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Isoflavones for hypercholesterolaemia in adults (Review)

Qin Y, Niu K, Zeng Y, Liu P, Yi L, Zhang T, Zhang QY, Zhu JD, Mi MT

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[Intervention Review]

Isoflavones for hypercholesterolaemia in adults

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ABSTRACT

Background

Hypercholesterolaemia is a significant risk factor for cardiovascular diseases. Isoflavones may be effective in improving hypercholesterolaemia.

Objectives

To assess the effects of isoflavones for hypercholesterolaemia.

Search methods

We searched the following databases: *The Cochrane Library* (Issue 9, 2012), MEDLINE, EMBASE, Chinese BioMedical Database and China National Knowledge Infrastructure (all to September 2012).

Selection criteria

We considered randomized controlled clinical trials in hypercholesterolaemic participants comparing isoflavones versus placebo, or soy isolated protein added with isoflavones versus soy isolated protein alone.

Data collection and analysis

Two review authors independently abstracted relevant population and intervention characteristics. We resolved any disagreements through discussion, or if required by a third party. We assessed the risk of bias of trials against key criteria: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting and other sources of bias.

Main results

We included five randomized trials (208 participants, 104 in the intervention group and 104 in the control group). Interventions ranged from three to six months. Four trials reported results in non-Asian populations published in English. One trial reported results in Chinese people published in Chinese. Overall, the risk of bias of included trials was high or unclear. There were no outcome data on death from any cause, morbidity, complications, health-related quality of life and costs. Two trials reported adverse effects, including gastrointestinal discomfort (bloating and constipation) and an increased number of hot flushes. None of the trials found serious adverse events. There was a slight significant effect on triglycerides in favour of isoflavones when compared with placebo (mean difference (MD) -0.46 mmol/L (95% confidence interval (CI) -0.84 to -0.09; P = 0.02; 52 participants; 2 trials). No statistically significant effects on total cholesterol, low-density lipoprotein cholesterol and high-density lipoprotein cholesterol were shown in favour of isoflavones.

Authors' conclusions

We found no evidence for effects of isoflavones on patient-important outcomes or lowering of cholesterol levels in people with hypercholesterolaemia. Our findings have to be interpreted with caution due to high or unclear risk of bias in several risk of bias domains, and low number of participants in trials.

PLAIN LANGUAGE SUMMARY

Isoflavones for hypercholesterolaemia

Hypercholesterolaemia is the presence of high levels of cholesterol in the blood. In humans, hypercholesterolaemia is often due to high low-density-lipoprotein (LDL)-cholesterol levels, the so-called 'bad' cholesterol. People with hypercholesterolaemia have a higher risk of developing cardiovascular diseases such as heart attacks or strokes. Isoflavones, which are chemicals in plants similar to phyto-oestrogen, may be helpful in improving hypercholesterolaemia. Soy and red clover are rich sources of isoflavones. Asian people consume more isoflavones from their regular diet than Western people.

To assess the effects of isoflavones for the treatment of hypercholesterolaemia, we examined five randomized controlled trials of isoflavones or soy protein containing isoflavones. The trials lasted three to six months and involved 208 participants. There were no outcome data on death from any cause, cardiovascular events such as heart attack or stroke, morbidity, complications, health-related quality of life and costs. Two trials reported adverse effects, including gastrointestinal discomfort (bloating and constipation) and an increased number of hot flushes. They observed no serious adverse events. In our included studies, we found no cholesterol-lowering effect of isoflavones. However, the quality of the included trials had some considerable limitations and the number of the participants was low. Further higher-quality and rigorously performed studies on patient-important outcome measures such as cardiovascular diseases and health-related quality of life are required.

SUMMARY OF FINDINGS

Summary of findings for the main comparison.

Isoflavones for hypercholesterolaemia in adults

Patient or population: postmenopausal women with hypercholesterolaemia

Settings: outpatients or not specified

Intervention: isoflavones or soy protein-containing isoflavones

Comparison: placebo or soy protein

Outcomes	Relative effect (95% CI)	No of participants (studies)	Quality of the evidence (GRADE)	Comments
Cardiovascular events	See comment	See comment	See comment	Not investigated
Death from any cause	See comment	See comment	See comment	Not investigated
Health-related quality of life	See comment	See comment	See comment	Not investigated
Adverse events [follow-up: 3 to 6 months]	See comment	208 (5)	⊕⊕⊖⊖ **low**[a]	No serious adverse events reported
Low-density lipoprotein (LDL) cholesterol [follow-up: 3 to 6 months]	See comment	132 (3)	⊕⊖⊖⊖ **very low**[b]	No statistically significant differences between groups
Costs	See comment	See comment	See comment	Not investigated

GRADE Working Group grades of evidence
High quality: Further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: We are very uncertain about the estimate.

[a]Due to serious risk of bias, low number of participants and studies, short duration of treatment.
[b]Due to serious risk of bias, serious indirectness, low number of participants and studies, short duration of treatment.

BACKGROUND

Description of the condition

Hypercholesterolaemia is the presence of abnormally high levels of cholesterol in the blood, and it has reached epidemic proportions worldwide. In 2008, Australasia, North America and Western Europe reported the highest serum total cholesterol (TC) concentrations worldwide (Farzadfar 2011). The regional mean of the serum TC levels was 5.24 mmol/L (95% confidence interval (CI) 5.08 to 5.39) for men and 5.23 mmol/L (95% CI 5.03 to 5.43) for women, which means that approximately 50% of the population in these countries had hypercholesterolaemia (Farzadfar 2011). In developing countries, the number of people with hypercholesterolaemia has risen since the early 1980s, particularly in Asia (Farzadfar 2011). For example, in 2001, the prevalence of hypercholesterolaemia was 33% in middle-aged and older-aged Chinese people (He 2004).

Hypercholesterolaemia is a significant risk factor for cardiovascular diseases (CVDs). In humans, hypercholesterolaemia is often due to high serum low-density-lipoprotein (LDL)-cholesterol levels, which are generally atherogenic. Atherosclerosis is the pathological basis for most CVDs and is often characterized as the progressive accumulation of lipids in the vessel wall. The subsequent rate of atherogenesis is positively associated with the severity of associated risk factors including serum cholesterol levels (Badimon 2011). High levels of serum cholesterol particles may increase vascular superoxide production in the blood, alter endothelium-dependent vasodilation (widening of the cavity in a blood vessel), and then promote the formation of atherosclerosis plaques, and finally facilitate CVDs. Thus, lowering the blood TC levels may be of benefit to people with CVDs. Several trials have demonstrated that treatment of high cholesterol levels played the most important role on more than half the decline in coronary heart disease mortality in the last decades (Ford 2007; Laatikainen 2005).

Description of the intervention

Isoflavones are plant-based chemicals related to phyto-oestrogen, which are found in soy and red clover. Asian people consume more isoflavones from their regular diet than Western people. Daily isoflavones intake in Chinese and Japanese people was estimated to be 15 to 50 mg/day (Kang 2010; Liu 2010; Shimazu 2010), whereas it was likely to be less than 3 mg/day in European and US populations (Chun 2007; Keinan 2002).

Hypercholesterolaemia can be modified by dietary changes including lowering cholesterol by diet or supplementing the diet with certain nutrients such as isoflavones. Isoflavones have many benefits for human health, such as improving osteoporosis and menopausal syndromes. In the 2000s, several systematic reviews and meta-analyses assessed the effects of isoflavones on lipid profiles in humans (Taku 2007; Taku 2008; Weggemans 2003; Zhan 2005; Zhuo 2004). However, the conclusions of these reviews were inconsistent, which may be due to the different isoflavones used, dose levels, styles and durations of the trials, and the various initial serum lipid concentrations of the participants. Natural food (including soy, tofu and red clover), soy protein containing isoflavones, isoflavones extracts and single compounds of isoflavones such as genistein, daidzein or glycetein were often supplemented with isoflavones. Other components such as soy protein may have contributed to and distorted the effects of isoflavones on the serum concentrations of total and LDL-cholesterol (Taku 2007; Taku 2008).

Adverse effects of the intervention

Isoflavones have a safe side-effect profile with moderately elevated rates of abdominal bloating (Albertazzi 2005; Garrido 2006), gastralgia (stomach pain) (Nikander 2004) and back pain (Albertazzi 2005). The rare, but serious, adverse effects include endometrial hyperplasia (Unfer 2004) and recurrence of breast cancer (Nikander 2004).

How the intervention might work

The activities of the phyto-oestrogen component of isoflavones may play an important role in the effects of isoflavones on serum lipid profiles. Clinical intervention studies have demonstrated that blood isoflavones metabolites (such as equol) levels were associated with the clinical effects of isoflavones, including several positive outcomes for vasomotor symptoms, increasing bone mineral density, and decreasing the cardiovascular risk factors LDL-cholesterol and C-reactive protein (Jackson 2011). The gut bacterial biotransformation of daidzein in certain individuals produces equol. There is a higher frequency of 'equol-producers' in Asian populations (50% to 60%) than in Western populations (25% to 30%) (Setchell 2010). Several clinical studies have concluded that isoflavones may produce better clinical effects in equol-producers than in non-equol-producers (Duncan 2000; Kreijkamp 2005; Setchell 2002).

The effects of isoflavones on serum lipid profiles may also contribute to their activities, independent of phyto-oestrogen. Cellular and animal studies demonstrated that genistein can act as an inhibitor of protein tyrosine kinase. In pancreatic beta cells, genistein acutely stimulates insulin secretion through a cyclic adenosine monophosphate (cAMP)-dependent protein kinase pathway (Liu 2006).

Why it is important to do this review

There were some limitations of previously published meta-analyses on this topic. First, only one meta-analysis searched databases such as MEDLINE, EMBASE, *The Cochrane Library*, Chinese BioMedical Database and China National Knowledge Infrastructure (CNKI) (Taku 2008). Other meta-analyses only reviewed studies published in PubMed and only those published in English (Taku 2007; Weggemans 2003; Zhan 2005; Zhuo 2004). Second, all of these meta-analyses focused on the short-term effects of isoflavones on lipid profiles (Taku 2007; Taku 2008; Weggemans 2003; Zhan 2005; Zhuo 2004). Some clinical trials that focused on the long-term effects of isoflavones on postmenopausal osteoporosis also evaluated blood lipid changes. However, the meta-analyses seldom analyzed these clinical trials. Third, only one meta-analysis mentioned adverse effects of isoflavones interventions (Taku 2008). We undertook this review to resolve these limitations and to provide a better understanding of the effects of isoflavones for hypercholesterolaemia. In the present meta-analysis, we screened the studies through PubMed, EMBASE, *The Cochrane Library*, CNKI and CBM, and primarily attempted to evaluate the long-term effects of isoflavones for hypercholesterolaemia.

OBJECTIVES

To assess the effects of isoflavones for hypercholesterolaemia.

METHODS

Criteria for considering studies for this review

Types of studies

Randomized controlled clinical trials.

Types of participants

Adults (aged 18 years or older) with hypercholesterolaemia.

Diagnostic criteria

A fasting blood TC greater than 5.20 mmol/L (200 mg/dL) is indicative of hypercholesterolaemia. We also accepted other definitions. We excluded people with familial hypercholesterolaemia and secondary hypercholesterolaemia.

We planned to subject diagnostic criteria to a sensitivity analysis.

Types of interventions

We investigated the following comparisons of intervention verus control/comparator where the same letters indicate direct comparisons.

Interventions

(a) Natural food, isoflavones extracts, single compound of isoflavones with total isoflavones amount of 15 mg/day or greater.

(b) Soy protein containing isoflavones with total isoflavone amount of 15 mg/day or greater.

Comparators

(a1) Placebo or other food with total isoflavones amount less than 5 mg/day.

(b1) Soy protein containing no or low isoflavones with total isoflavones amount less than 5 mg/day.

Types of outcome measures

Primary outcomes

- Death from any cause.
- Cardiovascular events (both fatal and non-fatal events, including myocardial infarction, angina pectoris, stroke, peripheral arterial disease, sudden death).
- LDL-cholesterol levels.

Secondary outcomes

- Adverse events.
- Health-related quality of life.
- TC levels.
- Other lipid levels (including high-density-lipoprotein (HDL) cholesterol, triglycerides, apolipoprotein A and B).
- Costs.

Timing of outcome measurement

The minimum treatment duration was three months. If one study had several results over time, we used the longest time point for overall effect analysis and used other time points for subgroup analysis (i.e. six months or less and longer than six months). For cardiovascular events, intervention duration had to be at least six months.

Search methods for identification of studies

Electronic searches

We searched the following sources from inception to the specified date.

- *The Cochrane Library* (Issue 9, 2012).
- MEDLINE (to September 2012).
- EMBASE (to September 2012).
- Chinese BioMedical Database (to September 2012).
- CNKI (to September 2012).

We also searched databases of ongoing trials (ClinicalTrials.gov (www.clinicaltrials.gov/), Current Controlled Trials metaRegister (www.controlled-trials.com/), the EU Clinical Trials register (www.clinicaltrialsregister.eu/).

For detailed search strategies see Appendix 1 (searches were not older than six months at the moment the final review draft was checked into the Cochrane Information and Management System for editorial approval).

If we had detected additional key words of relevance during any of the electronic or other searches, we would have modified the electronic search strategies to incorporate these terms. We included studies published in any language.

Searching other resources

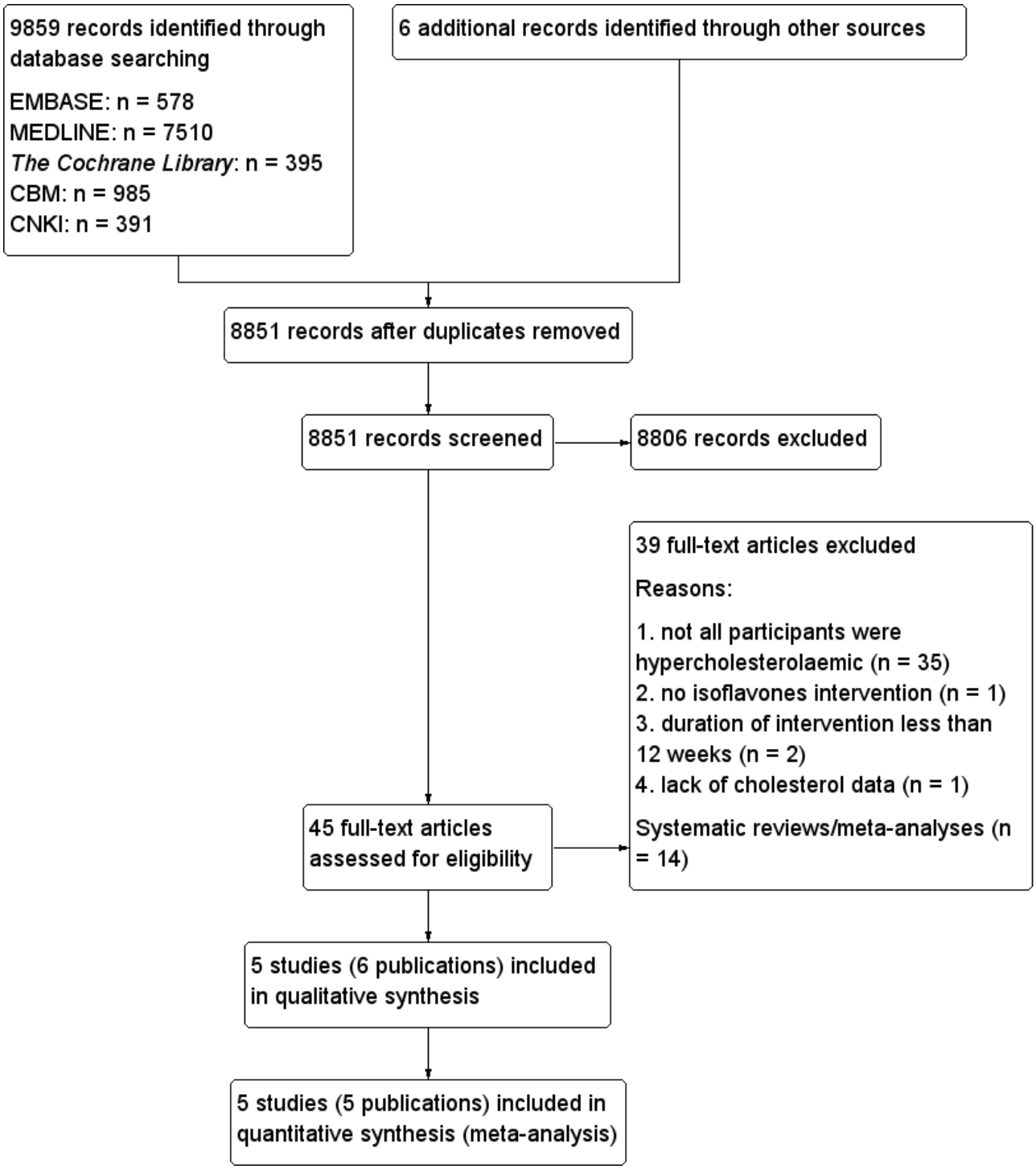
We tried to identify additional studies by searching the reference lists of included trials, systematic reviews, meta-analyses and health technology assessment reports found as a result of the searches.

Data collection and analysis

Selection of studies

Two review authors (YQ, KN) independently scanned the abstract, title or both sections of every record retrieved to determine the studies to be assessed further. We investigated all potentially relevant articles as full text. A third party resolved any differences in opinion. If resolving disagreement was not possible, we added the article to those 'awaiting assessment' and we contacted authors for clarification. We attach a PRISMA (preferred reporting items for systematic reviews and meta-analyses) flow-chart of study selection (Figure 1) (Liberati 2009).

Figure 1. Study flow diagram.



Data extraction and management

For studies that fulfilled inclusion criteria, two review authors (YQ, KN) independently abstracted relevant population and intervention characteristics using standard data extraction templates (for details see Characteristics of included studies; Table 1; Appendix 2; Appendix 3; Appendix 4; Appendix 5; Appendix 6; Appendix 7; Appendix 8; Appendix 9) with any disagreements resolved by discussion, or, if required, by a third party.

We sent an email request to authors of published studies to enquire whether they would answer questions regarding their trials. Appendix 10 shows the results of this survey. Thereafter, we sought relevant missing information on the trial from the study authors of the article, if required.

Dealing with duplicate publications

In the case of duplicate publications and companion papers of a primary study, we tried to maximize yield of information by simultaneous evaluation of all available data.

Assessment of risk of bias in included studies

Two review authors (YQ, KN) assessed each trial independently. Possible disagreements were resolved by consensus, or by consultation with a third party. In cases of disagreement, we consulted the rest of the group and made a judgement based on consensus.

We assessed risk of bias using The Cochrane Collaboration's tool (Higgins 2011). We used the following bias criteria.

- Random sequence generation (selection bias).
- Allocation concealment (selection bias).
- Blinding (performance bias and detection bias), separated for blinding of participants and personnel and blinding of outcome assessment.
- Incomplete outcome data (attrition bias).
- Selective reporting (reporting bias).
- Other bias.

We judged risk of bias criteria as 'low risk', 'high risk' or 'unclear risk' and use individual bias items as described in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011). We present a 'Risk of bias' figure and a 'Risk of bias' summary figure.

We assessed the impact of individual bias domains on study results at endpoint and study levels.

Measures of treatment effect

We expressed dichotomous data as odds ratios (OR) or risk ratios (RR) with 95% CIs. We expressed continuous data as mean differences (MD) with 95% CI.

Unit of analysis issues

We took into account the level at which randomization occurred, such as cross-over trials, cluster-randomized trials and multiple observations for the same outcome.

Dealing with missing data

We obtained relevant missing data from authors, if feasible and carefully performed evaluation of important numerical data such as screened, randomized participants as well as intention-to-treat (ITT), as-treated and per-protocol (PP) populations. We investigated attrition rates, for example, drop-outs, losses to follow up and withdrawals and critically appraised issues of missing data and imputation methods (e.g. last-observation-carried-forward (LOCF)).

Assessment of heterogeneity

In the event of substantial clinical or methodological or statistical heterogeneity, we did not report study results as meta-analytically pooled effect estimates.

We identified heterogeneity by visual inspection of the forest plots and by using a standard Chi^2 test with a significance level of $\alpha = 0.1$, in view of the low power of this test. We specifically examined heterogeneity employing the I^2 statistic, which quantifies inconsistency across studies to assess the impact of heterogeneity on the meta-analysis (Higgins 2002; Higgins 2003), where an I^2 statistic of 75% or more indicates a considerable level of inconsistency (Higgins 2011).

Had we found heterogeneity, we planned to determine potential reasons for it by examining individual study and subgroup characteristics.

We expected the following characteristics to introduce clinical heterogeneity.

- Asian and non-Asian populations (due to their different genotype, diet style, and equol metabolism phenotype).
- Degree of hypercholesterolaemia.
- Previous cardiovascular events.
- Other cardiovascular risk factors.

Assessment of reporting biases

We planned to use funnel plots in case we included more than 10 studies for a given outcome to assess small study bias. There are a number of explanations for the asymmetry of a funnel plot (Sterne 2001) and we planned to interpret results carefully (Lau 2006).

Data synthesis

We primarily summarized low risk of bias data by means of a random-effects model. We performed statistical analyses according to the statistical guidelines referenced in the most recent version of the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011).

Subgroup analysis and investigation of heterogeneity

We planned to carry out the following subgroup analyses of the primary outcome parameter(s) (see above) and investigate interaction.

- Asian and non-Asian population.
- Males and females.
- Hypercholesterolaemia with or without hypertriglyceridaemia.
- Equol and non-equol producers.

Sensitivity analysis

We planned to perform sensitivity analyses in order to explore the influence of the following factors on effect size.

- Restricting the analysis to published studies.
- Restricting the analysis taking into account risk of bias, as specified above.
- Restricting the analysis to very long or large studies to establish how much they dominate the results.
- Restricting the analysis to studies using the following filters: diagnostic criteria, language of publication, source of funding (industry versus other), country.

We also tested the robustness of the results by repeating the analysis using different measures of effect size (RR, OR, etc.) and different statistical models (fixed-effect and random-effects models).

RESULTS

Description of studies

Results of the search

The initial search using the electronic search strategies listed in Appendix 1 yielded 9859 records. We did not identify any unpublished studies. After we removed duplicates from different databases, we kept potentially relevant articles for further assessment. We identified 45 articles that could not be excluded by screening of the title, the abstract or both. Further investigation of the full-text articles revealed five included studies (six publications). We found no ongoing studies. We prepared a PRISMA flow diagram to describe the articles found from our searches (Figure 1). The review authors found no differences in their independent assessment of the eligibility of the studies.

Missing data

We sent emails to study authors to obtain relevant missing data, only one author replied and provided data. On the basis of this information, the study could be included (Aubertin-Leheudre 2008).

Dealing with duplicate publications/companion papers

One included study (Aubertin-Leheudre 2008) had one companion paper (Aubertin-Leheudre 2007a), and the publication presenting cholesterol data was used. Other included studies had no duplicate publications or companion papers.

Included studies

We included five randomized controlled trials (five publications) in this review (see Characteristics of included studies for further details). Four of the included trials (five articles) were published in English. One trial (one publication) was published in Chinese (Wang 2005). Of the five trials, two were conducted in the US (Dewell 2002; Gardner 2001), and the other three trials were conducted in Canada (Aubertin-Leheudre 2008), Australia (Mackey 2000) and China (Wang 2005). All trials employed a parallel design. Three studies compared isoflavones versus placebo (Aubertin-Leheudre 2008; Dewell 2002; Wang 2005), and the other two studies compared soy isolated protein plus isoflavones versus soy isolated protein alone or plus trace amounts of isoflavones.

Participants

The five trials included 208 participants with hypercholesterolaemia, 100 in US populations (Dewell 2002; Gardner 2001) and 108 in other countries. Sample size of the trials ranged from 19 to 64 participants per trial. The age of the participants ranged from 40 to 83 years. All participants were postmenopausal women. Two trials included outpatients (Gardner 2001; Wang 2005); others did not specify trial settings. Table 1, Appendix 2 and Appendix 3 provide detailed information.

Interventions

There were small variations in the formulations, dosages, duration of treatments and control interventions in the included trials among the isoflavones tested (see Appendix 2 and Characteristics of included studies). Three trials used isoflavones capsules (Aubertin-Leheudre 2008; Wang 2005) or tablets (Dewell 2002), and compared these with placebo. The other two trials used soy isolated protein plus isoflavones powers, and compared them to soy isolated protein plus less than 4 mg isoflavones/day. Treatment duration of isoflavones therapy was six months in two trials (Aubertin-Leheudre 2008; Dewell 2002) and three months in one trial (Wang 2005). The duration of soy protein containing isoflavones treatment was 12 weeks in two trials (Gardner 2001; Mackey 2000).

Outcomes

One trial reported serum TC, HDL-cholesterol, triglycerides and non-HDL-cholesterol levels, but did not report serum LDL-cholesterol levels (Dewell 2002). The other four trials reported plasma (Aubertin-Leheudre 2008), serum (Gardner 2001; Mackey 2000) or blood (Wang 2005) lipids and lipoprotein levels, including TC, triglycerides, LDL- and HDL-cholesterol. One trial assessed LDL-cholesterol by calculation according to the method of Friedewald (Gardner 2001), and another trial may have used the same method (Mackey 2000). No trial reported outcomes on cardiovascular events, such as myocardial infarction, angina pectoris or stroke, peripheral arterial disease or sudden death. One trial reported details of adverse events in the soy isolated protein added with isoflavones group (see Appendix 8), including gastrointestinal discomfort (bloating and constipation) and an increased number of hot flushes (Gardner 2001). One trial found no adverse effects (Aubertin-Leheudre 2008). Three trials did not report adverse events in the intervention groups (Dewell 2002; Mackey 2000; Wang 2005). The other outcomes that were reported included body weight, body mass index (BMI), androstenedione, estrone, oestradiol, follicle-stimulating hormone, luteinizing hormone, thyroid-stimulating hormone, sex hormone binding globulin (SHBG), osteocalcin and bone-specific alkaline phosphatase. All trials measured outcomes at the end of treatment. No trial reported health-related quality of life or socioeconomic costs.

Study details

Two trials had a run-in period (Gardner 2001; Mackey 2000). No studies stopped before the scheduled end.

Publication details

Four studies were published in English and one study in Chinese (Wang 2005). None of the studies reported on commercial funding. Three studies reported non-commercial funding (Aubertin-Leheudre 2008; Dewell 2002; Wang 2005). All studies were published in peer-reviewed journals (see Characteristics of included studies).

Excluded studies

We excluded 39 (reasons for exclusion are listed under Characteristics of excluded studies). The major exclusion reason was that not all participants were hypercholesterolaemic. Other exclusion reasons included less than three months' intervention time, improper control or improper intervention.

Risk of bias in included studies

Two trials provided limited information about design and methodology (Mackey 2000; Wang 2005). All trials were conducted in one study centre. Two trials reported a sample size calculation (Aubertin-Leheudre 2008; Gardner 2001). All the trials had prespecified inclusion criteria and four trials had prespecified exclusion criteria. One trial prespecified diagnostic criteria (Mackey

2000). No trial stated that they performed an ITT analysis to evaluate the data. Two studies may have performed ITT analyses (Dewell 2002; Wang 2005). Three trials did not report missing data. The Characteristics of included studies table shows the assessment of risk of bias. Review authors' judgements about each risk of bias item is presented as percentages across all included studies in Figure 2, and review authors' judgements about each risk of bias item for each included study is shown in Figure 3. The review authors had the same judgements on their risk of bias assessments of the included studies.

Figure 2. Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included studies.

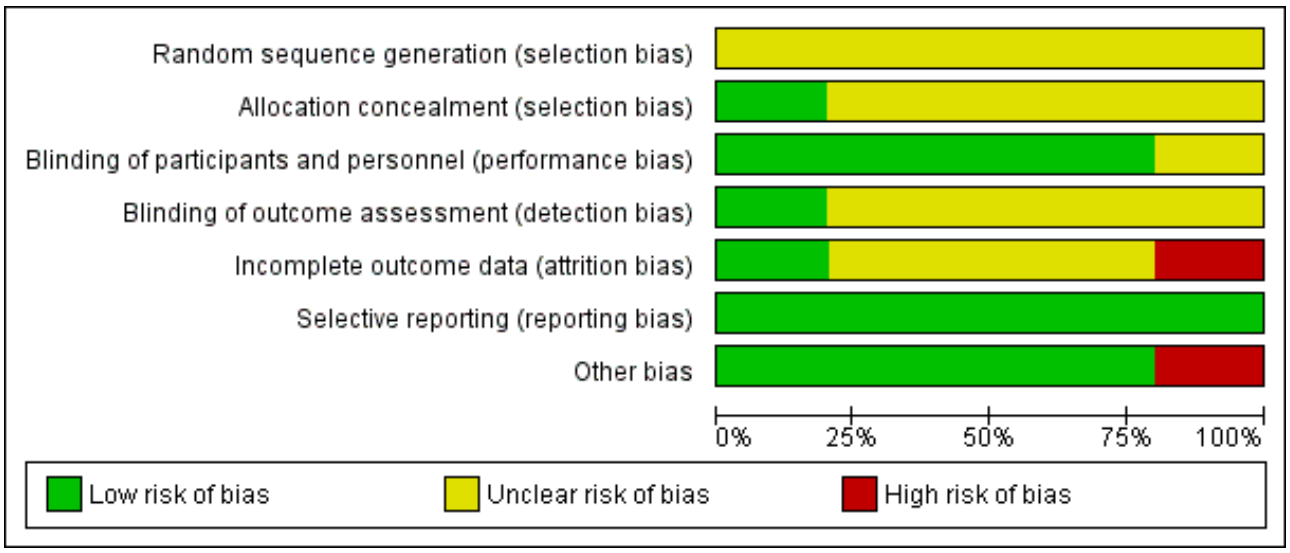
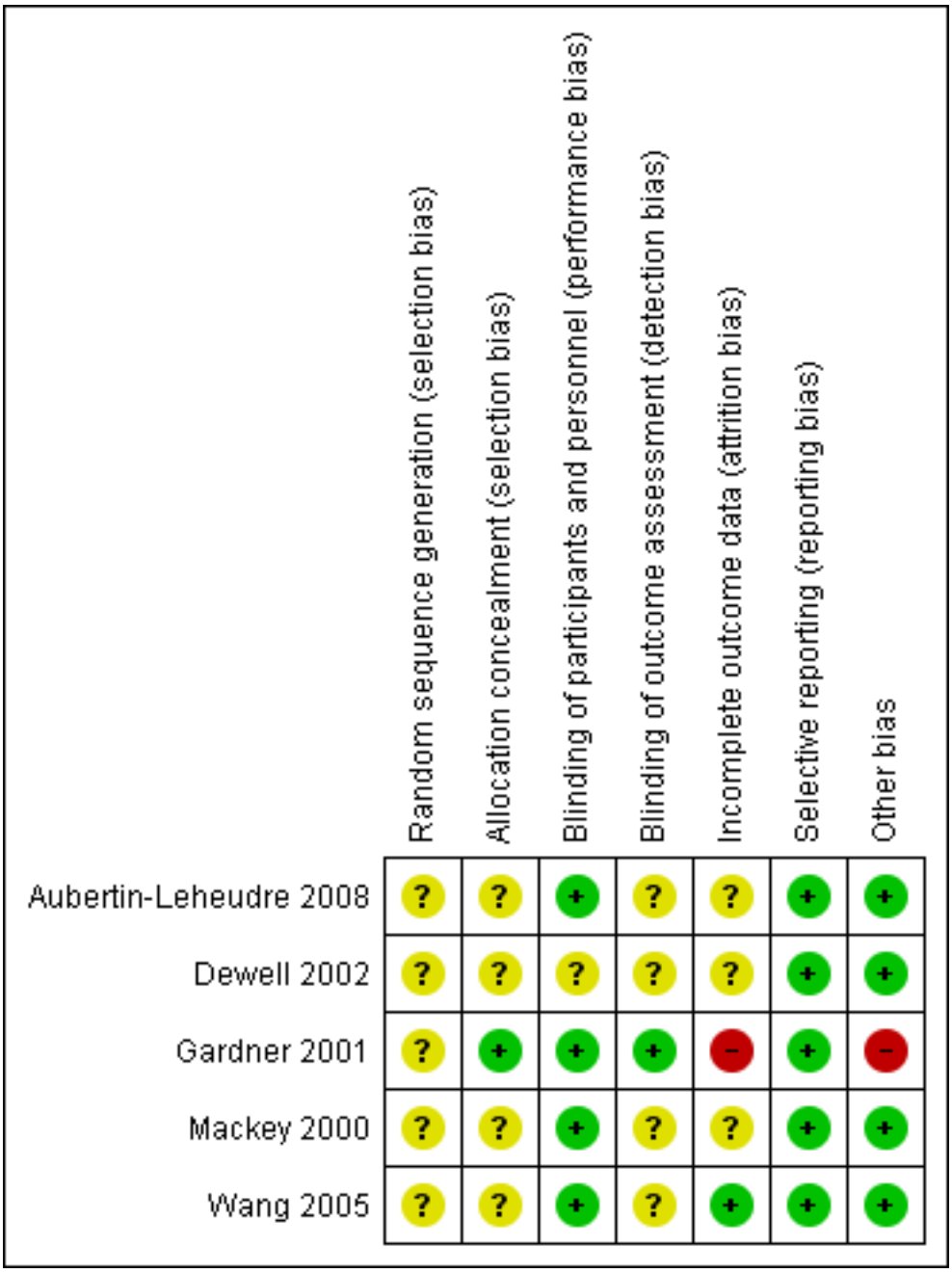


Figure 3. Risk of bias summary: review authors' judgements about each risk of bias item for each included study.



Allocation

One study reported an adequate allocation concealment (Gardner 2001).

Blinding

Four studies reported on blinding of participants and personnel (Aubertin-Leheudre 2008; Gardner 2001; Mackey 2000; Wang 2005) and one study on outcome assessment (Gardner 2001).

Incomplete outcome data

Only one study addressed incomplete outcome data adequately (Wang 2005).

Selective reporting

None of the trials reviewed had published protocols of their study. No reporting bias was detected according to the stated outcomes in the methods section and the reported outcomes in the results section of the publication.

Other potential sources of bias

We considered four trials to be free of other potential sources of bias with one trial showing high risk of bias (Gardner 2001).

Effects of interventions

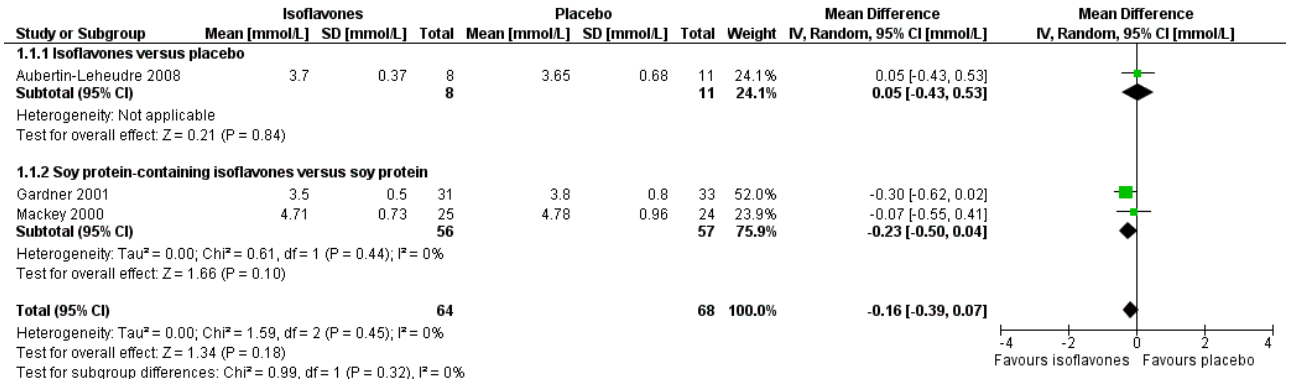
See: **Summary of findings for the main comparison**

We were only able to perform meta-analyses on four outcomes in this review. One trial performed in an Asian population demonstrated a major difference to the other studies with participants being postmenopausal women with abnormal endocrine function and showing very low baseline fasting serum HDL-cholesterol concentrations (0.28 to 0.42 mmol/L). Thus, we excluded this trial from meta-analysis.

Primary outcomes

There were no data on the primary outcomes death from any cause and cardiovascular events. One trial compared isoflavones with placebo in 19 participants including eight in the intervention and 11 in the control group (Aubertin-Leheudre 2008). Two trials compared soy protein containing isoflavones versus soy protein alone in 113 participants including 56 in the intervention and 57 in the control groups (Gardner 2001; Mackey 2000). For LDL-cholesterol, there were no statistically significant differences between isoflavones and placebo (MD -0.23 mmol/L, 95% CI -0.5 to 0.04; P value = 0.10; 113 participants; 2 trials; Analysis 1.1 subgroup 1.1.2; Figure 4).

Figure 4. Forest plot of comparison: 1 Isoflavones versus placebo, outcome: 1.1 LDL-cholesterol [mmol/L].

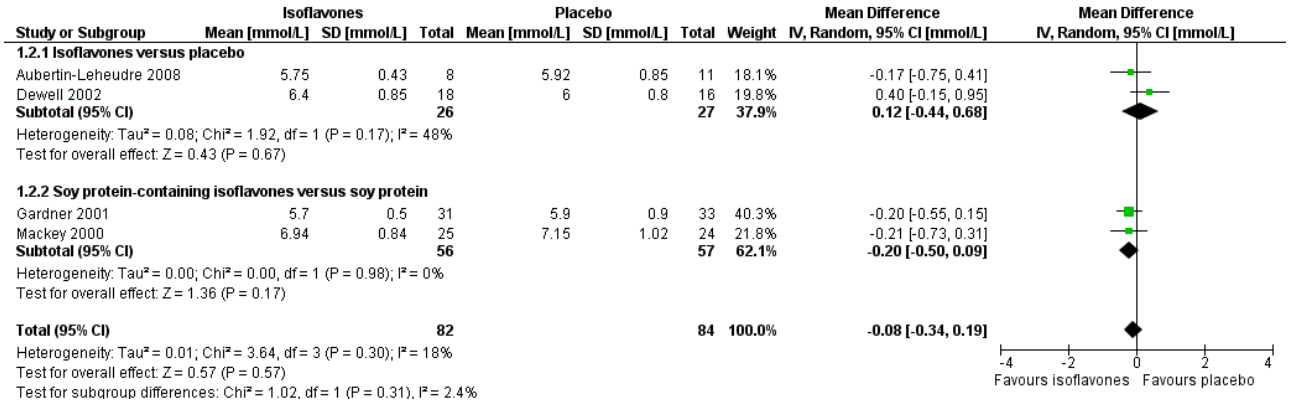


Secondary outcomes

There were no data on the secondary outcomes health-related quality of life and costs in any of the trials. The trials reported no serious adverse events (Appendix 8; Appendix 9).

A meta-analysis of four trials (Aubertin-Leheudre 2008; Dewell 2002; Gardner 2001; Mackey 2000) showed no statistically significant effects of isoflavones on TC (Analysis 1.2; Figure 5).

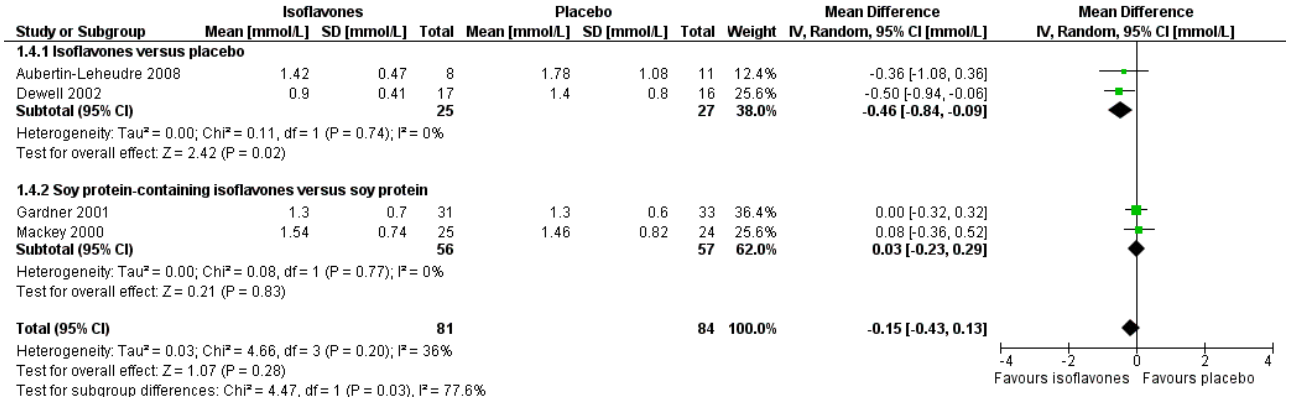
Figure 5. Forest plot of comparison: 1 Isoflavones versus placebo, outcome: 1.2 Total cholesterol [mmol/L].



A meta-analysis of three trials (Aubertin-Leheudre 2008; Gardner 2001; Mackey 2000) showed no statistically significant effects of isoflavones on HDL-cholesterol (Analysis 1.3).

Two trials compared isoflavones with placebo in 52 participants including 25 in the intervention and 27 in the control groups (Aubertin-Leheudre 2008; Dewell 2002) and showed a statistically significant reduction of triglycerides (MD -0.46 mmol/L, 95% CI -0.84 to -0.09; P value = 0.02; Analysis 1.4) in favour of isoflavones (Figure 6).

Figure 6. Forest plot of comparison: 1 Isoflavones versus placebo, outcome: 1.4 Triglycerides [mmol/L].



Reporting biases

We did not draw funnel plots due to the low number of included studies.

Subgroup analyses

All participants of the five included trials were postmenopausal women. We could not identify equol or non-equol producers, or participants with hypercholesterolaemia with or without hypertriglyceridaemia. We did not perform subgroup analyses due to lack of data.

Sensitivity analyses

We were not able to do sensitivity analyses on language of publication, source of funding and country due to the limited number of trials.

DISCUSSION

Summary of main results

Five randomized controlled clinical trials with 208 participants were included in this review, but we excluded one study (Wang 2005) from meta-analysis due to substantial differences in clinical characteristics of participants. Duration of treatment ranged from three to six months. There were no outcome data on death from any cause, morbidity, complications, health-related quality of life and costs.

Compared with placebo, there was a statistically significant effect of isoflavones alone on triglycerides and no statistically significant effect on TC, LDL-cholesterol or HDL-cholesterol in hypercholesterolaemic women. There was no statistically significant effect of soy protein containing isoflavones on hypercholesterolaemia. Compared with placebo, isoflavones also showed no statistically significant effects on body weight, BMI, waist circumference, total fat mass (FM), abdominal FM, visceral FM, resting energy expenditure, daily energy expenditure, diastolic and systolic blood pressure, HDL/TC, fasting plasma glucose, insulin and HOMA2-IR (no data were provided in these studies). Compared with soy protein, soy protein containing isoflavones showed no statistically significant effects on body weight, BMI, androstenedione, estrone, oestradiol, follicle-stimulating hormone, luteinizing hormone, SHBG, thyroid-stimulating hormone, osteocalcin, bone-specific alkaline phosphatase (with the exception of SHBG, no data of other variables were provided in these studies).

Overall completeness and applicability of evidence

The participants in the included trials were postmenopausal women. These populations may have been chosen due to the theory that isoflavones have phyto-oestrogen effects. However, these participants may not be representative of all people with hypercholesterolaemia. Most of the participants were recruited from non-Asian populations. The major source of isoflavones is soy, which is consumed largely by Asian populations. In addition, the included trials reported no long-term data on cardiovascular events and other patient-important outcomes. Therefore, the evaluation of the effects of isoflavones on hypercholesterolaemia especially in Asian populations and on patient-relevant end points should be addressed in future studies.

Quality of the evidence

All of the randomized trials included in this review were of rather poor quality in terms of their design, reporting and methodology. They provided only limited descriptions of study design, randomization, allocation concealment and baseline data. We identified no multicentre, large-scale randomized controlled clinical trials. Moreover, the included trials were heterogeneous in the populations (adults or elderly people, or hypercholesterolaemia with or without hypertriglyceridaemia), interventions and the reported outcomes.

Diagnostic criteria

Among the five included trials, only one trial diagnosed participants with hypercholesterolaemia (Mackey 2000). The other four trials did not describe exact diagnostic criteria.

Interventions

Three trials used isoflavones interventions and placebo controls (Aubertin-Leheudre 2008; Dewell 2002; Wang 2005). Two trials used soy protein-containing isoflavones and compared this with soy isolated protein plus less than 4 mg isoflavones/day (Gardner 2001; Mackey 2000). No trial lasted longer than six months

Surrogate outcomes

The primary target of hypercholesterolaemia treatment is to prevent cardiovascular events. No trial reported cardiovascular

events in people with hypercholesterolaemia. Other outcomes from the included trials were also surrogate outcomes, that is TC, LDL- and HDL-cholesterol, and triglycerides. We excluded 40 randomized trials from this review. The main reasons were participants not being hypercholesterolaemic, less than three-month interventions and improper control or intervention.

Adverse outcomes

There was inadequate reporting on adverse events in the included trials (Appendix 8; Appendix 9). Two trials reported results about adverse effects. Three trials did not report adverse events. In general, isoflavones were safe for postmenopausal women although there were gastrointestinal side effects.

Potential biases in the review process

Although we conducted comprehensive searches, we only identified and included five trials. Among the five trials, four trials were performed in non-Asian populations and one trial was performed in an Asian population. All studies were of small sample sizes. We tried to avoid location bias, but we could not exclude potential dissemination bias. We have undertaken extensive searches for unpublished material and identified no trials that qualified for inclusion.

Agreements and disagreements with other studies or reviews

Several systematic reviews and meta-analyses on isoflavones for dyslipidaemia have been published (Taku 2007; Taku 2008; Weggemans 2003; Zhan 2005; Zhuo 2004). Only one review assessed the effects of extracted soy isoflavones alone on lipids (Taku 2008). Two reviews assessed the effects of soy protein-containing isoflavones on lipids (Weggemans 2003; Zhan 2005). Two reviews assessed the effects of soy isoflavones alone and soy protein-containing isoflavones on lipids, and performed subgroup analysis by normal cholesterolaemia and primary hypercholesterolaemia (Taku 2007; Zhuo 2004). All reviews included participants with normal cholesterolaemia and primary hypercholesterolaemia. These reviews included 36 trials. Of these trials, only three were included in our review (Dewell 2002; Gardner 2001; Mackey 2000). The other included trials in these reviews were excluded from this review, mostly because the intervention durations were less than three months or the participants were not hypercholesterolaemic. We included one trial in this review because of information provided by contacting the study author and another was published in Chinese. The conclusions of the above-mentioned reviews were inconsistent due to various factors. Two reviews performed similar meta-analyses to our review and concluded that supplementation of soy protein-containing isoflavones for one to three months only slightly decreased TC (Taku 2007) and LDL-cholesterol (Zhuo 2004), but had no statistically significant effects on HDL-cholesterol or triglycerides compared to soy protein-containing no or traces of isoflavones in hypercholesterolaemia. In contrast to these latter two reviews, our systematic review found that three to six months' consumption of isoflavones compared with placebo, or soy protein-containing isoflavones compared with soy protein had no statistically significant effects on TC, LDL-cholesterol or HDL-cholesterol. Our review only found that isoflavones alone significantly lowered triglycerides compared with placebo in hypercholesterolaemia.

AUTHORS' CONCLUSIONS

Implications for practice

We found no evidence for effects of isoflavones on patient-important outcomes or lowering of cholesterol levels in people with hypercholesterolaemia.

Implications for research

From the results of the present review, it would be interesting to test isoflavones compared with placebo in Asian hypercholesterolaemic populations. International, multicentre, rigorously designed, adequately powered, long-term, high-quality studies are required to provide better evidence. Long-term trials should consider the definition of cardiovascular events measures, the incidence of adverse events and other patient-important endpoints in long-term trials.

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Zhuo XG, Melby MK, Watanabe S. Soy isoflavone intake lowers serum LDL cholesterol: a meta-analysis of 8 randomized controlled trials in humans. *Journal of Nutrition* 2004;**134**(9):2395-400. [PUBMED: 15333734]

* Indicates the major publication for the study

CHARACTERISTICS OF STUDIES

Characteristics of included studies *[ordered by study ID]*

Aubertin-Leheudre 2008

Methods	**Parallel randomised controlled clinical trial, randomisation ratio 1 : 1**
Participants	**Inclusion criteria:** postmenopausal women aged 50-70 years, obese (fat mass > 40%), without major physical incapacity, without hormone therapy (at the time of the study, women had never been on hormone therapy or were off hormone therapy for at least 1 year), sedentary (practiced 3 hours/wk of physical activities), weight stable (2 kg) for the last 6 months, non-smoker, moderate drinking (maximum 15 g of alcohol/d, the equivalent of 1 alcoholic beverage/d), no medication that could influence body composition and metabolism, and absence of menses for the past 12 months **Exclusion criteria:** no **Diagnostic criteria:** not specified
Interventions	**Number of study centres:** 1 **Treatment before study:** no **Titration period:** 6 months
Outcomes	**Outcomes reported in abstract of publication:** body composition, medical and social characteristics, daily energy expenditure, dietary intake and blood biochemical analyses (lipid profile, insulin, glucose)
Study details	**Run-in period:** no **Study terminated before regular end (for benefit/because of adverse events):** no
Publication details	**Language of publication:** English **Commercial funding:** not reported **Non-commercial/other funding:** supported by the Canadian Institutes of Health Research (CIHR) and the Research Centre on Aging **Publication status:** peer-reviewed journal
Stated aim of study	Quote from publication: "To investigate whether 6 months of isoflavone supplementation, which has been shown to be sufficient to improve menopausal symptoms, could also improve clinical cardiovascular disease (CVD) risk factors in obese postmenopausal women, compared with a placebo"
Notes	Not all participants were hypercholesterolaemic. The authors provided the data of participants with the baseline cholesterol higher than 5.2 mmol/L. 8 and 11 women with hypercholesterolaemia in the isoflavones and placebo groups, respectively, completed the trial. Thus, this trial was included

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Quote from publication: "Women were randomly assigned to one of two groups, isoflavones (ISO) or placebo (PLA)" Comment: no information about sequence generation
Allocation concealment (selection bias)	Unclear risk	Comment: no information about concealment

Aubertin-Leheudre 2008 *(Continued)*

Blinding of participants and personnel (performance bias) All outcomes	Low risk	Quote from publication: "Identical active and placebo capsules were supplied and encapsulated by Arkopharma Ltd. (Carros, France)"
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Comment: no information about blinding
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Quote from publication: "Among these 50 participants, 39 completed the study (21 in ISO vs. 18 in PLA)" Comment: number of participants analyzed was less than number of participants randomized
Selective reporting (reporting bias)	Low risk	Comment: none detected
Other bias	Low risk	Comment: no other sources of bias were found in this study

Dewell 2002

Methods	**Parallel randomised controlled clinical trial, randomisation ratio intervention: control = 5 : 4**
Participants	**Inclusion criteria:** healthy moderately hypercholesterolaemic (mean TC 6.6 ± 1.3 mmol/L) postmenopausal women (mean age 69 ± 4 years) **Exclusion criteria:** receiving hormone replacement therapy, clinical or biochemical evidence of diabetes or renal, hepatic or cardiovascular disease **Diagnostic criteria:** not specified
Interventions	**Number of study centres:** 1 **Treatment before study:** two individuals were taking medication known to affect carbohydrate or lipid metabolism. 1 woman was taking simvastatin and 1 woman fluvastatin for hypercholesterolaemia. Both women had been on a stable dose for at least 1 year before the study, and medications were not altered during the study period **Titration period:** 6 months
Outcomes	**Outcomes reported in abstract of publication:** triacylglycerol, TC and HDL-C
Study details	**Run-in period:** no **Study terminated before regular end (for benefit/because of adverse events):** no
Publication details	**Language of publication:** English **Commercial funding:** not reported **Non-commercial/other funding:** yes; a small research grant from the College of Applied Sciences and Arts at San Jose State University and a research award from the Circle of Friends/Department of Nutrition and Food Science of San Jose State University **Publication status:** peer-reviewed journal

Dewell 2002 *(Continued)*

Stated aim of study	Quote from publication: "to investigate the effects of PE supplementation (150 mg) on serum lipids and lipoproteins in moderately hypercholesterolemic, elderly, postmenopausal women"
Notes	The trial reported the outcomes of HDL-C and non-HDL-C at baseline and at 2 months, but no data at 6 months Abbreviations: HDL: high-density-lipoprotein

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Quote from publication: "Thirty-six subjects were randomized into two groups" Comment: no information about sequence generation
Allocation concealment (selection bias)	Unclear risk	Comment: no information about concealment
Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	Quote from publication: "Subjects were recruited initially as part of a larger randomized, double blind, placebo-controlled trial with a parallel design to assess the role of PE supplementation on bone mineral health" Comment: no information about blinding
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Comment: no information about blinding
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Quote from publication: "Because of the unavailability of serum, determinations of triacylglycerol and cholesterol concentrations at 6 months were performed only on 17 and 18 of the 20 subjects in the PE-treated group, respectively" Comment: number of participants analyzed was less than number of participants randomized
Selective reporting (reporting bias)	Low risk	Comment: none detected
Other bias	Low risk	Comment: no other sources of bias were found in this study

Gardner 2001

Methods	**Parallel randomised controlled clinical trial, randomisation ratio 1 : 1**
Participants	**Inclusion criteria:** the fasting plasma LDL-cholesterol concentration of 3.37-4.92 mmol/L (130-190 mg/dL) and a triacylglycerol concentration < 2.82 mmol/L (< 250 mg/dL); postmenopausal (≥ 1 year since their last menstrual cycle), age < 80 years, and a BMI of 20–31 kg/m^2 **Exclusion criteria:** smokers, had been taking hormone replacements or lipid-lowering medication during the previous 3 months, had a history of cardiovascular disease or diabetes, or had breast, endometrial or ovarian cancer in the previous 10 years **Diagnostic criteria:** not specified

Gardner 2001 *(Continued)*

Interventions	**Number of study centres:** 1 **Treatment before study:** no **Titration period:** 12 wks
Outcomes	**Outcomes reported in abstract of publication:** TC, LDL-C, HDL-C and triacylglycerol
Study details	**Run-in period:** yes **Study terminated before regular end (for benefit/because of adverse events):** no
Publication details	**Language of publication:** English **Commercial/non-commercial/other funding:** not reported **Publication status:** peer-reviewed journal
Stated aim of study	Quote from publication: "to determine the effect of soy protein and isoflavones on plasma lipid concentrations in postmenopausal, moderately hypercholesterolemic women"
Notes	"LDL-cholesterol was calculated according to the method of Friedewald unless the triacylglycerols were > 4.52 mmol/L (> 400 mg/dL), in which case the LDL-C value was considered missing data (3 LDL-cholesterol data points were excluded: 1 in the Milk group, 0 in the Soy– group, and 2 in the Soy+ group)" Abbreviations: HDL: high-density-lipoprotein; LDL: low-density-lipoprotein

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Quote from publication: "The subjects were randomly assigned to 12 wk of dietary protein supplementation (42 g/d) with either a milk protein (Milk group) or 1 of 2 soy proteins containing either trace amounts of isoflavones (Soy– group) or 80 mg aglycone isoflavones (Soy+ group)", "Randomization was performed in blocks of 30 participants" Comment: no details provided
Allocation concealment (selection bias)	Low risk	Quote from publication: "Dietary supplements containing a mixture of protein, carbohydrate, and calcium in powder form (Shaklee Corporation, Hayward, CA) were provided in sealed packets, each containing one-half of the daily dose (21 g protein/packet 2 packets/d)", "All supplements were formulated to be identical in taste, color, and odor"
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Quote from publication: "Participants, investigators, study staff, and laboratory technicians were blinded to treatment assignments until the conclusion of the trial"
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Quote from publication: "Participants, investigators, study staff, and laboratory technicians were blinded to treatment assignments until the conclusion of the trial"
Incomplete outcome data (attrition bias) All outcomes	High risk	Quote from publication: "These analyses showed a stable composition of isoflavone concentrations throughout the study (data not shown)", "Of the 115 women who entered the study, 21 withdrew before study completion", "Statistical analyses were performed on data"

Gardner 2001 *(Continued)*

		Comment: number of participants analyzed was less than number of participants randomized
Selective reporting (reporting bias)	Low risk	Comment: none detected
Other bias	High risk	Quote "At the onset of the study, the participants were instructed to take the full dose of the protein supplements. However, we had a higher than anticipated dropout rate early in the study because of adverse gastrointestinal responses to the acute dietary change. Therefore, gradual adaptation to the protein supplements was recommended for the latter four-fifths of participants. These participants began the 4-wk run-in phase by consuming one-half of the goal dose rather than the full dose. These participants were then encouraged to increase their intake to the full dose by week 3 of the run-in phase". "Exceptions to group comparability at randomization included a higher average age and a higher number of years since menopause in the Soy+ group and a lower percentage of married women in the Soy– group than in the Soy+ group" Comment: not all participants performed the run-in phase; several baseline characteristics of participants were not equally distributed between the intervention and placebo groups

Mackey 2000

Methods	**Parallel randomised controlled clinical trial, randomisation ratio not reported**
Participants	**Inclusion criteria:** postmenopausal women aged 45-65 years **Exclusion criteria:** a history of allergy to soy or if any of them were taking cholesterol-lowing agents **Diagnostic criteria:** a fasting TC > 5.5 mmol/L
Interventions	**Number of study centres:** 1 **Treatment before study:** no **Titration period:** 12 wks
Outcomes	**Outcomes reported in abstract of publication:** TC, LDL-C, HDL-C, SHBG and LH
Study details	**Run-in period:** yes **Study terminated before regular end (for benefit/because of adverse events):** no
Publication details	**Language of publication:** English **Commercial funding:** yes **Publication status:** peer-reviewed journal
Stated aim of study	Quote from publication: "We performed a series of studies in men and women using soy protein with or without isoflavones to study the effect on the lipoprotein profile as well as other biochemical indices such as sex hormones, pituitary hormones, markers of bone turnover and glucose tolerance"
Notes	The female study was a prospective, double-blind, randomised controlled study. The male study was an open prospective observational pilot study. Thus, only female study was included. The concentrations of LDL-C maybe calculated

Mackey 2000 *(Continued)*

Abbreviations: HDL: high-density-lipoprotein; LDL: low-density-lipoprotein

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Quote from publication: "Fifty four female subjects were randomised to receive 28 g of protein powder; either a) a soy protein with an isoflavone content of 65 mg isoflavones daily (ISP+) or b) a soy protein isolate with less than 4 mg isoflavones per daily (ISP-)" Comment: no information about sequence generation
Allocation concealment (selection bias)	Unclear risk	Comment: no information about concealment
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Quote from publication: "The female study was a prospective, double-blind, randomised controlled study"
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Comment: no information about blinding
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Quote: "Of the 54 women who were randomised into the study, 49 women completed the study" Comment: number of participants analyzed was less than number of participants randomized
Selective reporting (reporting bias)	Low risk	Comment: none detected
Other bias	Low risk	Comment: no other sources of bias were found in this study

Wang 2005

Methods	**Parallel randomised controlled clinical trial, randomisation ratio 1 : 1**
Participants	**Inclusion criteria:** postmenopausal women (≥ 6 months since their last menstrual cycle) with perimenopausal symptoms, dyslipidaemia and abnormal endocrine function, aged 45-55 years **Exclusion criteria:** use of steroid drugs **Diagnostic criteria:** not specified
Interventions	**Number of study centres:** 1 **Treatment before study:** no **Titration period:** 3 month
Outcomes	**Outcomes reported in abstract of publication:** serum TC, HDL-C, LDL-C and triacylglycerol
Study details	**Run-in period:** no

Wang 2005 *(Continued)*

	Study terminated before regular end (for benefit/because of adverse events): no
Publication details	**Language of publication:** Chinese **Commercial/non-commercial/other funding:** not reported **Publication status:** peer-reviewed journal
Stated aim of study	Quote from publication: "To study the effects of soybean isoflavones on lipids metabolism in the peri-menopausal female"
Notes	Baseline serum HDL-C concentrations were very low

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Quote from publication: "Subjects were randomised to 3 groups" Comment: no information about sequence generation
Allocation concealment (selection bias)	Unclear risk	Comment: no information about concealment
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Quote from publication:"The placebo group consumed the placebo capsules that looked like the isoflavones capsules" Comment: participants and personnel were potentially masked
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Comment: no information about blinding
Incomplete outcome data (attrition bias) All outcomes	Low risk	Comment: number of participants analyzed was equal to the number of participants randomized
Selective reporting (reporting bias)	Low risk	Comment: none detected
Other bias	Low risk	Comment: no other sources of bias were found in this study

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Atkinson 2004	Randomized clinical trial comparing 43.5 mg red clover-derived isoflavones versus placebo in 177 women. It was excluded because not all participants were hypercholesterolaemic
Badeau 2007	56 postmenopausal women were treated with either isoflavone or placebo tablets for 3 months in a cross-over design, separated by a 2-month washout period. It was excluded because not all participants were hypercholesterolaemic
Blakesmith 2003	Randomized clinical trial comparing isoflavone versus placebo in 25 healthy premenopausal women. It was excluded because not all participants were hypercholesterolaemic

Study	Reason for exclusion
Cancellieri 2007	Multicentre, randomized, double-blind, placebo-controlled clinical investigation on 125 menopausal women randomly assigned to 2 groups treated for 6 months with placebo or 1 tablet daily of an herbal product containing 72 mg/dose of isoflavones of different plants origin and other plant extracts. It was excluded because not all participants were hypercholesterolaemic
Chedraui 2008	Randomized controlled cross-over clinical trial conducted in 60 postmenopausal women. It was excluded because not all participants were hypercholesterolaemic
Cheng 2007	Randomized clinical trial comparing isoflavone versus placebo in 60 healthy postmenopausal women. It was excluded because not all participants were hypercholesterolaemic
Cianci 2012	120 women with a mean age of 54.8 ± 0.6 years were enrolled and randomized to treatment with isoflavones and berberine or calcium and vitamin D(3). It was excluded because 1) not all participants were hypercholesterolaemic, and 2) intervention was isoflavones and berberine
Colacurci 2005	Randomized clinical trial comparing isoflavone versus placebo in 60 healthy postmenopausal women. It was excluded because not all participants were hypercholesterolaemic
Dent 2001	Randomized clinical trial comparing isoflavone-rich soy versus isoflavone-poor soy in perimenopausal women. It was excluded because not all participants were hypercholesterolaemic
Fornaro 2006	54 healthy postmenopausal women were randomly allocated to 60 mg/d of both genistein and daidzein for 6 months (active group, n = 27) or no therapy (control group, n = 27). It was excluded because not all participants were hypercholesterolaemic
Gallagher 2004	A total of 65 women, with a mean age of 55 years and 7.5 years since menopause, were randomized to 1 of 3 groups; soy protein with 96 mg isoflavones/d, soy with 52 mg isoflavones/d, or soy without isoflavones (< 4 mg isoflavones/d). It was excluded because not all participants were hypercholesterolaemic
Garrido 2006	29 healthy postmenopausal women were invited to take part in a randomized study to receive either 100 mg/d isoflavone supplement (n = 15) or identical placebo capsules (n = 14). It was excluded because not all participants were hypercholesterolaemic
Gonzalez 2007	A randomized, double-blind, placebo-controlled, cross-over study was conducted in 32 Caucasian, postmenopausal women with diet-controlled type 2 diabetes. It was excluded because not all participants were hypercholesterolaemic
Han 2002	Randomized clinical trial comparing isoflavone versus placebo in postmenopausal women. It was excluded because not all participants were hypercholesterolaemic
Hidalgo 2005	60 postmenopausal women aged > 40 years, non-users of hormone therapy, with Kupperman index score 15, were randomized in a double-blind method to receive either a commercially available red clover isoflavone supplement (80 mg/d) or placebo for 90 d. It was excluded because not all participants were hypercholesterolaemic
Ho 2007	A double-blind, randomized, placebo-controlled trial was conducted in 203 postmenopausal Chinese women aged 48-62 years. They were randomly assigned to receive daily doses of 500 mg calcium, and 0 mg isoflavones (placebo, n = 67), 40 mg isoflavones (n = 68) and 80 mg isoflavones (n = 68). It was excluded because not all participants were hypercholesterolaemic
Jiang 2008	Randomized clinical trial comparing isoflavone versus placebo in 189 normal cholesterolaemic and hypercholesterolaemic perimenopausal women. It was excluded because lack of cholesterol data in hypercholesterolaemic women in the placebo group
Lee 2012	In this randomized, double-blind, placebo-controlled trial, 51 subjects with a body mass index of 23 kg/m^2 or greater and a waist-to-hip ratio of 0.90 or greater for men or 0.85 or greater for women

Study	Reason for exclusion
	were randomly assigned to take 9.9 g/d of either a placebo or doenjang for 12 weeks. It was excluded because 1) not all participants were hypercholesterolaemic, and 2) doenjang may contain isoflavones and soy protein
Liang 2007	It was excluded because the intervention was soy lecithin
Llaneza 2010	116 postmenopausal women with insulin resistance were randomly assigned to a group of Mediterranean diet and physical exercise (control group) or a group of Mediterranean diet, physical exercise and daily oral ingestion of 40 mg of soy isoflavones (soy isoflavones group). It was excluded because not all participants were hypercholesterolaemic
Marini 2010	Randomized clinical trial comparing genistein versus placebo in postmenopausal women. It was excluded because not all participants were hypercholesterolaemic
Nikander 2004	Randomized clinical trial comparing isoflavone versus placebo in 56 non-diabetic postmenopausal women with a history of breast cancer. It was excluded because not all participants were hypercholesterolaemic
Oliveira 2012	In a prospective, randomized and single-blinded clinical trial, we compared people with chronic hepatitis C who had casein as a supplement (n = 80) (control group), with people who consumed a soy supplement diet (n = 80) (intervention group). It was excluded because 1) not all participants were hypercholesterolaemic, and 2) the intervention was soy protein and isoflavones, but the control was placebo
Ozturk 2009	Randomized clinical trial comparing isoflavone versus placebo in 22 postmenopausal women. It was excluded because not all participants were hypercholesterolaemic
Petri Nahas 2004	Randomized clinical trial comparing isoflavone versus placebo in 50 postmenopausal women. It was excluded because not all participants were hypercholesterolaemic
Rios 2008	In this double-blind, placebo-controlled study, 47 postmenopausal women 47-66 years of age received 40 mg of isoflavone (n = 25) or 40 mg of casein placebo (n = 22). It was excluded because not all participants were hypercholesterolaemic
Schult 2004	Randomized clinical trial comparing isoflavones versus placebo in 252 menopausal women aged 45-60 years. It was excluded because not all participants were hypercholesterolaemic
Swain 2002	Perimenopausal women (n = 69) were randomly assigned (double blind) to treatment: isoflavone-rich soy-protein isolate (n = 24), isoflavone-poor soy-protein isolate (n = 24) or whey protein (control; n = 21). Each subject consumed 40 g soy or whey protein daily for 24 weeks. It was excluded because not all participants were hypercholesterolaemic
Terzic 2009	Randomized clinical trial comparing a red clover-derived isoflavone versus placebo in 40 healthy postmenopausal women with a mean age of 56 years. It was excluded because not all participants were hypercholesterolaemic
Törmälä 2006	30 postmenopausal women were treated in a randomized, placebo-controlled, cross-over trial with isoflavones or placebo for 3 months interrupted by a 2-month washout period. It was excluded because not all participants were hypercholesterolaemic
Uesugi 2003	Randomized clinical trial comparing isoflavone versus placebo in postmenopausal women. It was excluded because not all participants were hypercholesterolaemic
Villa 2009	A randomized placebo controlled study was conducted in 50 postmenopausal women. It was excluded because not all participants were hypercholesterolaemic

Study	Reason for exclusion
Wong 2012	Randomized controlled cross-over clinical trial conducted in 41 participants (23 men, 18 women). It was excluded because the intervention duration was 4 weeks
Woo 2003	Randomized clinical trial comparing isoflavone versus placebo in postmenopausal women. It was excluded because not all participants were hypercholesterolaemic
Wu 2006a	Randomized clinical trial comparing isoflavone versus placebo, and isoflavone plus walking versus walking in 136 postmenopausal women at < 5 years after the onset of menopause. It was excluded because the mean daily dietary isoflavones intakes of participants in all groups at baseline and during the trial were greater than 37 mg/d, and not all participants were hypercholesterolaemic. This paper was also republished
Wu 2006b	Randomized clinical trial comparing isoflavone versus placebo, and isoflavone plus walking versus walking in 128 postmenopausal women at < 5 years after the onset of menopause. It was excluded because the mean daily dietary isoflavones intakes of participants in all groups at baseline and during the trial were greater than 37 mg/d, and not all participants were hypercholesterolaemic. This paper was also republished
Xiao 2003	It was excluded because the intervention duration was 8 weeks
Ye 2012	A randomized placebo-controlled trial. 90 early postmenopausal Chinese women, aged 45-60 years, were randomly assigned to 3 treatment groups (30 each) receiving daily doses of 0 (placebo), 84 and 126 mg of soy germ isoflavones. It was excluded because most of participants were not hypercholesterolaemic
Yildiz 2005	Randomized clinical trial comparing 40 mg of genistein versus placebo in postmenopausal women. It was excluded because not all participants were hypercholesterolaemic

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DATA AND ANALYSES

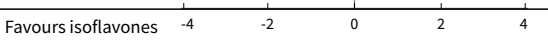
Comparison 1. Isoflavones versus placebo or soy protein+isoflavones versus soy protein

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 LDL-cholesterol	3	132	Mean Difference (IV, Random, 95% CI)	-0.16 [-0.39, 0.07]
1.1 Isoflavones versus placebo	1	19	Mean Difference (IV, Random, 95% CI)	0.05 [-0.43, 0.53]
1.2 Soy protein-containing isoflavones versus soy protein	2	113	Mean Difference (IV, Random, 95% CI)	-0.23 [-0.50, 0.04]
2 Total cholesterol	4	166	Mean Difference (IV, Random, 95% CI)	-0.08 [-0.34, 0.19]
2.1 Isoflavones versus placebo	2	53	Mean Difference (IV, Random, 95% CI)	0.12 [-0.44, 0.68]

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
2.2 Soy protein-containing isoflavones versus soy protein	2	113	Mean Difference (IV, Random, 95% CI)	-0.20 [-0.50, 0.09]
3 HDL-cholesterol	3	132	Mean Difference (IV, Random, 95% CI)	-0.03 [-0.22, 0.16]
3.1 Isoflavones versus placebo	1	19	Mean Difference (IV, Random, 95% CI)	-0.07 [-0.38, 0.24]
3.2 Soy protein-containing isoflavones versus soy protein	2	113	Mean Difference (IV, Random, 95% CI)	-0.03 [-0.30, 0.25]
4 Triglycerides	4	165	Mean Difference (IV, Random, 95% CI)	-0.15 [-0.43, 0.13]
4.1 Isoflavones versus placebo	2	52	Mean Difference (IV, Random, 95% CI)	-0.46 [-0.84, -0.09]
4.2 Soy protein-containing isoflavones versus soy protein	2	113	Mean Difference (IV, Random, 95% CI)	0.03 [-0.23, 0.29]
5 Sex hormone binding globulin	1	46	Mean Difference (IV, Random, 95% CI)	-4.22 [-15.62, 7.18]

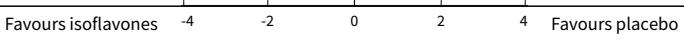
Analysis 1.1. Comparison 1 Isoflavones versus placebo or soy protein+isoflavones versus soy protein, Outcome 1 LDL-cholesterol.

Study or subgroup	Isoflavones N	Isoflavones Mean(SD)	Placebo N	Placebo Mean(SD)	Mean Difference Random, 95% CI	Weight	Mean Difference Random, 95% CI
1.1.1 Isoflavones versus placebo							
Aubertin-Leheudre 2008	8	3.7 (0.4)	11	3.7 (0.7)		24.13%	0.05[-0.43,0.53]
Subtotal ***	**8**		**11**			**24.13%**	**0.05[-0.43,0.53]**
Heterogeneity: Not applicable							
Test for overall effect: Z=0.21(P=0.84)							
1.1.2 Soy protein-containing isoflavones versus soy protein							
Gardner 2001	31	3.5 (0.5)	33	3.8 (0.8)		51.97%	-0.3[-0.62,0.02]
Mackey 2000	25	4.7 (0.7)	24	4.8 (1)		23.9%	-0.07[-0.55,0.41]
Subtotal ***	**56**		**57**			**75.87%**	**-0.23[-0.5,0.04]**
Heterogeneity: Tau^2=0; Chi^2=0.61, df=1(P=0.44); I^2=0%							
Test for overall effect: Z=1.66(P=0.1)							
Total ***	**64**		**68**			**100%**	**-0.16[-0.39,0.07]**
Heterogeneity: Tau^2=0; Chi^2=1.59, df=2(P=0.45); I^2=0%							
Test for overall effect: Z=1.34(P=0.18)							
Test for subgroup differences: Chi^2=0.99, df=1 (P=0.32), I^2=0%							



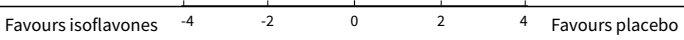
Analysis 1.2. Comparison 1 Isoflavones versus placebo or soy protein +isoflavones versus soy protein, Outcome 2 Total cholesterol.

Study or subgroup	Isoflavones N	Isoflavones Mean(SD)	Placebo N	Placebo Mean(SD)	Mean Difference Random, 95% CI	Weight	Mean Difference Random, 95% CI
1.2.1 Isoflavones versus placebo							
Aubertin-Leheudre 2008	8	5.8 (0.4)	11	5.9 (0.9)		18.13%	-0.17[-0.75,0.41]
Dewell 2002	18	6.4 (0.9)	16	6 (0.8)		19.81%	0.4[-0.15,0.95]
Subtotal ***	**26**		**27**			**37.94%**	**0.12[-0.44,0.68]**
Heterogeneity: Tau^2=0.08; Chi^2=1.92, df=1(P=0.17); I^2=48%							
Test for overall effect: Z=0.43(P=0.67)							
1.2.2 Soy protein-containing isoflavones versus soy protein							
Gardner 2001	31	5.7 (0.5)	33	5.9 (0.9)		40.26%	-0.2[-0.55,0.15]
Mackey 2000	25	6.9 (0.8)	24	7.2 (1)		21.8%	-0.21[-0.73,0.31]
Subtotal ***	**56**		**57**			**62.06%**	**-0.2[-0.5,0.09]**
Heterogeneity: Tau^2=0; Chi^2=0, df=1(P=0.98); I^2=0%							
Test for overall effect: Z=1.36(P=0.17)							
Total ***	**82**		**84**			**100%**	**-0.08[-0.34,0.19]**
Heterogeneity: Tau^2=0.01; Chi^2=3.64, df=3(P=0.3); I^2=17.58%							
Test for overall effect: Z=0.57(P=0.57)							
Test for subgroup differences: Chi^2=1.02, df=1 (P=0.31), I^2=2.38%							



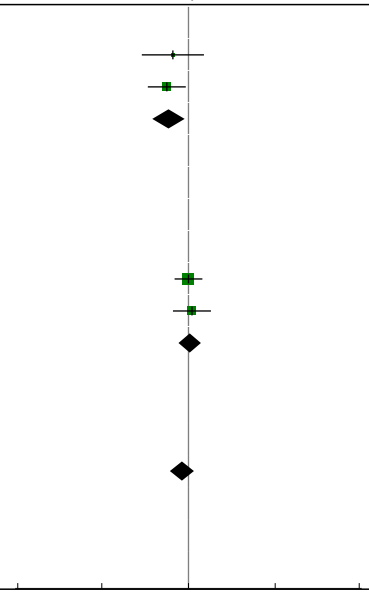
Analysis 1.3. Comparison 1 Isoflavones versus placebo or soy protein+isoflavones versus soy protein, Outcome 3 HDL-cholesterol.

Study or subgroup	Isoflavones N	Isoflavones Mean(SD)	Placebo N	Placebo Mean(SD)	Mean Difference Random, 95% CI	Weight	Mean Difference Random, 95% CI
1.3.1 Isoflavones versus placebo							
Aubertin-Leheudre 2008	8	1.4 (0.3)	11	1.5 (0.4)		22.72%	-0.07[-0.38,0.24]
Subtotal ***	**8**		**11**			**22.72%**	**-0.07[-0.38,0.24]**
Heterogeneity: Not applicable							
Test for overall effect: Z=0.44(P=0.66)							
1.3.2 Soy protein-containing isoflavones versus soy protein							
Gardner 2001	31	1.6 (0.3)	33	1.5 (0.2)		44.79%	0.1[-0.03,0.23]
Mackey 2000	25	1.5 (0.3)	24	1.7 (0.5)		32.49%	-0.18[-0.4,0.04]
Subtotal ***	**56**		**57**			**77.28%**	**-0.03[-0.3,0.25]**
Heterogeneity: Tau^2=0.03; Chi^2=4.78, df=1(P=0.03); I^2=79.09%							
Test for overall effect: Z=0.18(P=0.86)							
Total ***	**64**		**68**			**100%**	**-0.03[-0.22,0.16]**
Heterogeneity: Tau^2=0.02; Chi^2=5.13, df=2(P=0.08); I^2=61.05%							
Test for overall effect: Z=0.3(P=0.76)							
Test for subgroup differences: Chi^2=0.04, df=1 (P=0.83), I^2=0%							



Analysis 1.4. Comparison 1 Isoflavones versus placebo or soy protein+isoflavones versus soy protein, Outcome 4 Triglycerides.

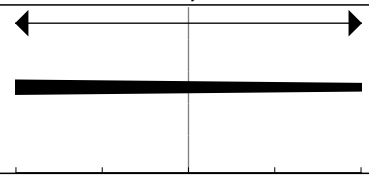
Study or subgroup	Isoflavones N	Isoflavones Mean(SD)	Placebo N	Placebo Mean(SD)	Mean Difference Random, 95% CI	Weight	Mean Difference Random, 95% CI
1.4.1 Isoflavones versus placebo							
Aubertin-Leheudre 2008	8	1.4 (0.5)	11	1.8 (1.1)		12.37%	-0.36[-1.08,0.36]
Dewell 2002	17	0.9 (0.4)	16	1.4 (0.8)		25.62%	-0.5[-0.94,-0.06]
Subtotal ***	**25**		**27**			**37.99%**	**-0.46[-0.84,-0.09]**
Heterogeneity: Tau^2=0; Chi^2=0.11, df=1(P=0.74); I^2=0%							
Test for overall effect: Z=2.42(P=0.02)							
1.4.2 Soy protein-containing isoflavones versus soy protein							
Gardner 2001	31	1.3 (0.7)	33	1.3 (0.6)		36.4%	0[-0.32,0.32]
Mackey 2000	25	1.5 (0.7)	24	1.5 (0.8)		25.61%	0.08[-0.36,0.52]
Subtotal ***	**56**		**57**			**62.01%**	**0.03[-0.23,0.29]**
Heterogeneity: Tau^2=0; Chi^2=0.08, df=1(P=0.77); I^2=0%							
Test for overall effect: Z=0.21(P=0.83)							
Total ***	**81**		**84**			**100%**	**-0.15[-0.43,0.13]**
Heterogeneity: Tau^2=0.03; Chi^2=4.66, df=3(P=0.2); I^2=35.57%							
Test for overall effect: Z=1.07(P=0.28)							
Test for subgroup differences: Chi^2=4.47, df=1 (P=0.03), I^2=77.61%							



Favours isoflavones — Favours placebo

Analysis 1.5. Comparison 1 Isoflavones versus placebo or soy protein +isoflavones versus soy protein, Outcome 5 Sex hormone binding globulin.

Study or subgroup	Isoflavones N	Isoflavones Mean(SD)	Control N	Control Mean(SD)	Mean Difference Random, 95% CI	Weight	Mean Difference Random, 95% CI
Mackey 2000	22	38.9 (20.2)	24	43.1 (19.2)		100%	-4.22[-15.62,7.18]
Total ***	**22**		**24**			**100%**	**-4.22[-15.62,7.18]**
Heterogeneity: Not applicable							
Test for overall effect: Z=0.73(P=0.47)							



Favours isoflavones — Favours control

ADDITIONAL TABLES

Table 1. Overview of study populations

Characteristic Study ID	Intervention and comparator	[N] Screened / eligible	[N] Randomised	[N] Safety	[N] ITT	[N] Finishing study	[%] Randomised finishing study
Aubertin-Leheudre 2008	Isoflavones (70 mg)	-	-	-	-	8	N/A
	Placebo		-	-	-	11	N/A
total:			-	-	-	19	N/A
Dewell 2002	Isoflavones (150 mg)	-	20	-	20	20	100
	Maltodextrin with 10% caramel		16	-	16	16	100
total:			36	-	36	36	100
Gardner 2001	Isoflavones (80 mg)	-	34	-	-	31	91.2
	Trace amounts of isoflavones		34	-	-	33	97.1
total:			68	-	-	64	94.1
Mackey 2000	Isoflavones (65 mg)	-	-	-	-	25	N/A
	Less than 4 mg isoflavones		-	-	-	24	N/A
total:			54	-	-	49	90.7
Wang 2005	Isoflavones (158 mg)	-	20	-	20	20	100
	Placebo		20	-	20	20	100
total:			40	-	40	40	100
Total	***All interventions***		***-***			***104***	
	All controls		***-***			***104***	
	All interventions and comparators		***-***			***208***	

"-" denotes not reported.
ITT: intention-to-treat; N/A: not applicable.

APPENDICES

Appendix 1. Search strategies

Search terms and databases
Unless otherwise stated, search terms are free text terms.
Abbreviations:
'$': stands for any character; '?': substitutes one or no character; adj: adjacent (i.e. number of words within range of search term); exp: exploded MeSH; MeSH: medical subject heading (MEDLINE medical index term); pt: publication type; sh: MeSH; tw: text word.
The Cochrane Library
#1 MeSH descriptor Hypercholesterolemia explode all trees
#2 MeSH descriptor Hyperlipoproteinemias explode all trees
#3 MeSH descriptor Cholesterol, HDL explode all trees
#4 MeSH descriptor Cholesterol, LDL explode all trees
#5 MeSH descriptor Lipoproteins, HDL explode all trees
#6 MeSH descriptor Lipoproteins, LDL explode all trees
#7 (hypercholesterolaemi* in All Text or hypercholesterolemi* in All Text or hyperlipoproteinaemi* in All Text or hyperlipoproteinemi* in All Text or hyperlipaemi* in All Text or hyperlipemi* in All Text)
#8 (HDL in All Text or LDL in All Text or cholesterol in All Text)
#9 (#1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8)
#10 MeSH descriptor Isoflavones explode all trees
#11 MeSH descriptor Soybeans explode all trees
#12 (biochanin* in All Text or formononetin* in All Text or prunetin* in All Text)
#13 ((red in All Text and glover* in All Text) or (alfalfa in All Text and sprout* in All Text))
#14 soybean* in All Text
#15 (coumestrol in All Text or pterocarpans in All Text or rotenone in All Text)
#16 (daidzin* in All Text or daidzein* in All Text or genistin* in All Text or genistein* in All Text or glycetin* in All Text or glycetein* in All Text)
#17 (#10 OR #11 OR #12 OR #13 OR #14 OR #15 OR #16)
#18 (#9 AND #17)
MEDLINE
1 exp hypercholesterolemia/ or exp hyperlipoproteinemias/
2 (hypercholesterol?emi* or hyperlipoprotein?emi* or hyperlip?emi*).tw,ot.
3 (HDL or LDL or cholesterol).tw,ot.
4 exp Lipoproteins, HDL/ or exp Cholesterol, HDL/

(Continued)

5 exp Cholesterol, LDL/ or exp Lipoproteins, LDL/

6 or/1-5

7 exp Isoflavones/

8 exp Genistein/ or exp Soybeans/

9 (daidzin* or daidzein* or genistin* or genistein* or glycetin* or glycetein*).tw,ot.

10 (biochanin* or formononetin* or prunetin*).tw,ot.

11 (coumestrol or pterocarpans or rotenone).tw,ot.

12 (red glover* or alfalfa sprout* or soybean*).tw,ot.

13 or/7-12

14 randomized controlled trial.pt.

15 controlled clinical trial.pt.

16 randomi?ed.ab.

17 placebo.ab.

18 drug therapy.fs.

19 randomly.ab.

20 trial.ab.

21 groups.ab.

22 or/14-21

23 Meta-analysis.pt.

24 exp Technology Assessment, Biomedical/

25 exp Meta-analysis/

26 exp Meta-analysis as topic/

27 hta.tw,ot.

28 (health technology adj6 assessment$).tw,ot.

29 (meta analy$ or metaanaly$ or meta?analy$).tw,ot.

30 ((review$ or search$) adj10 (literature$ or medical database$ or medline or pubmed or embase or cochrane or cinahl or psycinfo or psyclit or healthstar or biosis or current content$ or systemat$)).tw,ot.

31 or/23-30

32 22 or 31

33 (comment or editorial or historical-article).pt.

34 32 not 33

35 (animals not (humans and animals)).sh.

36 34 not 35

37 6 and 13 and 36

(Continued)

EMBASE

1 exp hyperlipidemia/

2 exp high density lipoprotein/

3 exp low density lipoprotein/

4 exp high density lipoprotein cholesterol/

5 exp low density lipoprotein cholesterol/

6 (hypercholesterol?emi* or hyperlipoprotein?emi* or hyperlipid?emi*).tw,ot.

7 exp cholesterol/

8 ((HDL or LDL) adj6 (lipoprotein* or cholesterol*)).tw,ot.

9 or/1-8

10 exp isoflavone derivative/

11 exp isoflavone/

12 exp genistein/

13 exp soybean/

14 exp daidzin/

15 exp daidzein/

16 exp genistin/

17 exp glycitein/

18 exp biochanin A/

19 exp formononetin/

20 exp prunetin/

21 isoflavone*.tw,ot.

22 (coumestrol or pterocarpans or rotenone).tw,ot.

23 (genistein* or genistin* or soybean* or daidzein* or glycitein* or biochanin* or formononetin* or prunetin*).tw,ot.

24 (red glover* or alfalfa sprout*).tw,ot.

25 or/10-24

26 exp Randomized Controlled Trial/

27 exp Controlled Clinical Trial/

28 exp Clinical Trial/

29 exp Comparative Study/

30 exp Drug comparison/

31 exp Randomization/

32 exp Crossover procedure/

33 exp Double blind procedure/

(Continued)

34 exp Single blind procedure/

35 exp Placebo/

36 exp Prospective Study/

37 ((clinical or control$ or comparativ$ or placebo$ or prospectiv$ or randomi?ed) adj3 (trial$ or stud$)).ab,ti.

38 (random$ adj6 (allocat$ or assign$ or basis or order$)).ab,ti.

39 ((singl$ or doubl$ or trebl$ or tripl$) adj6 (blind$ or mask$)).ab,ti.

40 (cross over or crossover).ab,ti.

41 or/26-40

42 exp meta analysis/

43 (metaanaly$ or meta analy$ or meta?analy$).ab,ti,ot.

44 ((review$ or search$) adj10 (literature$ or medical database$ or medline or pubmed or embase or cochrane or cinahl or psycinfo or psyclit or healthstar or biosis or current content$ or systematic$)).ab,ti,ot.

45 exp Literature/

46 exp Biomedical Technology Assessment/

47 hta.tw,ot.

48 (health technology adj6 assessment$).tw,ot.

49 or/42-48

50 41 or 49

51 (comment or editorial or historical-article).pt.

52 50 not 51

53 9 and 25 and 52

54 limit 53 to human

China National Knowledge Infrastructure

#1 (soy or soya):ti,ab,kw

#2 (tofu):ti,ab,kw

#3 (red clover):ti,ab,kw

#4 (isoflavone or isoflavones):ti,ab,kw

#5 (daidzin or daidzein):ti,ab,kw

#6 (genistin or genistein):ti,ab,kw

#7 (glycetin or glycetein):ti,ab,kw

#8 #1 or #2 or #3 or #4 or #5 or $6 or #7

#9 (cholesterol):ti,ab,kw

#10 (triglyceride):ti,ab,kw

#11 (lipid or lipids):ti,ab,kw

(Continued)

#12 (LDL-C or HDL-C):ti,ab,kw
#13 #9 or #10 or #11 or #12
#14 #8 and #13

Chinese BioMedical Database
#1 (soy or soya):ti,ab,kw
#2 (tofu):ti,ab,kw
#3 (red clover):ti,ab,kw
#4 (isoflavone or isoflavones):ti,ab,kw
#5 (daidzin or daidzein):ti,ab,kw
#6 (genistin or genistein):ti,ab,kw
#7 (glycetin or glycetein):ti,ab,kw
#8 #1 or #2 or #3 or #4 or #5 or $6 or #7
#9 (cholesterol):ti,ab,kw
#10 (triglyceride):ti,ab,kw
#11 (lipid or lipids):ti,ab,kw
#12 (LDL-C or HDL-C):ti,ab,kw
#13 #9 or #10 or #11 or #12
#14 #8 and #13

Appendix 2. Description of interventions

Characteristic **Study ID**	**Intervention** **[route, frequency, total dose/day]**	**Comparator** **[route, frequency, total dose/day]**
Aubertin-Leheudre 2008	Isoflavones capsules (orally, 4 times daily, 70 mg/day (44 mg daidzein, 16 mg glycetein, 10 mg genistein))	Placebo capsules (orally, 4 times daily)
Dewell 2002	Soy-derived isoflavones tablets (orally, 3 times daily, 150 mg/day)	Maltodextrin with 10% caramel colour tablets, (orally, 3 times daily, 150 mg/day)
Gardner 2001	42 g soy proteins containing 80 mg aglycone isoflavones (orally, twice daily, 80 mg/day)	42 g soy proteins containing trace amounts of isoflavones (orally, twice daily, 3 mg/day)
Mackey 2000	28 g soy protein powder with 65 mg isoflavones	28 g soy protein powder with less than

(Continued)

	(orally, once daily, 65 mg/day)	4 mg isoflavones (orally, once daily, < 4 mg/day)
Wang 2005	Isoflavones capsules (orally, 3 times daily, 157.5 mg/day)	Placebo capsules (orally, 3 times daily)

Appendix 3. Baseline characteristics (I)

Characteristic Study ID	Intervention and comparator	Duration of intervention	Duration of follow-up	Participating population	Country	Setting	Ethnic groups (%)	Duration of disease [mean years (SD)/ range]
Aubertin-Leheudre 2008	Isoflavones (70 mg) Placebo	6 months	6 months	Hypercholesterolaemic postmenopausal women	Canada	-	-	-
Dewell 2002	Isoflavones (150 mg) Maltodextrin with 10% caramel	6 months	6 months	Moderately hypercholesterolaemic postmenopausal women	US	-	-	-
Gardner 2001	Isoflavones (80 mg) Trace amounts of isoflavones	12 weeks	16 weeks	Moderately hypercholesterolaemic postmenopausal women	US	Outpatients	White, non-Hispanic (69) White, non-Hispanic (87)	-
Mackey 2000	Isoflavones (65 mg) Less than 4 mg isoflavones	12 weeks	22 weeks	Hypercholesterolaemic postmenopausal women	Australia	-	-	-
Wang 2005	Isoflavones (158 mg) Placebo	3 months	3 months	Hypercholesterolaemic postmenopausal women	China	Outpatients	-	-

Footnotes

"-" denotes not reported.

SD: standard deviation.

Appendix 4. Baseline characteristics (II)

Characteristic Study ID	Intervention and comparator	Sex [female %]	Age [mean years (SD)/range]	TC [mean mmol/L (SD)]	BMI [mean kg/m^2 (SD)]	Co-medications / Co-interventions	Co-morbidities
Aubertin-Leheudre 2008	Isoflavones (70 mg)	100	50-70	5.90 (0.59)	31.2 (4.5)	-	-
	Placebo	100	50-70	6.02 (0.90)	32.8 (4.8)		
Dewell 2002	Isoflavones (150 mg)	100	69 (4) 64-83	6.8 (0.9)	25 (4)	1 participant took simvastatin and 1 took fluvastatin	-
	Maltodextrin with 10% caramel	100	70 (4) 65-77	6.3 (2.0)	25 (4)		
Gardner 2001	Isoflavones (80 mg)	100	62.6 (7.3)	6.1 (0.6)	25.6 (4.4)	-	-
	Trace amounts of isoflavones	100	58.4 (7.2)	6.2 (0.9)	25.4 (3.6)		
Mackey 2000	Isoflavones (65 mg)	100	56.4 (4.9)	7.29 (0.90)	-	-	-
	Less than 4 mg isoflavones	100	56.8 (4.2)	7.47 (1.04)			
Wang 2005	Isoflavones (158 mg)	100	45-55	7.63 (0.18)	-	-	-
	Placebo	100	45-55	7.74 (0.23)			

Footnotes

"-" denotes not reported.

BMI: body mass index; SD: standard deviation; TC: total cholesterol.

Appendix 5. Matrix of study endpoints (publications)

Characteristic Study ID	Primary[a] endpoint(s)	Secondary[b] endpoint(s)	Other[c] endpoint(s)	Time points for outcome measurement
Aubertin-Leheudre 2008	Fasting plasma LDL-C	Adverse events, fasting plasma TC, HDL-C, triglycerides	BMI, body weight, waist circumference, total FM, abdominal FM, visceral FM, REE, DEE, diastolic blood pressure, systolic blood pressure, HDL-C/TC, fasting plasma glucose and insulin, HOMA2-IR	6 months
Dewell 2002	-	Fasting serum TC, triglycerides	-	6 months
Gardner 2001	Fasting plasma LDL-C	Adverse events, fasting plasma TC, HDL-C, triglycerides	Androstenedione, BMI, oestradiol, estrone, FSH	12 weeks
Mackey 2000	Fasting serum LDL-C	Fasting serum TC,HDL-C, triglycerides	Body weight, FSH, LH, TSH, SHBG, osteocalcin, bone-specific alkaline phosphatase	12 weeks
Wang 2005	Fasting blood LDL-C	Fasting blood TC, HDL-C, triglycerides	-	3 months

Footnotes

[a,b] verbatim statement in the publication; [c] not explicitly stated as primary or secondary endpoint(s) in the publication.

"-" denotes not reported.

BMI: body mass index; DEE: daily energy expenditure; FFM: fat-free mass; FM: fat mass; FSH: follicle-stimulating hormone; HDL-C: high-density lipoprotein cholesterol; HOMA2-IR: homeostasis model assessment; LDL-C: low-density lipoprotein cholesterol; LH: luteinizing hormone; REE: resting energy expenditure; SHBG: sex hormone binding globulin; TC: total cholesterol; TSH: thyroid-stimulating hormone.

Appendix 6. Matrix of study endpoints (protocol/trial documents)

Characteristic Study ID	Trial identifier
Aubertin-Leheudre 2008	-
Dewell 2002	-
Gardner 2001	-
Mackey 2000	-
Wang 2005	-

Footnotes

(Continued)
"-" denotes no protocol documents available

Appendix 7. Definition of endpoint measurement

Characteristic Study ID	Cardiovascular events	Health-related quality of life	Costs	Severe/serious adverse events
Aubertin-Leheudre 2008	-	-	-	-
Dewell 2002	-	-	-	-
Gardner 2001	-	-	-	-
Mackey 2000	-	-	-	-
Wang 2005	-	-	-	-

Footnotes

"-" denotes not reported.

Appendix 8. Adverse events (I)

Characteristic Study ID	Intervention and comparator	Deaths [n/N]	All adverse events [n/N]	Severe/serious adverse events [n/N]	Left study due to adverse events [n/N]
Aubertin-Leheudre 2008	Isoflavones (70 mg)	0	0	0	0
	Placebo	0	0	0	0
Dewell 2002	Isoflavones (150 mg)	0	-	-	-
	Maltodextrin with 10% caramel	0	-	-	-
Gardner 2001	Isoflavones (80 mg)	0	2/34 gastrointestinal discomfort (bloating and constipation); an increased number of hot flushes	0	2/34
	Trace amounts of isoflavones	0	1/34	0	1/34

(Continued)

			gastrointestinal discomfort (bloating and constipation)		
Mackey 2000	Isoflavones (65 mg)	0	-	-	-
	Less than 4 mg isoflavones	0	-	-	-
Wang 2005	Isoflavones (158 mg)	0	-	-	-
	Placebo	0	-	-	-

Footnotes

"-" denotes not reported.

Appendix 9. Adverse events (II)

Characteristic Study ID	Intervention and comparator	Hospitalization [n/N]	Outpatient treatment [n/N]	Symptoms [n/N]
Aubertin-Leheudre 2008	Isoflavones (70 mg)	0	0	0
	Placebo	0	0	0
Dewell 2002	Isoflavones (150 mg)	-	-	-
	Maltodextrin with 10% caramel	-	-	-
Gardner 2001	Isoflavones (80 mg)	-	-	-
	Trace amounts of isoflavones	-	-	-
Mackey 2000	Isoflavones (65 mg)	-	-	-
	Less than 4 mg isoflavones	-	-	-
Wang 2005	Isoflavones (158 mg)	-	-	-
	Placebo	-	-	-

Footnotes

"-" denotes not reported.

Appendix 10. Survey of authors providing information on included trials

Characteristic Study ID	Study author contacted	Study author replied	Study author provided data	Comments
Aubertin-Leheudre 2008	Y	Y	Y	Authors provided data of participants with baseline cholesterol levels higher than 5.2 mmol/L; therefore, this trial was included
Dewell 2002	Y	N	N	The study did not provide the email address of the authors. We tried to find the email address by searching other publications by the same authors in PubMed, but the email address was invalid
Gardner 2001	Y	Y	N	We asked the corresponding author for the results of estrone, oestradiol, androstendione and follicle-stimulating hormone measurements of participants at weeks 0 and 12. He replied but provided no data (due to no access to 12-year-old data)
Mackey 2000	Y	N	N	-
Wang 2005	Y	N	N	-

Footnotes

N: no; Y: yes.

CONTRIBUTIONS OF AUTHORS

Yu Qin (YQ): protocol draft, search strategy development, acquiring trial reports, trial selection, data extraction, data analysis, data interpretation, review draft and update draft.

Kai Niu (KN): search strategy development, acquiring trial reports, trial selection, data extraction, data analysis, data interpretation, review draft and update draft.

Yuan Zeng (YZ): acquiring trial reports, trial selection and data extraction.

Peng Liu (PL): acquiring trial reports and data extraction.

Long Yi (LY): acquiring trial reports and data extraction.

Ting Zhang (TZ): data analysis and data interpretation.

Qian Yong Zhang (QYZ): data analysis and data interpretation.

Jun Dong Zhu (JDZ): data analysis and data interpretation.

Man Tian MI (MTM): search strategy development, data analysis, data interpretation and review draft.

DECLARATIONS OF INTEREST

None known.

SOURCES OF SUPPORT

Internal sources

- No sources of support supplied

External sources

- National Natural Science Foundation of China (No. 81072288), China.
- Chongqing Science and Technology Commission, China.

  (No. CSTC, 2011AB5040)

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

Review authors have changed since the published protocol and we added six more review authors (Yuan Zeng, Peng Liu, Long Yi, Ting Zhang, Qian Yong Zhang, Jun Dong Zhu). We changed the contact person to Man Tian Mi.

We added the Chinese BioMedical Database (to September 2012) to the search.

INDEX TERMS

Medical Subject Headings (MeSH)

Hypercholesterolemia [*drug therapy]; Isoflavones [adverse effects] [*therapeutic use]; Randomized Controlled Trials as Topic; Soybean Proteins [*therapeutic use]

MeSH check words

Adult; Aged; Aged, 80 and over; Female; Humans; Middle Aged