outcome measures were differences of serum total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C) and triglycerides (TG) between post-randomization baselines and after treatments. Eligible interventions were isoflavones tablets of single isolated component or any mixtures of genisteins, daidzein, formononectin and biochanin A. Isoflavones interventions in forms of soy protein supplements or diets were also included as long as they compared isoflavones containing treatments with isoflavones depleting controls. Treatments with soy polysaccharides, fiber, and phytosterols as their primary interventions were excluded.

Validity assessment and data abstraction

Two independent investigators reviewed the articles obtained without masking. There were no scorings to the included trials. Data were entered twice to reduce input errors. Inter-rater reliability was not performed. Data were abstracted with a designed form before analysis. Duplicated trials or studies with the same population were counted once to reduce the duplicated publication bias. Data disagreement between the two reviewers was resolved by discussion.

Study characteristics

The characteristics of the obtained studies were tabulated with subtypes of isoflavones interventions, subjects' serum lipids status, dosages of isoflavones and lengths of treatment. Sub-group analysis was performed with different forms of isoflavones interventions, such as isolated genistein or mixtures of isoflavones tablets versus placebo, isoflavones containing versus depleting soy protein diets. Sensitivity analysis was also made across different population characteristics and lengths of treatment. Funnel plots were used to detect possible publication bias or treatment heterogeneity across sample sizes.

Quantitative data synthesis

We obtained the risk differences (RD) from the post-randomization baselines and after-treatment values in each trial and calculated the pooled standard deviation of the RD as:

$$\sqrt{\frac{(n_1-1)SD_1^{\,2}+(n_2-1)SD_2^{\,2}}{(n_1-1)+(n_2-1)}}$$

where n_1 and SD_1 were sample size and standard deviation from treatment and n_2 and SD_2 were from control. Inverse variance method was used to pool all trial results [6] with software 'Review Manager 4.2 [31]. Fixed-effects model was used as the method of combination and it was supplemented with random-effects model if necessary. Since log odds ratios were not available in the trials, funnel plots were plotted with standard errors against risk (mean) differences.

Results

Trial flow

The trial flow chart was illustrated in Figure 1. Seventeen studies [7-23] (21 comparisons) with 853 subjects were included in this meta-analysis. Reasons for exclusion were non-randomization, lack of control [24-27], insufficient original data and baseline values [28,29].

Study characteristics

The characteristics of the trials included were shown in Table 1. The populations being studied were adults of age between 18 and 73. Soy phytoestrogens interventions varied from isoflavones tablets to isoflavones containing soy diets. Isoflavones tablets were introduced in 11 trials, soy capsule in 1 trial, isolated soy protein (ISP) in 3 trials, and soy foods in 2 trials. The average intake of isoflavones was 73 mg per day (ranging from 28.5 to 150 mg) and length of treatment was 10 weeks (ranging from 2 to 26 weeks). Three trials recruited hyperlipidemic subjects while 8 included participants with normolipidemia. Thirteen studies recruited female, two recruited male and two recruited subjects with both genders.

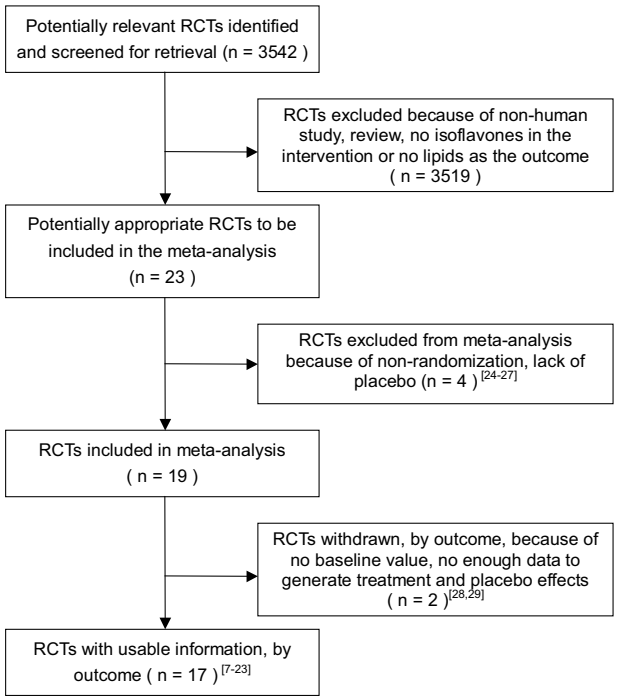

Figure 1

Trial flow chart of including isoflavones studies. Databases generated 3542 potential eligible randomized controlled trials (RCT). After careful review, seventeen RCT (reference [7-23]) were included in this meta-analysis.

Quantitative data synthesis

We found that isoflavones tablets insignificantly increased serum TC by 0.01 mmol/L (95% CI = -0.17 to 0.18; heterogeneity p = 1.0) (Figure 2); LDL by 0.00 mmol/L (95% CI = -0.14 to 0.15; heterogeneity p = 0.9); HDL by 0.01 mmol/L (95% CI = -0.05 to 0.06; heterogeneity p = 1.0); and triglyceride by 0.03 mmol/L (95% CI = -0.06 to 0.12; heterogeneity p = 0.9). Isoflavones containing soy biscuits or burger had insignificant effects on serum TC with reference to isoflavones depleting soy biscuits or burger. Only isoflavones containing isolated soy protein (ISP) or soy protein (SP) capsule reduced serum TC when compared with isoflavones depleting ISP or SP capsule (Table 2). There was only one trial [11] introduced SP capsule as treatment. Mixtures of the four soy phytoestrogens (genistein, daidzein, formononectin and biochanin A) or isolated genistein did not have significant effects on serum TC. The significant result from intervening the mixtures of genistein and daidzein had shown to be statistically heterogeneous to combine (heterogeneity p = 0.06) as genistein and daidzein tablets were introduced among 3 studies [9,10,16] while Sanders [19] chose genistein and daidzein soy burger and Urban [22] used genistein and daidzein ISP. In subgroup analysis, the effect of genistein and daidzein tablets on serum TC from the 3 studies was shown to be insignificant, 0.1 mmol/L (95% CI = -0.25 to 0.45; heterogeneity p = 0.87). Dose-response effect was not found in any forms of interventions in this meta-analysis. The results remained insignificant when the length of treatment increased. Although one trial [22] (without washout period) produced significant effect, it seemed that study design had no influence on the serum TC. There were insignificant decreases of serum TC among pre- and postmenopausal women. Interestingly, cholesterol lowering effects could only be found among normolipidemic subjects but not participants with hyperlipidemia and men were shown to be benefited with isoflavones treatment. Isoflavones in the forms of tablets and ISP were all insignificant on serum low density lipoprotein cholesterol (LDL), high density lipoprotein cholesterol (HDL) and triglycerides (TG). These results were shown in Table 3. Funnel plots of isoflavones tablets were asymmetrical and the results of the published trials tended to favor treatment.

Discussion

Main findings

Phytoestrogens with either isolated genistein or isoflavones mixtures of genistein, daidzein, formononectin and biochanin A were found to be statistically insignificant in lowering serum total cholesterol (Table 2). Resolving the heterogeneity from pooling the mixtures of genistein and daidzein, we found that tablets prescription with these two isoflavones [9,10,16] yielded insignificant benefits over serum TC, 0.1 mmol/L (95% CI = -0.25 to 0.45; heterogeneity p-value = 0.87). One trial [19], introducing soy burger as the prescription of these 2 isoflavones, found insignificant benefits. Another trial [22], prescribing them in the form of isolated soy protein (ISP), had shown a decrease in serum TC by 0.43 mmol/L (95% CI = -0.73 to -0.13). It may be a result of bias as this trial lacked a washout period. When dose-response effect and treatment length were taken into account, phytoestrogens seemed to have insignificant effects over serum cholesterol (Table 2). Isoflavones in the forms of tablets, soy burger or biscuits seemed to have insignificant benefits over serum TC. Only Han [11] reported a significant benefit for isoflavones in the form of soy protein capsule. Isoflavones in the forms of tablets or ISP could not be shown to have significant benefits over serum low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol and triglycerides (TG) (Table 3).

We could not demonstrate significant benefits with all types of prescriptions when the dosages or treatment lengths increased. No dose-response effect was detected nor benefits on serum cholesterol with any forms of prescription among pre- and post-menopausal women and

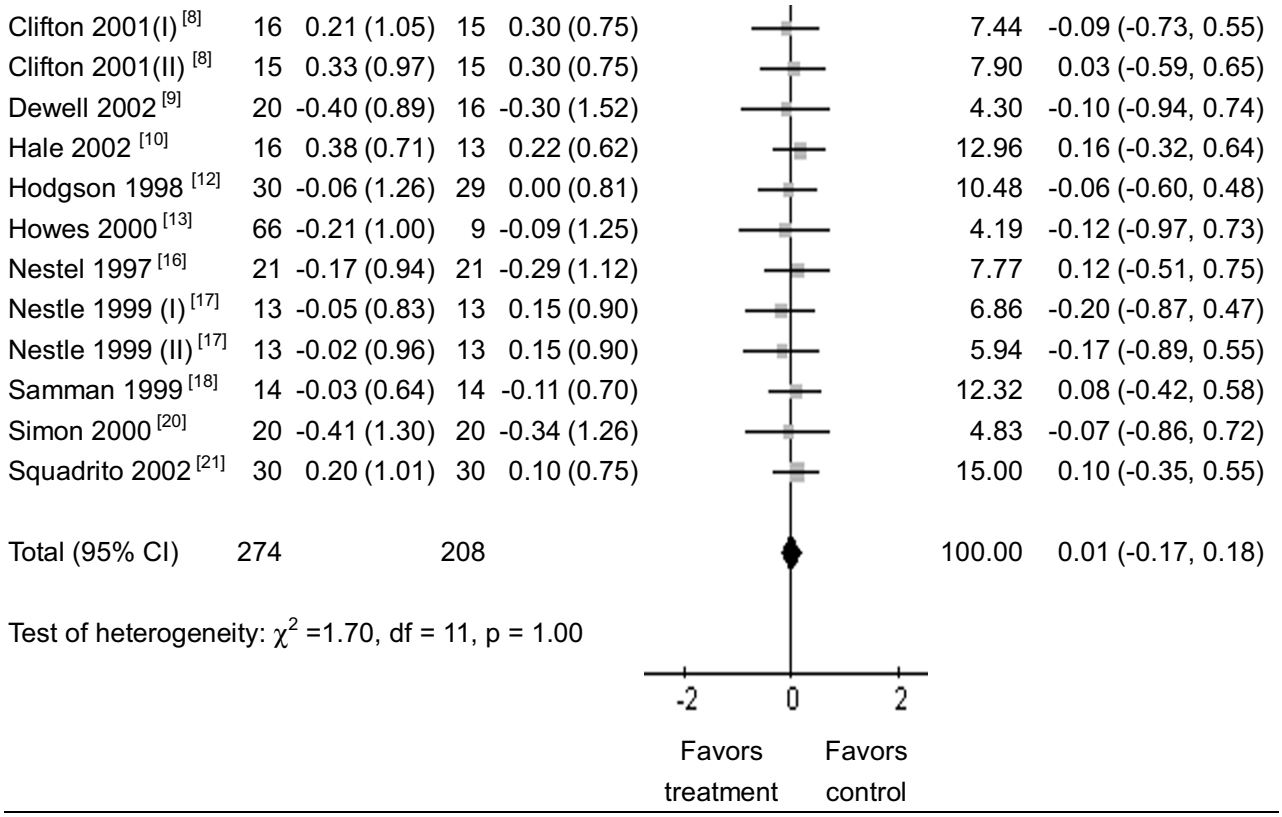

Figure 2

Funnel plot of risk difference of isoflavones tablets versus placebo on serum total cholesterol. Total treatment size was 274 and total control was 208. The pooled risk difference of isoflavones tablets versus placebo was 0.01 with 95% confidence interval between -0.17 and 0.18. Heterogeneity p-value was 1.00. Note: 'N' = sample size; 'SD' = standard deviation; 'RD' = risk difference; '95% CI' = 95% confidence interval

hyperlipidemic subjects. The benefit over normolipidemic subjects was heterogeneous (p = 0.08). It may be again the result of prescription diversity. Separated analysis found that isoflavones tablets [12,18,21] had insignificant effect on serum TC among normolipidemic subjects, 0.05 mmol/L (95% CI = -0.23 to 0.33; heterogeneity p = 0.9). Only isoflavones in the form of soy protein capsule [11] or ISP [22] reported benefits on participants with normolidemia. In fact, some suggest that the beneficial effects of soy protein require synergistic reactions between isoflavones and other soy components [32]. This hypothesis was supported by some animal experiments [33-35]. It seemed that isoflavones in the form of tablets, up to 150 mg per day (genistein about 50 mg, daidzein about 50 mg), had no significant benefits on serum cholesterols in this meta-analysis. Phytoestrogens treatments in forms of soy protein supplements or soy protein capsule may need further investigations, especially interactions between other chemically active components inside prescriptions.

Validity and limitation

In subgroup analysis, the sample sizes were inevitably reduced and there might be insufficient power to detect small significant differences. Isoflavones treatments with dosages beyond 150 mg per day or treatment lengths beyond 26 weeks could not be shown in this study. The effects on serum cholesterols among subjects with hypertension, diabetes mellitus or other cardiovascular risk factors were also beyond this meta-analysis. Generalizability over other phytoestrogens such as coumestans, lignans

Table 1: Characteristics of the 17 included studies. There were 9 crossover and 8 parallel studies. The isoflavones tablets introduced were mainly in aglycone form. The abbreviations were listed below table.

Study	Treatment (Rx) †	Isoflavones content in Rx (mg/d) ‡	Control	Isoflavones content in control (mg/d)	No. of subjects	Lipid status §	Subject gender ‖	Length of treatment (weeks)
*Calvert 1981 [7]	soy biscuits	NA	soy biscuits	0	10	H	male	4
Clifton-Bligh 2001(I) [8]	T	57	T					
Clifton-Bligh 2001(II) [8]	T	85.5		28.5	46	B	female	26
Dewell 2002 [9]	T	150 G = 40; D = 50; glycosides = 60	placebo	0	36	H	female	24
Hale 2002 [10]	T	80 G = 40; D = 40	placebo	0	29	NS	post	2
Han 2002 [11]	soy protein capsule	100 G = 70; D = 18; glycitein = 12	soy protein capsule	0	78	N	peri	16
Hodgson 1998 [12]	T	55 G = 30; D = 1; B = 16; F = 8	placebo	0	59	N	male & female	8
Howes 2000 [13]	T	40 G = 1; D = 0.5; B = 26; F = 16	placebo	0	75	NS	post	5
Mackey 2000 [14]	ISP	65	ISP	< 4	49	H	post	12
*Merz-Demlow 2000(I) [15]	ISP	113–144	ISP					
*Merz-Demlow 2000(II) [15]	ISP	55–74		9–11	13	N	female	3
*Nestel 1997 [16]	T	40–80 G= 22–43; D = 17–33; glycitein= 1–3	placebo	0	21	B	female	5
*Nestel 1999(I) [17]	T	80 G = 8; D = 7; B = 49; F = 16	placebo					
*Nestel 1999(II) [17]	T	40 G = 4; D = 3.5; B = 24.5; F = 8		0	13	B	female	5
*Samman 1999 [18]	T	86 G = 8.6; D = 7.4; B = 51.4; F = 18.6	placebo	0	14	N	pre	16
*Sanders 2002 [19]	soy burger	56 G = 34.8; D = 21.2	soy burger	2	22	N	male & female	2
*Simons 2000 [20]	T	80	placebo	0	20	N	post	8
Squadrito 2002 [21]	T	54 G= 54	placebo	0	60	N	female	24
*Urban 2001 [22]	T	70 G = 42; D = 27	ISP	3	28	N	elderly men	6
*Wangen 2001(I) [23]	ISP	110–154	ISP					
*Wangen 2001(II) [23]	ISP	54–76		6–8.2	18	NS	post	4

* crossover design † 'T' = isoflavone tablet; 'ISP' = isolated soy protein ‡ 'G' = Genistein; 'D' = Daidzein; 'B' = Biochanin A; 'F' = Formononectin §'H' = hyperlipidemic subjects, 'N' = normolipidemic subjects; 'B' = both hyper- and normolipidemic subjects; 'NS' = not specified ‖ 'pre' = premenopausal women; 'peri' = perimenopausal women; 'post' = postmenopausal women

and resveratrol may need further trials to clarify the questions. No funnel plots were shown in this study because the choice of risk differences as the horizontal axes may lead to funnel plot asymmetry [36]. Publication bias was still possible although vigorous searching had been attempted.

Biological plausibility

It was suggested that isoflavones readily bind estrogen receptors – beta (ER-beta) which are an important receptors in both the central nervous and cardiovascular systems [37-39]. Isoflavones may also have anti-oxidant effects on blood vessels [40-42]. However, compared with estradiol, isoflavones bind estrogen receptors with 100 and 1,000 times less affinity [30]. Both conjugated and aglycone forms of daidzein and genistein are rapidly cleared from the plasma following a single dose of isoflavones [43] and urinary excretion is virtually nil within 48 hours after ingestion [44,45]. Biological effects of soy phytoestrogens are still controversial. The hypothesis from an influential meta-analysis [1] stating soy estrogens may be responsible for the lipid reduction effects was criticized for the fact that the trials involved largely isoflavone-free soy protein products [46]. Apart from phytoestrogens, in fact, some other components such as phytic acid, saponins and fibers were potential candidates responsible for the hypocholesterolemic effects [32,48,49].

Suggestion to future trials

Tablets of single isolated isoflavone with considerable dosages may be important in future randomized trials. Intervention with isoflavones mixtures may be helpful but interactions between components should be handled with extra cares. Introduction of phytoestrogens in forms of soy diets is not suggested because we found much heterogeneity inside the diets, ranging from soy biscuits to burger, which may be difficult to combine and analyze. Confounding factors such as fiber, fatty acids, amino acids and energy intake are especially hard to control. Complications associated with hyperlipidemia, such as coronary

Table 2: Subgroup analysis of the effects of isoflavones on serum total cholesterol. Isoflavones were shown to have significant benefits when given in the form of ISP or soy protein capsule. Regardless the forms of prescription, isoflavones decreased serum total cholesterol among normolipidemic but not hyperlipidemic subjects.

Subgroup outcome	No. of comparisons	No. of subjects ||	Treatment effect on TC (mmol/L) ¶	Heterogeneity p-value §
Form of intervention †:				
a.) isoflavones tablets	12 [8-10,12,13,16-18,20,21]	482	0.01 (-0.17, 0.18)	1.00
b.) ISP(+)	8 [14,15,22,23]	229	-0.11 (-0.21, -0.01)*	0.34
c.) soy protein capsule	1 [11]	78	-0.69 (-1.19, -0.19)*	NA
d.) soy biscuit	1 [7]	20	-0.05 (-0.47, 0.37)	NA
e.) soy burger	1 [19]	44	0.09 (-0.38, 0.56)	NA
f.) Overall	21 [7-23]	853	-0.09 (-0.18, -0.01)*	0.77
Isoflavones mixture ‡:				
G&D&B&F	5 [12,13,17,18]	214	-0.06 (-0.34, 0.21)	0.96
G&D	6 [9-11,16,19,22]	285	-0.22 (-0.41, -0.03)*	0.06
G	1 [21]	60	0.10 (-0.35, 0.55)	NA
Isoflavones intake (mg per day):				
< 50	3 [8,13,17]	132	-0.12 (-0.54, 0.29)	0.99
51–100	12 [8,10,12,14,15,17-23]	484	-0.10 (-0.21,0.01)	0.74
101 – 150	4 [9,11,15,23]	176	-0.10 (-0.24, 0.05)	0.09
Design :				
parallel crossover	9 [8-14,21]	447	-0.08 (-0.27, 0.10)	0.48
a.) no washout	1 [22]	56	-0.43 (-0.73, -0.13)*	NA
b.) washout mentioned	6 [15,19,20,23]	208	-0.07 (-0.17, 0.03)	0.91
Gender:				
male	2 [7,22]	76	-0.30 (-0.54, -0.05)*	0.15
female	17 [8-11,13-18,20,21,23]	674	-0.07 (-0.17, 0.02)	0.89
Treatment length:				
2–10 weeks	14[7,10,12,13,15-17,19,20,22,23]	541	-0.09 (-0.18, 0.00)	0.84
11–20 weeks	3 [11,14,18]	155	-0.22 (-0.52, 0.07)	0.07
21–30 weeks	4 [8,9,21]	157	0.02 (-0.28, 0.31)	0.96
Menopausal status:				
pre-menopausal	3 [15,18]	80	-0.06 (-0.17, 0.06)	0.58
peri-menopausal	1 [11]	78	-0.69 (-1.19, -0.19)*	NA
post-menopausal	13 [8-10,13,14,16,17,20,21,23]	516	-0.05 (-0.20, 0.10)	1.00
Subjects with:				
normolipidemia	8 [11,12,15,18,19,21,22]	377	-0.11 (-0.21, -0.01)*	0.08
hyperlipidemia	3 [7,9,14]	105	-0.05 (-0.36, 0.26)	1.00

* statistically significant † 'ISP (+)' = isoflavone containing isolated soy protein; 'ISP (-)' = isoflavone depleting isolated soy protein ‡ 'G' = Genistein; 'D' = Daidzein; 'B' = Biochanin A; 'F' = Formononectin § NA = not applicable || Since subjects acted as their own controls in a crossover trial, the 'calculated' total number was thus doubled in a single pair comparison. ¶ 95% confidence interval in parenthesis

heart disease (CHD) or cardiovascular accident (CVA), may be selected as other endpoints in future trials. It may help constructing convincing funnel plots with less heterogeneity as trials accumulate.

Conclusions

Isoflavones tablets, up to 150 mg per day, had insignificant effects in lowering serum total cholesterol, LDL-cholesterol and triglyceride. There was also insignificant benefit over serum HDL-cholesterol. The results were consistent when tablets were introduced as isolated genistein, mixture of genistein and daidzein, or mixture of genistein, daidzein, formononectin and biochanin A. No significant effects were found among participants with normo- or hyperlipidemia and women with pre- or postmenopausal status. Isoflavones interventions in the forms of soy proteins, such as isolated soy protein (ISP), soy diets or soy protein capsule, were inconclusive due to inadequate sample size, heterogeneity and presence of potentially uncontrolled confounders.

List of abbreviations

'ISP' = isolated soy protein; 'TC' = total cholesterol; 'LDL' = Low density lipoprotein cholesterol; 'HDL' = High density lipoprotein cholesterol; 'TG' = Triglycerides

Competing interests

None declared.

Table 3: Effects of isoflavones on serum LDL-cholesterol, HDL-cholesterol and triglycerides levels. Isoflavones in the forms of tablets or isolated soy protein (ISP) did not show significant benefits over serum LDL-cholesterol, HDL-cholesterol and triglycerides levels. The results were not heterogeneous to combine.

Type of intervention*	No. of trials	No. of comparisons	No. of subjects	Treatment effect (mmol/L) †	Heterogeneity p-value
1.) Isoflavones tablets					
LDL	9	11[8,10,12,13,16-18,20,21]	446	0.00 (-0.14, 0.15)	0.94
HDL	10	12[8-10,12,13,16-18,20,21]	482	0.01 (-0.05, 0.06)	0.98
TG	9	12[8-10,12,13,16,17,20,21]	482	0.03 (-0.06, 0.12)	0.93
2.) ISP (+)					
LDL	3	5 [14,15,23]	173	-0.06 (-0.16, 0.03)	0.84
HDL	3	5 [14,15,23]	173	-0.01 (-0.07, 0.05)	0.97
TG	3	5 [14,15,23]	173	0.02 (-0.05, 0.09)	1.00

* 'ISP (+)' = isoflavones containing isolated soy protein; 'LDL' = Low density lipoprotein cholesterol; 'HDL' = High density lipoprotein cholesterol; 'TG' = Triglycerides † 95% confidence interval in parenthesis

Authors' contributions

JY participated in the design of this manuscript. JY and YTF participated in abstracted the data and performed statistical analysis. All authors read and approved the final manuscript.

References

1. Anderson JW, Johnstone BM, Cook-Newell ME: **Meta-analysis of the effects of soy protein intake on serum lipids.** *N Eng J Med* 1995, **333:**276-282.
2. Hasler CM: **The cardiovascular effects of soy products.** *J Cardiovasc Nurs* 2002, **16(4):**50-63.
3. Clarkson TB: **Fourth international symposium on the role of soy in preventing and treating chronic disease: Soy, soy phytoestrogens and cardiovascular disease.** *J Nutr* 2002, **suppl 132:**566-569S.
4. Dickersin K, Scherer R, Lefebvre C: **Systematic Reviews: Identifying relevant studies for systematic reviews.** *BMJ* 1994, **309:**1286-1291.
5. Phipps WR, Duncan AM, Kurzer MS: **A Critical Review: Isoflavones and Postmenopausal Women.** *Treat Endocrinol* 2002, **1(5):**293-311.
6. Deeks JJ, Altman DG, Bradburn MJ: **Statistical methods for examining heterogeneity and combining results from several studies in meta-analysis.** In *Systematic Reviews in Health Care: Meta-analysis in Context* 2nd edition. Edited by: *Egger M, Smith GD, Altman DG. London: BMJ Publishing Group*; 2001:285-312.
7. Calvert GD, Blight L, Illman RJ, Topping DL, Potter JD: **A trial of the effects of soya-bean flour and soya-bean saponins on plasma lipids, faecal bile acids and neutral sterols in hypercholesterolaemic men.** *Br J Nutr* 1981, **45:**277-281.
8. Clifton-Bligh PB, Baber RJ, Fulcher GR, Nery ML, Moreton T: **The effect of isoflavones extracted from red clover (Rimostil) on lipid and bone metabolism.** *Menopause* 2001, **8(4):**259-265.
9. Dewell A, Hollenbeck CB, Bruce B: **The effects of soy-derived phytoestrogens on serum lipids and lipoproteins in moderately hypercholesterolemic postmenopausal women.** *J Clin Endocrinol Metab* 2002, **87:**118-121.
10. Hale G, Paul-Labrador M, Dwyer JH, Merz CNB: **Isoflavone supplementation and endothelial function in menopausal women.** *Clin Endocrinol* 2002, **56:**693-701.
11. Han KK, Soares JM, Haidar MA, de Lima GR, Baracat EC: **Benefits of soy isoflavone therapeutic regimen on menopausal symptoms.** *Obstet Gynecol* 2002, **99:**389-394.
12. Hodgson JM, Puddey IB, Beilin LJ, Mori TA, Croft KD: **Supplementation with isoflavonoid phytoestrogens does not alter serum lipid concentrations: a randomized controlled trial in humans.** *J Nutr* 1998, **128:**728-732.
13. Howes JB, Sullivan D, Lai N, Nestel P, Pomeroy S, West L, Eden JA, Howes LG: **The effects of dietary supplementation with isoflavones from red clover on the lipoprotein protein profiles of post menopausal women with mild to moderate hypercholesterolaemia.** *Atherosclerosis* 2000, **152:**143-147.
14. Mackey R, Ekangaki A, Eden JA: **The effects of soy protein in women and men with elevated plasma lipids.** *Biofactors* 2000, **12:**251-257.
15. Merz-Demlow BE, Duncan AM, Wangen KE, Xu X, Carr TP, Phipps WR, Kurzer MS: **Soy isoflavones improve plasma lipids in normocholesterolemic, premenopausal women.** *Am J Clin Nutr* 2000, **71:**1462-1469.
16. Nestel PJ, Yamashita T, Sasahara T, Pomeroy S, Dart A, Komesaroff P, Owen A, Abbey M: **Soy isoflavones improve systemic arterial compliance but not plasma lipids in menopausal and perimenopausal women.** *ArterioscIerThromb Vasc Biol* 1997, **17:**3392-3398.
17. Nestel PJ, Pomeroy S, Kay S, Komesaroff P, Behrsing J, Cameron JD, West L: **Isoflavones from red clover improve systemic arterial compliance but not plasma lipids in menopausal women.** *J Clin Endocrinol Metab* 1999, **84:**895-898.
18. Samman S, Wall PML, Chan GSM, Smith SJ, Petocz P: **The effect of supplementation with isoflavones on plasma lipids and oxidisability of low density lipoprotein in premenopausal women.** *Atherosclerosis* 1999, **147:**277-283.
19. Sanders TAB, Dean TS, Grainger D, Miller GJ, Wiseman H: **Moderate intakes of intact soy protein rich in isoflavones compared with ethanol-extracted soy protein increase HDL but do not influence transforming growth factor beta concentrations and hemostatic risk factors for coronary heart disease in healthy subjects.** *Am J Clin Nutr* 2002, **76:**373-377.
20. Simons LA, von Konigsmark M, Simons J, Celermajer DS: **Phytoestrogens do not influence lipoprotein levels or endothelial function in healthy, postmenopausal women.** *Am J Cardiol* 2000, **85:**1297-1301.
21. Squadrito F, Altavilla D, Morabito N, Crisafulli A, D'Anna R, Corrado F, Ruggeri P, Campo GM, Calapai G, Caputi AP, Squadrito G: **The effect of the phytoestrogen genistein on plasma nitric oxide concentrations, endothelin-1 levels and endothelium dependent vasodilation in postmenopausal women.** *Atherosclerosis* 2002, **163:**339-347.
22. Urban D, Irwin W, Kirk M, Markiewicz MA, Myers R, Smith M, Weiss H, Grizzle WE, Barnes S: **The effect of isolated soy protein on plasma biomarkers in elderly men with elevated serum prostate specific antigen.** *J Urol* 2001, **165:**294-300.
23. Wangen KE, Duncan AM, Xu X, Kurzer MS: **Soy isoflavones improve plasma lipids in normocholesterolemic and mildly hypercholesterolemic postmenopausal women.** *Am J Clin Nutr* 2001, **73:**225-231.

24. Cassidy A, Bingham S, Setchell KDR: **Biological effects of a diet of soy protein rich in isoflavones on the menstrual cycle of premenopausal women.** *Am J Clin Nutr* 1994, **60:**333-340.
25. Cassidy A, Bingham S, Setchell K: **Biological effects of isoflavones in young women: importance of the chemical composition of soyabean products.** *Br J Nutr* 1995, **74:**587-601.
26. Tikkanen MJ, Wahala K, Ojala S, Vihma V, Adlercreutz H: **Effect of soybean phytoestrogen intake on low density lipoprotein oxidation resistance.** *Proc Natl Acad Sci USA* 1998, **95:**3106-3110.
27. Wong WW: **Effects of soy isoflavones on blood lipids, blood pressure and biochemical markers of bone metabolism in postmenopausal women.** *J Nutr* 2000, **suppl 130:**686S.
28. Gooderham MJ, Adlercreutz H, Ojala ST, Wahala K, Holub BJ: **A soy protein isolate rich in genistein adn daidzein and its effects on plasma isoflavone concentrations, platelet aggregation, blood lipids and fatty acid composition of plasma phospholipid in normal men.** *J Nutr* 1996, **126:**2000-2006.
29. Potter SM, Baum JA, Teng H, Stillman RJ, Shay NF, Erdman JW: **Soy protein and isoflavones: their effects on blood lipids and bone density in postmenopausal women.** *Am J Clin Nutr* 1998, **suppl 68:**1375-1379S.
30. Adlercruetz H, Goldin BR, Gorbach SL, Hockerstedt KA, Watanabe S, Hamalainen EK, Markkanen MH, Makela TH, Wahala KT, Adlercreutz T: **Soybean phytoestrogen intake and cancer risk.** *J Nutr* 1995, **suppl 125:**757-770S.
31. **Review Manager (RevMan) [Computer program]. Version 4.2 for Windows.** *Oxford, England: The Cochrane Collaboration* 2003.
32. Erdman JW Jr: **AHA science advisory: soy protein and cardiovascular disease: a statement for healthcare professionals from the Nutrition Committee of the AHA.** *Circulation* 2000, **102:**2555-2559.
33. Clarkson TB: **Soy, soy phytoestrogens and cardiovascular disease.** *J Nutr* 2002, **suppl 132:**566-569S.
34. Clarkson TB, Anthony MS, Morgan TM: **Inhibition of postmenopausal atherosclerosis progression: a comparison of the effects of conjugated equine estrogens and soy phytoestrogens.** *J Clin Endocrinol Metab* 2001, **86:**41-47.
35. Clarkson TB, Kaplan JR, Wagner JD *et al.*: **Lessons from animal models.** In *Cardiovascular disease and disease in women* 2nd edition. Edited by: *Douglas PS. New York: WB Saunders Company*; 2002:231-256.
36. Sterne JAC, Egger M: **Funnel plots for detecting bias in meta-analysis: Guidelines on choice of axis.** *Journal of Clinical Epidemiology* 2001, **54:**1046-1055.
37. Kuiper GG, Lemmen JG, Carlsson B, Corton JC, Safe SH, van der Saag PT, van der Burg B, Gustafsson JA: **Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor b.** *Endocrinology* 1998, **139:**4252-4263.
38. Dornstauder E, Jisa E, Unterrieder I, Krenn L, Kubelka W, Jungbauer A: **Estrogenic activity of two standardized red clover extracts (Menoflavon) intended for large scale use in hormone replacement therapy.** *J SteroidBiochem Mol Biol* 2001, **78:**67-75.
39. Morito K, Hirose T, Kinjo J, Hirakawa T, Okawa M, Nohara T, Ogawa S, Inoue S, Muramatsu M, Masamune Y: **Interaction of phytoestrogens with estrogen receptors alpha and beta.** *Biol Pharm Bull* 2001, **24:**351-356.
40. Mendelsohn ME: **Nongenomic, ER-mediated activation of endothelial nitric oxide synthase: how does it work? What does it mean?** *Circ Res* 2000, **87:**956-960.
41. Gruber CJ, Tschugguel W, Schneeberger C, Huber JC: **Production and actions of estrogens.** *N Engl J Med* 2002, **346:**340-352.
42. Jayachandran M, Miller VM: **Molecular and cellular mechanisms of estrogen's actions.** In *Cardiovascular disease and disease in women* 2nd edition. Edited by: *Douglas PS. New York: WB Saunders Company*; 2002:207-230.
43. Busby MG, Jeffcoat AR, Bloedon LT, Koch MA, Black T, Dix KJ, Heizer WD, Thomas BF, Hill JM, Crowell JA, Zeisel SH: **Clinical characteristics and pharmacokinetics of purified soy isoflavones: single-dose administration to healthy men.** *Am J Clin Nutr* 2002, **75:**126-36.
44. Lu LJ, Anderson KE: **Sex and long-term soy diets affect the metabolism and excretion of soy isoflavones in humans.** *Am J Clin Nutr* 1998, **suppl 68:**1500-1504S.
45. Xu X, Wang HJ, Murphy PA, Hendrich S: **Neither background diet nor type of soy food affects short-term isoflavone bioavailability in women.** *J Nutr* 2000, **130:**798-801.
46. Sirtori CR, Gianazza E, Manzoni C, Lovati MR, Murphy PA: **Role of isoflavones in the cholesterol reduction by soy proteins in the clinic.** *Am J Clin Nutr* 1997, **65:**166-167.
47. Potter SM: **Soy protein and cardiovascular disease: the impact of bioactive components in soy.** *Nutr Rev* 1998, **56:**231-235.
48. Potter SM: **Overview of proposed mechanisms for the hypocholesterolemic effect of soy.** *J Nutr* 1995, **suppl 125:**606-611S.