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Effects of isoflavones on breast density in pre- and post-menopausal women: a systematic review and meta-analysis of randomized controlled trials

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BACKGROUND: Isoflavones from soy and red clover exert modest hormonal effects in women, but the relevance to risk of breast cancer is unclear. The aim of this meta-analysis was to assess the effects of isoflavone-rich foods or supplements on a biomarker of breast cancer risk, women's mammographic density.

METHODS: Electronic searches were performed on The Cochrane Library, Medline and EMBASE (to June 2009), and reference lists and trial investigators were consulted to identify further studies. Randomized controlled trials (RCTs) of isoflavone-rich foods or supplements versus placebo with a duration of at least 6 months were included in our analysis. Inclusion/exclusion, data extraction and validity assessment were carried out independently in duplicate, and meta-analysis used to pool study results. Subgrouping, sensitivity analysis, assessment of heterogeneity and funnel plots were used to interpret the results.

RESULTS: Eight RCTs (1287 women) compared isoflavones with placebo for between 6 months and 3 years. Meta-analysis suggested no overall effect of dietary isoflavones on breast density in all women combined [mean difference (MD) 0.69%, 95% confidence interval (CI) −0.78 to 2.17] or post-menopausal women (MD −1.10%, 95% CI −3.22 to 1.03). However, there was a modest increase in

mammographic density in premenopausal women (MD 1.83%, 95% CI 0.25–3.40) without heterogeneity but this effect was lost in one of three sensitivity analyses.

CONCLUSIONS: Isoflavone intake does not alter breast density in post-menopausal women, but may cause a small increase in breast density in premenopausal women. Larger, long-term trials are required to determine if these small effects are clinically relevant.

Key words: isoflavone / mammographic density / breast cancer risk / meta-analysis / menopause

Introduction

The structural similarity of plant-derived isoflavones to human 17β estradiol has stimulated significant interest in their importance to women's health (Balk *et al.*, 2005; Messina *et al.*, 2006), with some evidence that dietary isoflavones can influence hormonal levels in pre- and post-menopausal women (Hooper *et al.*, 2009). However, the safety and efficacy of soy-derived isoflavones from different sources has not been fully evaluated, particularly in relation to breast cancer risk.

Increased circulating estradiol concentrations are associated with increased risk of breast cancer in post-menopausal women (Endogenous Hormones Breast Cancer Collaborative Group, 2002), whereas higher soy isoflavone intakes are associated with lower breast cancer risk in epidemiological studies. Cross-cultural studies suggest 3-fold lower rates of breast cancer in Asian populations, where soy consumption is high (Messina *et al.*, 2006; Trock *et al.*, 2006). A recent meta-analysis of cohort and case–control data on soy intake and breast cancer showed soy to be associated with a small reduction in risk (Trock *et al.*, 2006) whereas another found a significant trend of reduced breast cancer risk with increasing soy food intake (Wu *et al.*, 2008). In a large recent analysis of breast cancer patients from the Shanghai Breast Cancer Survival Study, soy food consumption (with average intakes of 47 mg/day of isoflavones) was inversely associated with risk of death [hazard ratio (HR) 0.71, 95% confidence interval (CI) 0.54–0.92 for the highest compared with the lowest quartiles of soy intake] and breast cancer recurrence (HR 0.68, 95% CI 0.54–0.87; Shu *et al.*, 2009). Despite these epidemiological data, results from the available intervention trials have been equivocal. No large, long-term randomized controlled trials (RCTs), powered to assess breast cancer as an outcome, have been published. It remains unclear whether isoflavones from different sources increase or decrease breast cancer risk in humans. A recent meta-analysis of side effects of phytoestrogens observed only 16 cases of breast cancer diagnoses, insufficient to assess effects on this outcome (Tempfer *et al.*, 2009).

The biological effects induced by isoflavones may result in long-term genomic actions mediated by intracellular estrogen-receptor (ER) induced changes in gene expression or as rapid non-genomic actions that modify a wide array of intracellular signal transduction cascades (Setchell and Cassidy, 1999; Losel *et al.*, 2003; Messina, 2007). Available evidence suggest that their ER binding potential may be more important *in vivo* (Losel *et al.*, 2003; Penttinen-Damdimopoulou *et al.*, 2009) since the isoflavone concentrations required to stimulate certain non-genomic activities, such as inhibition of cellular protein tyrosine kinases (>10 μM) and topoisomerase-II typically exceed the plasma levels that can be attained via a habitual dietary intake of soy-rich foods (~2–5 μM; Messina *et al.*, 2009).

There are conflicting experimental data on the effects of isoflavones on breast cancer cells *in vitro* and estrogen-sensitive induced mammary tumours *in vivo* (Hsieh *et al.*, 1998; Allred *et al.*, 2001; Kanno *et al.*, 2003; Messina *et al.*, 2006, 2009; Cline and Wood, 2009; Power *et al.*, 2006a, b, 2007; Saarinen *et al.*, 2009). Isoflavones are not estrogenic in cynomolgus monkeys, even following high isoflavone intake; these reduced estrogen-induced proliferation responses occur via effects on estrogen metabolism, or are mediated through ER interactions (Cline and Wood, 2009). Paradoxically, in rodents and *in vitro* cell models isoflavones induce estrogen-like effects in breast cancer cells lines and cause uterine enlargement in rodents (Wang *et al.*, 1996; Hsieh *et al.*, 1998; Allred *et al.*, 2001; Kanno *et al.*, 2003; Power *et al.*, 2006a, b, 2007; Seo *et al.*, 2006; Saarinen *et al.*, 2009). This mixed estrogen agonist/antagonist activity of isoflavones was recently reported in an estrogen reporter mouse model, where ER signalling was modulated following ingestion of isoflavones (Penttinen-Damdimopoulou *et al.*, 2009); soy isoflavones exerted antiestrogenic effects in the presence of estradiol and estrogenic effects in the absence of estradiol. Rodent studies and epidemiological data suggest that these differential biological effects may be explained by timing of exposure or dose of isoflavones in relation to endogenous estrogens (Hsieh *et al.*, 1998; Lamartiniere *et al.*, 1998, 2002; Lamartiniere, 2000; Wu *et al.*, 2002; Korde *et al.*, 2009). These observed estrogenic effects in animal models have heightened concerns over the potential estrogenic effects of isoflavone consumption in humans.

Although there are currently no reliable biomarkers of breast cancer risk, mammographic density has consistently been one of the best independent biomarkers; moderate to high-density confers a 1.8 to 5-fold risk for developing breast cancer compared with low-density in both pre- and post-menopausal women (McCormack and dos Santos Silva, 2006; Vachon *et al.*, 2007; Cummings *et al.*, 2009). Despite the evidence that it is an established risk factor for breast cancer development, there remains some uncertainty about the use of mammographic density as an intermediate marker of risk for the disease (Becker and Kaaks, 2009). Available data suggest that several hormonal and dietary factors can modify breast density; density increases with estrogen and progestin therapy and decreases following exposure to tamoxifen or ovarian suppression (Boyd *et al.*, 1997, 2001; Greendale *et al.*, 2003).

Given the current controversies and complex relationship between isoflavone intake and breast cancer risk and the lack of RCTs on the effects of isoflavones on breast cancer incidence, we conducted a systematic review and meta-analysis of the available randomized controlled studies of isoflavones using mammographic breast density as a surrogate marker. The primary objective was to assess the effect of isoflavone-rich foods and isoflavone extracts from soy and red clover on breast density in women. Secondary

objectives included understanding effects in pre- and post-menopausal women, by baseline breast cancer risk, by duration of exposure and by source and dose of isoflavone. No protocol for this review has been published.

Methods

Search and inclusion criteria

An electronic search was performed on The Cochrane Library, Medline and EMBASE (from inception of each database until June 2009). The search was in the format: [isoflavones or soy or red clover] and [RCT filter] and [breast density or breast cancer]. The search was not limited to English language publications but no relevant non-English publications were located. In addition to the electronic searches the reference lists of included studies and relevant related systematic reviews were checked for further trials. Attempts were made to contact authors of all included studies for information about ongoing trials, as well as further details on their published studies.

Included studies were randomized controlled parallel arm trials of at least 6 months duration which compared increased intake of traditional soy foods, isoflavone-rich soy products or isolated isoflavones from soy or red clover compared with usual or control/placebo diet. Included participants were women of any age, at any baseline risk of breast cancer, with or without a history of breast cancer and outcomes included breast density.

All potential titles and abstracts were assessed for their relevance by two independent reviewers. Potentially relevant papers were collected in full text for further assessment of inclusion. One reviewer initially excluded studies that were clearly not relevant (such as *in vitro* or case–control studies) then the remainder were assessed independently for inclusion by two reviewers using an inclusion/exclusion form designed for the review. Reviewers met to discuss differences in data extraction, all differences were decided by discussion.

Data collection and assessment of validity

Data extraction and validity assessment were carried out together onto a data extraction form developed for the review. Extracted data included bibliographic details, participants' characteristics (menopausal status, mean age, baseline cancer risk, country), type of intervention (source, isoflavone dose, type of placebo, compliance), duration of intervention, numbers of participants randomized to and completing each study arm, method used to assess breast density, side effects and breast cancer diagnoses. In addition, details of the number of participants, mean breast density change (absolute change or as change per year) and variance of that change (or end breast density and its variance where change data were not available) were collected for each arm of each included study at the latest time point available. Baseline risk of breast cancer was defined as follows: high risk included participants with family history of breast cancer, presence of genetic risk markers or high risk according to the Gail model, the standard tool for assessing a woman's future risk for breast cancer (Gail *et al.*, 1989), high mammographic density or Wolfe parenchymal pattern [based on patterns of ducts, nodularity and densities seen on mammography (Wolfe, 1976)]; moderate risk was participants with a history of any type of cancer or with first degree relatives with breast cancer; low risk was all other participants (Vachon *et al.*, 2007). Where more than one method was used to asses breast density, as in the study by Atkinson (Atkinson *et al.*, 2004; Kataoka *et al.*, 2008), data for the main analysis were used from the semi-automated assessment, rather than the entirely visual or entirely automated assessments, as semi-automated assessment appeared to be most commonly used through the studies, although the visual and fully automated data were also used in subgroup analyses. Data for the main analysis were taken from the latest follow-up in each trial on the basis that any effect of isoflavones are likely to be cumulative and so any differences are more likely to be observed after a longer duration. Where studies did not provide data on the change in breast density from baseline to study end, but only data on mean breast density at study end, the end data were used within the meta-analysis (as is considered appropriate in the Higgins and Green, 2008a), to make best use of the available data. Authors were contacted for further data or clarification where questions arose.

Validity assessment of included studies was based on allocation concealment, masking of participants, masking of outcome assessors, industry funding or involvement, whether compliance with the intervention was measured and reported, whether isoflavone doses in intervention and control arms were reported, and whether dropouts were clearly reported. These characteristics [based on the method used by the Higgins and Green (2008a)] were:

(i) Allocation concealment (concealment of the ability of those recruiting participants to assess which arm participants will be randomized into before recruitment is complete, coded as adequate, unclear or inadequate);
(ii) participant blinding (concealment of the participants to whether they are part of the intervention or control condition, coded as yes, unclear or no);
(iii) outcome assessor blinding (concealment of the outcome assessor, here the reader of the mammogram, to whether participants are part of the intervention or control condition, coded as yes, unclear or no);
(iv) industry funding or involvement [level of financial involvement of industries that may have a financial interest in the study results, coded as yes (study mainly funded by industry, or at least one author is employed by industry), partly (other impartial funding, but includes some industry funding that may include free provision of supplements) or no];
(v) compliance assessed and reported [measurement of the degree to which participants complied with taking the intervention and control foods or supplements, and reporting of these data, coded as yes (both reported), partly (one or the other) or no]; and
(vi) reporting of withdrawals (numbers of withdrawals in each group clear and reasons reported coded as done, partial and not done).

Statistical analysis

Characteristics of included studies and study validity were tabulated (Tables I and II). Differences in percentage mammographic density between the isoflavone-rich intervention and control periods were combined across studies using mean differences (MD) using a random effects model in Review Manager 5.0 software (2008).

Where a study provided data in several different ways the data were dealt with as follows for the main analysis: the data used were from all available participants (so that if there were two intervention groups and one control group the data from the two intervention groups were combined using the methods recommended in the Cochrane Handbook); from the longest duration available; and using the semi-automated system of assessment of breast density. In subgroup analyses, we used all available data; the Powles study (Powles *et al.*, 2008) which provided data at 1, 2 and 3 years provided data into each of the three subgroups; the Atkinson study (Atkinson *et al.*, 2004; Kataoka *et al.*, 2008) which used three methods of assessment of breast density provided data into all three assessment type subgroups; and the OPUS study (Maskarinec *et al.*, 2009) which provided two doses of isoflavones provided data to two dose subgroups, with the full control group used twice, so subgroups were not pooled.

Table 1 Characteristics of included studies.

Study	Participants	Interventions	Outcomes
Atkinson 2004, UK (Atkinson *et al.*, 2004; Kataoka *et al.*, 2008)	Participants: pre-, post- and peri- menopausal women without breast cancer but with Wolfe P2 or DY mammographic patterns Randomized: int 102, cont 103 Analysed: int 76, cont 84 Mean age (sd): int 55.1 (4.7), cont 55.2 (4.9) Baseline risk: high	Form: tablet Intervention: 1 tablet/day, Promensil (isolated isoflavones from red clover) versus 1 tablet/day placebo Total isoflavone dose: int ~44 mg/day, including 26 mg/day biochanin A, 16 mg/day formonetin, 1 mg/day genistein, 0.5 mg/day daidzein, cont unclear (nil?)	Duration: 52 weeks Method of assessment: three methods used: visual assessment by two independent radiologists, computer-assisted using Cumulus and fully automated using SMF—data on Cumulus used in our analysis (% density)
Marini 2008, Italy (Marini *et al.*, 2007, 2008; D'Anna *et al.*, 2009; Atteritano *et al.*, 2009)	Participants: post-menopausal osteopenic women aged 49–67 Randomized: int 198, cont 191 Analyzed: at 2 years int 150, cont 154; At 3 years int 71, cont 67 Mean age (sd): int 53.8 (2.9), cont 53.5 (2.0) Baseline risk: low	Form: tablets Intervention: 2 tablets/day purified genistein (isolated aglycone genistein) versus 2 tablets/day placebo Total isoflavone dose: int 54 mg/day genistein (aglycone units), cont nil	Duration: 156 weeks Method of assessment: computer-assisted assessment (IMI)
Maskarinec 2002, USA (Maskarinec *et al.*, 2002, 2003)	Participants: premenopausal women aged 35–46 with recent normal mammogram Randomized: int 17, cont 17 Analyzed: unclear, int 15, cont 15?? Mean age: int 41.1 (3.1), cont 43.3 (1.7) Baseline risk: low	Form: tablet Intervention: 2 × 50 mg tablets/day soy extract (isolated isoflavones) versus placebo (maltodextrin) Total isoflavone dose: int 76 mg/day (aglycone equivalents, 100 mg isoflavones) of total isoflavones, including 38.8 mg/day daidzein, 33.4 mg/day genistein, 3.8 mg/day glyceitin, cont unclear (nil?)	Duration: 52 weeks Method of assessment: computer-assisted assessment (% density)
Maskarinec 2004a,b, USA (Maskarinec *et al.*, 2004a, b)	Participants: premenopausal women with clear mammograms Randomized: int 109, cont 111 Analyzed: int 98, cont 103 Mean age (sd): int 43.2 (3.1), cont 42.8 (2.9) Baseline risk: low	Form: soy foods Intervention: 2 servings/day soy foods versus usual diet Total isoflavone dose: int ~50 mg/day soy isoflavones (aglycone equivalents), cont unclear (usual diet)	Duration: 104 weeks Method of assessment: computer-assisted assessment (% density)

Maskarinec 2009 (OPUS), USA (Maskarinec *et al.*, 2009)	Participants: early post-menopausal women aged 40–60 Randomized: int A 136, int B 135, cont 135 Analyzed (2 yrs): int A 109, int B 100, cont 116 Mean age (sd): int A 54.7 (3.8), int B 55.2 (4.0), cont 54.8 (3.6) Baseline risk: low	Form: tablets Intervention: 80 or 120 mg/day isoflavones from soy germ (isolated isoflavones) versus placebo tablets Total isoflavone dose: int A 120 mg/day isoflavones (aglycone equivalents), including 1.2 mg/day genistein, 15.6 mg/day genistin, 2.4 mg/day daidzein, 50.4 mg/day daidzein, 46.8 mg/day glyceitin, 3.6 mg/day glyceitein, int B 80 mg/day isoflavones, 2/3 content int A for subfractions, cont unclear	Duration: 104 weeks Method of assessment: computer-assisted assessment by one assessor (% density)
Powles 2008, UK (Powles *et al.*, 2008)	Participants: pre-, post-, and peri-menopausal women aged 35–69 with a first degree relative with breast cancer Randomized: int 199, cont 202 Analyzed: int 119 (111 pre-, 8 post-menopausal), cont 112 (111 pre- and 11 post-menopausal) Mean age: mean unclear, medians: int 45, cont 45 Baseline risk: moderate	Form: tablet Intervention: 1/day red clover isolate tablet, Promensil (isolated isoflavones) versus placebo tablet Total isoflavone dose: 40 mg/day total isoflavones	Duration: 156 weeks Method of assessment: visual assessment (% density)
Tice 2009 (PREVENT), USA (Tice *et al.*, 2005; Tice, 2006)	Participants: otherwise healthy premenopausal women with breast density ≥50% Randomized: int 24, cont 23 Analyzed: int 20, cont 20 Mean age (sd): int 44.8 (sd unclear), cont 44.6 (sd unclear) Baseline risk: moderate	Form: protein powder (taken in smoothies or added to other foods and drinks) Intervention: 25 g/day soy protein (ISP) versus 25 g/day milk protein Total isoflavone dose: int 50 mg/day isoflavones (aglycone equivalents), cont 0 mg/day isoflavones	Duration: 26 weeks Method of assessment: computer-assisted threshold method, using craniocaudal view (% density)
Verheus 2008 (Finesse) Netherlands (Kok *et al.*, 2004, 2005a, b; Kreijkamp-Kaspers *et al.*, 2005, 2009; Verheus *et al.*, 2009)	Participants: healthy post-menopausal women aged 60–75 with a recent normal mammogram Randomized: int 100, cont 102 Analyzed: int 70, cont 56 Mean age (sd): int 66.3 (4.3), cont 65.3 (4.0) Baseline risk: low	Form: powder to be mixed with food or drink Intervention: 25.6 g/day Solae soy protein (ISP) versus 25.6 g/day milk protein Total isoflavone dose: int 99 mg/day isoflavones (aglycone units), including 52 mg/day genistein, 41 mg/day daidzein, 6 mg/day glyceitin, cont nil	Duration: 52 weeks Method of assessment: computer-assisted assessment (% density)

ISP, isolated soy protein; Cont, control group; Int, intervention group; Isoflav, isoflavone/s; FP, food provided; DO, dropouts; sd, standard deviation; ISP, isolated soy protein; % density, percent density; SMF, Standard Mammogram Form, which is a fully automated volumetric computer method.

Table II **Validity of included studies.***

Study	Allocation concealment	Masking of participants	Masking of outcome assessors	Industry funding or involvement	Compliance assessed and reported	Reporting of withdrawals
Atkinson 2004	Yes	Yes	Yes	Partly (some funding from non-industry sources, some from Novogen Ltd)	Done, urinary isoflavones	Partial
Marini 2008	Unclear	Yes	Yes	Partly (some funding from non-industry sources, some from Primus Pharmaceuticals, two authors work for Primus Pharmaceuticals)	Done, serum genistein	Done
Maskarinec 2002	Unclear	Yes	Yes	Yes, funding from Pharmavite Corporation	Done, urinary isoflavones and tablet count	Done
Maskarinec 2004	Unclear	No	Yes	Partly (funding from non-industry source, food donations from Aloha Tofu, Dr Soy, Solae Company)	Done, urinary isoflavones, intake logs, 24 h recalls	Partial
Maskarinec 2009 OPUS	Unclear	Yes	Yes	Unclear (funded by NIH but unclear if study paid for the tablets or not)	Partly done, blood isoflavones and pill counts, results not reported	Done
Powles 2008	Unclear	Yes	Unclear	Partly (funding from non-industry source, supplement supplied by Novogen Ltd)	Not done	Partial
Tice 2009 (PREVENT)	Yes	Yes	Yes	None (US Army)	Done, packet count at 6 mo.	Done
Verheus 2008 (Finesse)	Yes	Yes	Yes	Partly (some funding from non-industry sources, some from DuPont Protein Technologies)	Done, serum genistein, counts of powder bags, diary	Done

*Trial quality characteristics assessed included: (i) allocation concealment (concealment of the ability of those recruiting participants to assess which arm participants will be randomized into before recruitment is complete, coded as adequate, unclear or inadequate); (ii) participant masking (concealment of the participants to whether they are part of the intervention or control condition, coded as 'yes' where there was a clear and realistic attempt to mask, 'no' where not, or 'unclear'); (iii) outcome assessor blinding (concealment of the outcome assessor, here the reader of the mammogram, to whether participants are part of the intervention or control condition, coded as 'yes' where there was a clear and realistic attempt to mask, 'no' where not, or 'unclear'); (iv) industry funding or involvement [level of financial involvement of industries that may have a financial interest in the study results, coded as yes (study mainly funded by industry, or at least one author is employed by industry), partly (other impartial funding, but includes some industry funding that may include free provision of supplements) or no]; (iv) compliance assessed and reported (measurement of the degree to which participants complied with taking the intervention and control foods or supplements, and reporting of these data, coded as 'done' when compliance was both assessed and reported, 'partly done' when it was assessed but not reported or reported without any indication of the method used, and 'not done' when neither was addressed adequately); and (v) reporting of withdrawals [numbers of withdrawals in each group clear and reasons reported coded as done, reported as 'done' when numbers randomized, completed and analysed all clear, plus reasons for dropouts given (by intervention arm), 'partially done' when some of the above, 'not done' when not].

The main analysis included all included trials with relevant outcome data, but data on no participants were included twice. Subgrouping was used to explore the effects of the following factors on breast density:

(i) source of isoflavones (soy-based foods, soy protein, soy germ, isoflavones isolated from soy, isoflavones isolated from red clover, pure genistein, pure daidzein);
(ii) participants baseline risk of breast cancer (high, moderate or low);
(iii) isoflavone dose (in aglycone equivalents, <50, 50 to <100, 100+ mg/day)
(iv) duration of intervention (6 to <18, 18 to <30 and 30+ months); and
(v) method of assessment of breast density (visual assessment, semi-automated and fully automated assessment).

It was intended that we would also subgroup by participants who were equol producers compared with those who were non-equol producers, however, there were insufficient data on breast density by equol producer status for this to be feasible. The equol producer phenotype is thought to be important as levels of this gut metabolite of the soy isoflavone daidzein have been inversely associated with breast cancer risk. In humans only 30–50% of the population harbour the bacteria capable of converting daidzein to equol, and this metabolite has biological activities that differ from its parent compound, e.g. relative binding affinity to estrogen receptors (Cassidy *et al*., 1994; Duncan *et al*., 1999; Lampe, 2009).

Sensitivity analysis was used to assess robustness of results. Sensitivity analyses were carried out to:

(i) include all breast density measures using standardized mean difference analysis [which allowed the Marini data (Marini *et al*., 2008), not measured using percentage breast density but using image mean index (IMI) which cannot be translated into percent breast density, to be included],
(ii) exclude studies wholly funded by industry (Lexchin *et al*., 2003), and
(iii) use the *P*-values provided for the Powles study ((Powles *et al*., 2008), as the 95% CIs did not appear to correlate with the *P*-values provided in the same table. As we were not able to initiate discussion with the authors, both the CIs and the *P*-values were used to compute standard deviations used in the analyses (2008a), the *P*-value data were used as a sensitivity analysis).

Type and frequency of side effects, diagnosis of breast cancer, deaths and study withdrawals were tabulated and compared between different studies. Heterogeneity was assessed using Cochran's test and the I^2 test (and assumed to be present when $I^2 > 50\%$; Higgins *et al*., 2003). Funnel plots were used to assess for evidence of publication bias (Egger *et al*., 1997). This manuscript follows the criteria suggested for systematic reviews in the PRISMA guidelines (Moher *et al*., 2009).

Results

Description of included studies

A total of 678 titles and abstracts were screened following the electronic and bibliographic searches. Of these 74 appeared potentially relevant and were ordered as full text papers to be assessed for inclusion independently in duplicate. Eight of these RCTs (published in 18 full text papers, one unpublished manuscript and an abstract) were included in the review (Fig. 1).

The eight included studies randomized 1904 women between them, and analysed data on 1287 after study durations of between 6 months and 3 years (Table 1). Five of the studies included postmenopausal women, and five included premenopausal women (two studies included both groups). The baseline risk of breast cancer was low in most studies, moderate in one (including pre-, post- and peri-menopausal women with a first degree relative with breast cancer (Powles *et al*., 2008), and high in two studies (including premenopausal women with a Gail risk of at least 1.67 and >50% breast density at initial mammogram (Tice, 2006), and pre-, post- and perimenopausal women with Wolfe P2 or DY mammographic patterns but without breast cancer (Atkinson *et al*., 2004). Four studies were conducted in Europe, four in the USA.

Two studies provided red clover-based isoflavone supplements, three soy-based isoflavone supplements (one pure genistein, one soy germ based isoflavones and one mixed soy isoflavones), one provided additional soy foods and two provided soy protein powder compared with milk protein powder. Isoflavone doses ranged from 40 to 120 mg/d. Control groups received other 'inert' or ill-defined substances low in isoflavones, advice to eat their usual diet or milk protein in place of soy protein.

All studies calculated mammographic percentage density except one (Marini *et al*., 2008) which calculated IMI, in arbitrary units. This outcome was assessed visually in one study, using a computer-assisted method in six and assessed using three methods (a visual method, a computer-assisted method and a fully computerized method) in one study.

All included studies were fully published in at least one journal article, except for the study by Tice which was informally published (Tice *et al*., 2005; Tice, 2006). The unpublished study by Tice was a double blinded, randomized trial of 6 months of daily soy protein containing 50 mg of isoflavones with a primary outcome of change in percentage mammographic breast density timed to the menopause cycle. For this trial data on the study were extracted from the study protocol and final report to the funders (Tice, 2006). As with other included studies the author was asked for further information.

Risk of bias

Allocation concealment was unclear in all studies except those by Atkinson, Verheus and Tice (Atkinson *et al*., 2004; Tice, 2006; Verheus *et al*., 2009), where it was clearly concealed. Attempts were made to mask participants in all studies except one (Maskarinec *et al*., 2004a, b), which was a food-based intervention. Masking of outcome assessors was attempted in all of the studies, except that by Powles *et al*. (2008) where masking of outcome assessors was unclear. Most studies were funded jointly by industry and non-industry sources, except for one of the Maskarinec trials (Maskarinec *et al*., 2003) which was funded solely by industry, the Tice study (Tice, 2006) which was exclusively funded by non-industry sources and OPUS (Maskarinec *et al*., 2009) which was unclear (though appeared to be mainly funded by non-industry sources). Most studies assessed compliance with the intervention and placebo, and reported results of this assessment, except that OPUS (Maskarinec *et al*., 2009) reported the method of assessment but not any results, and the Powles study (Powles *et al*., 2008) did not report either a methodology for assessing compliance or any results. Reporting of withdrawals was done for five studies and partially done for three (Atkinson *et al*., 2004; Maskarinec *et al*., 2004a, b; Powles *et al*., 2008).

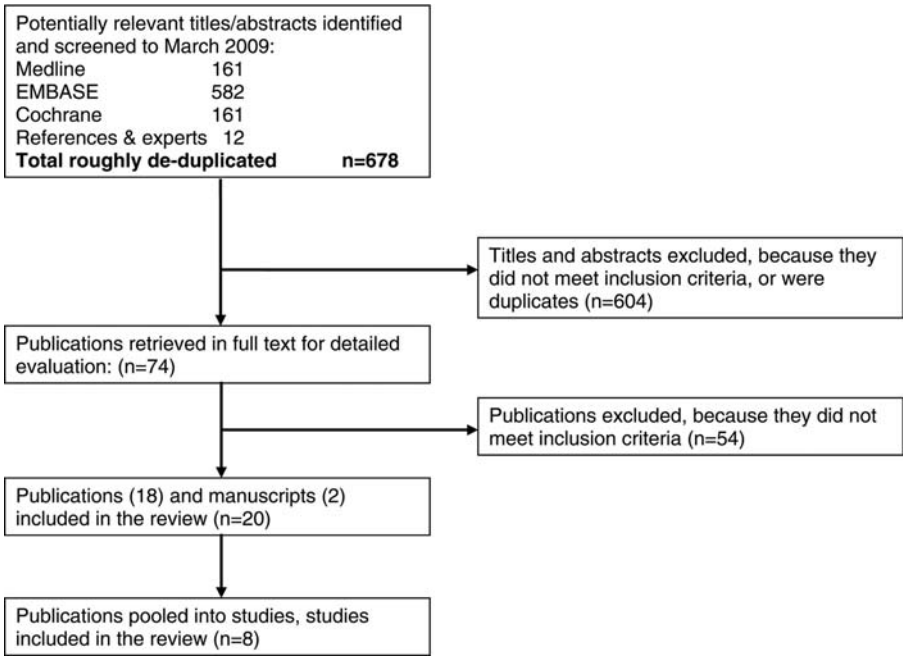

Figure 1 Flowchart illustrating movement of papers from search to inclusion.

Effect of isoflavones on breast density

There was no evidence of an overall effect of isoflavones on percentage breast density from seven studies including 1149 participants for at least 6 months each (mean difference 0.69%, 95% CI −0.78 to 2.17) and no evidence of heterogeneity [Fig. 2; this analysis excluded the eighth study, that did not use percentage breast density as a measure (Marini *et al*., 2008)]. Sensitivity analyses including the Marini study [the study assessing breast density using a different measure (Marini *et al*., 2008)], or excluding studies wholly funded by industry, or using Powles *P*-value data [(Powles *et al*., 2008) rather than the CIs presented] similarly did not suggest any overall effect (Table III).

Analysis subgrouping by menopausal status resulted in an analysis that included slightly fewer women than were included in the overall analysis as some women from the Atkinson study had not been clearly allocated to a particular menopausal status. In premenopausal women isoflavone intake resulted in a modest increase in mammographic percent density compared with controls (mean difference 1.83%, 95% CI 0.25–3.40, $n = 519$, 5 trials) with no evidence of heterogeneity ($P = 0.85$, $I^2 = 0\%$; Fig. 3). In contrast, in post-menopausal women, isoflavone intervention had no effect on breast density (mean difference −1.10%, 95% CI −3.22 to 1.03, $n = 592$, 4 trials) and there was no evidence of heterogeneity ($P = 0.23$, $I^2 = 30\%$). There was no significant effect in the few peri-menopausal women (mean difference −0.37%, 95% CI −6.12 to 5.38, $n = 16$, 1 trial).

Sensitivity analyses, using the *P*-value data from the Powles study (Powles *et al*., 2008), rather than the CI data (as these did not correspond, see the statistical analysis section of the Methodology above), resulted in marginal loss of statistical significance in this subgroup ($P = 0.05$). Including, or not, the Marini study data [(Marini *et al*., 2008), not expressed as breast density] did not alter these findings (the effects in premenopausal women were still statistically significant, see Table III). Removal of studies with some industry funding would remove almost all studies, but removal of the one study which was solely industry funded (Maskarinec *et al*., 2002) did not result in loss of the statistically significant effect of isoflavones on breast density in premenopausal women.

Subgrouping by duration suggested that there may possibly be an increase in breast density compared with controls in the long-term trials (3 years) although the significance was only marginal (mean difference 3.22%, 95% CI −0.18 to 6.63, $P = 0.06$, 2 trials including 241 participants, with no evidence of heterogeneity; Fig. 4).

From the available evidence, there was little suggestion of differential effects of isoflavone source, isoflavone dose, baseline risk of breast cancer or type of assessment technique for breast density (Table III). The funnel plot was ineffective in assessing whether there was a risk of publication bias as most studies were of a similar size, and so the plot is difficult to interpret (Fig. 5).

Effect of isoflavones on breast cancer, dropouts and adverse events

As anticipated there were too few cases of breast cancer or deaths reported to draw conclusions on the effects of isoflavones on these outcomes (Table IV).

Gastrointestinal complaints were commonly reported side effects, and meta-analysis of studies reporting dropouts due to gastrointestinal problems did not suggest a statistically significant difference between intervention and control arms (RR 1.49, 95% CI 0.69–3.24, 4 studies, 870 participants). However, there was strong heterogeneity between studies (*P* 0.07, I^2 63%), which will have been accommodated

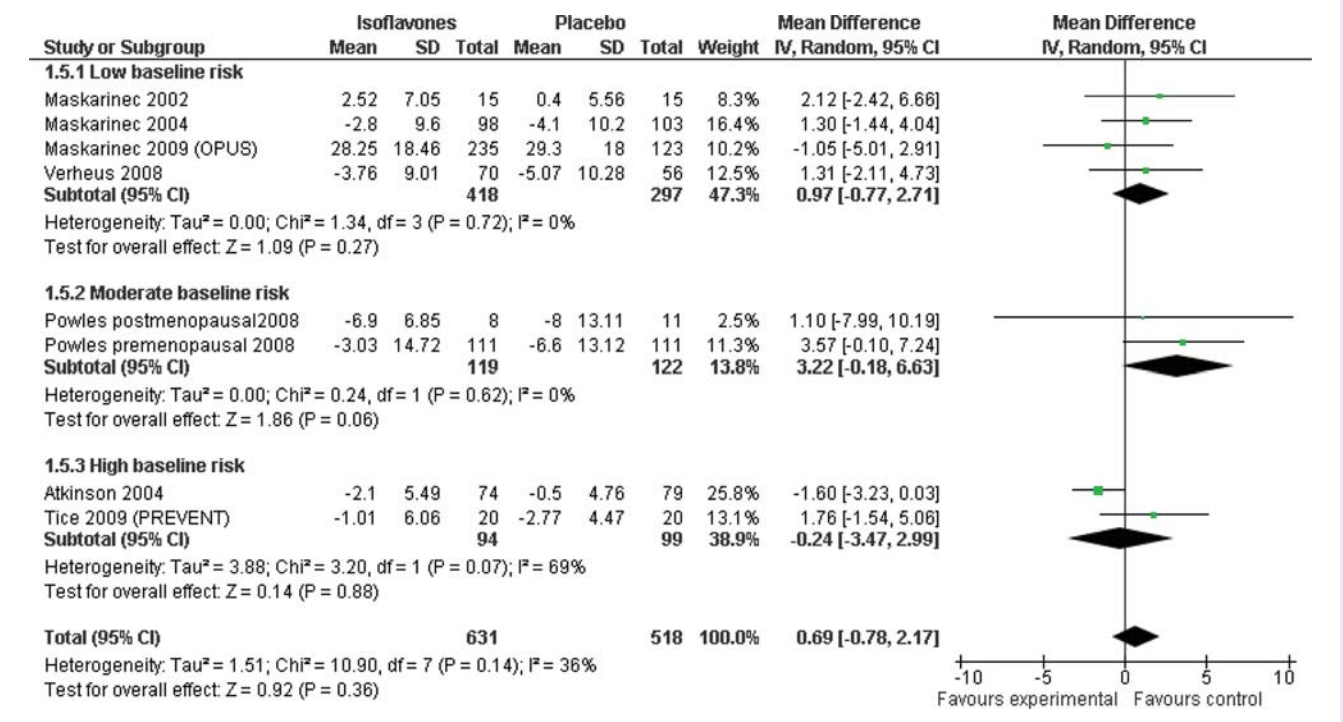

Figure 2 Main analysis, subgrouping by baseline risk of breast cancer, using only percentage breast density data (mean difference analysis).

through use of random effects meta-analysis. Individually, one study suggested significantly greater numbers of dropouts due to gastrointestinal problems in the intervention group (Marini *et al*., 2008), although the others suggested no significant differences (Maskarinec *et al*., 2004a, b; Powles *et al*., 2008; Verheus *et al*., 2009).

Other side effects were reported by Powles *et al.* (2008) (they noted breast abnormalities, weight gain, skin problems and lethargy, of which only weight gain appeared to be different between intervention and control arms—those on the intervention were less likely to report weight gain than those in the control group). Weight gain caused one participant to drop out of the control group of the Tice study (Tice, 2006), but did not affect other participants.

There was no significant difference in dropouts due to any cause between intervention and control arms (RR 1.14, 95% CI 0.96–1.35, 7 studies, 1870 participants, no significant heterogeneity).

Discussion

We included eight RCTs of isoflavones versus placebo or control diet with a duration of at least 6 months that assessed effects on breast density in women. These analyses included 1287 participants included in dietary intervention trials for between 6 months and 3 years. Although the available studies individually suggest that there is no effect overall on breast density following dietary isoflavone intervention, data from this meta-analysis provide evidence of a modest increase in breast density in premenopausal women taking isoflavones compared with control, and the effect may be greater in longer studies. These effects were small (a 1.83% increase in breast density compared with controls for premenopausal women) and although the clinical relevance of this potential relationship merits further investigation there are no immediate implications for practice.

In contrast, no significant effect was observed in post-menopausal women and to date only one trial has conducted analysis on a small number of peri-menopausal women (Atkinson *et al*., 2004; Kataoka *et al*., 2008). There were insufficient data to directly assess effects of isoflavones on breast cancer or mortality.

The doses of isoflavone fed in the trials ranged from 40 to 120 mg/day (aglycone equivalents). However, across this wide range in intake, there was little suggestion of differential effects by dose. The source of isoflavone did not significantly alter the observed effect.

The method used to assess breast density varied across the trials and this may affect the relative readings of breast densities in different groups. Kataoka assessed breast density using three separate techniques and found that the methods were not equivalent, confirming previous studies (Kataoka *et al*., 2008; Yaffe, 2008). We used the semi-automated assessment data from Atkinson and Kataoka within the main analyses (as this was the most common type of assessment across the different studies—this was chosen by a researcher blinded to the outcome measures), however, these suggest a larger effect of isoflavones on breast density than either of the other methods. Development of more robust methodology would be helpful in allowing assessment of effects in future studies (Yaffe, 2008).

Other dietary components and hormonal factors have previously been shown to modify breast density (Boyd *et al*., 1997, 2001; Greendale *et al*., 2003) and our previous systematic review of isoflavone and hormonal status suggested modest effects of isoflavones on gonadotrophins and estradiol in women (Hooper *et al*., 2009). However, to our knowledge this is the first systematic review of

Table III Subgrouping and sensitivity analyses.

Factor	Subgroup	Number of studies	Number of participants	Mean difference (95% CI)	*P*-value for heterogeneity, I^2
Main analysis	Overall analysis (no subgroups)	7	1149	0.69 (−0.78 to 2.17)	0.14, 36%
Menopausal status*	Premenopausal	5	519	1.83 (0.25 to 3.40)	0.85, 0%
	Post-menopausal	4	592	−1.10 (−3.22 to 1.03)	0.23, 30%
	Peri-menopausal	1	16	−0.37 (−6.12 to 5.38)	NR
Baseline risk of breast cancer	Low risk	4	715	0.97 (−0.77 to 2.71)	0.72, 0%
	Moderate risk	2	241	3.22 (−0.18 to 6.63)	0.62, 0%
	High risk	2	194	−0.24 (−3.47 to 2.99)	0.07, 69%
Isoflavone source	Isolated red clover isoflavones	3	394	0.70 (−3.37 to 4.76)	0.04, 69%
	Isolated soy isoflavones	1	30	2.12 (−2.42 to 6.66)	NR
	Soy foods	2	559	0.54 (−1.71 to 2.79)	0.34, 0%
	Soy protein	2	166	1.54 (−0.83 to 3.92)	0.85, 0%
	Any soy intervention	5	755	1.14 (−0.40 to 2.68)	0.82, 0%
Dose, mg/day total isoflavones**	<50	3	394	0.70 (−3.37 to 4.76)	0.04, 69%
	50 to <100	5	635	0.90 (−0.74 to 2.54)	0.36, 8%
	100+	1	243	1.30 (−3.38 to 5.98)	NR
Duration**	6 to <18 months	7	1014	−0.20 (−1.29 to 0.88)	0.33, 13%
	18 to <30 months	4	846	0.08 (−1.34 to 1.51)	0.65, 0%
	30+ months	2	241	3.22 (−0.18 to 6.63)	0.62, 0%
Type of density assessment**	Visual assessment	1	160	0.70 (−2.95 to 4.35)	NR
	Semi-automated	7	1023	0.67 (−1.01 to 2.36)	0.11, 43%
	Fully automated	2	277	−0.22 (−1.57 to 1.13)	0.34, 0%
Sensitivity analyses					
SMD analysis including all breast density measures	Overall analysis	8	1287	0.06 (−0.09 to 0.21)	0.09, 39%
	Premenopausal	4	479	0.21 (0.04 to 0.38)	0.95, 0%
	Post-menopausal	5	730	−0.07 (−0.33 to 0.18)	0.05, 57%
	Peri-menopausal	1	16	−0.07 (−1.05 to 0.92)	NR
Excluding studies fully funded by industry	Overall analysis	7	1119	0.60 (−1.00 to 2.19)	0.12, 41%
	Premenopausal	4	489	1.79 (0.11 to 3.47)	0.72, 0%
	Post-menopausal	5	730	−0.07 (−0.33 to 0.18)	0.05, 57%
	Peri-menopausal	1	16	−0.07 (−1.05 to 0.92)	NR
Using Powles *P*-value data	Overall analysis (no subgroups)	7	1149	0.43 (−0.91 to 1.77)	0.24, 23%
	Premenopausal	5	519	1.63 (−0.03 to 3.30)	0.94, 0%
	Post-menopausal	4	592	−0.89 (−3.04 to 1.26)	0.18, 38%
	Peri-menopausal	1	16	−0.37 (−6.12 to 5.38)	NR

CI, confidence intervals; NR, Not relevant (assessment of heterogeneity is not relevant when only one study is included).
*As the menopausal status of some of the Atkinson study participants was not known the total numbers of participants are smaller when subgrouped by menopausal status than in the overall analysis.
**In some studies data were measured at more than one time point or using more than one technique, so numbers do not add up to total numbers of study participants. One study used two different dose levels and as these arms fell in separate dose subgroupings the full control group was used once in each subgroup, increasing the apparent number of participants.

the available RCTs examining the effects of isoflavones on mammographic density.

In epidemiological settings, studies in high soy-consuming Asian populations suggest a reduction in breast cancer risk with increased habitual intake of soy; risk was lowest in those consuming ≥20 mg/day although the data were largely based on case–control studies and an inverse association was observed in both pre- and post-menopausal women (Wu *et al.*, 2008). Recent data from Chinese breast cancer patients, where the range in soy intake is large enough to provide heterogeneity in isoflavone intake, showed that soy food consumption was significantly associated with decreased risk of death and recurrence and a linear dose–response effect was observed up to an isoflavone intake of 40 mg/day (Shu *et al.*, 2009). However, these protective effects stem from consumption of traditional soy food and there is currently no epidemiological evidence for beneficial effects of dietary supplements derived from soy or red clover, such as were tested in many of our included studies.

The beneficial effects seen in observational studies may relate to lifetime or early life exposure to soy isoflavones, and the strongest and most consistent effect is related to childhood soy intake (Wu *et al.*, 2002; Korde *et al.*, 2009). Isoflavones may exert their potential protective effects early in life by stimulating breast cell differentiation (Lamartiniere, 2000; Lamartiniere *et al.*, 2002) but these positive epidemiological data are in contrast to the conflicting experimental data from *in vitro* models and studies in animal models including the ovarectomized athymic nude mouse model implanted with MCF-7 cells (an estrogen-sensitive breast cancer cell line; Wang *et al.*, 1996; Hsieh *et al.*, 1998; Allred *et al.*, 2001, 2004a, b; Ju *et al.*, 2001; Kanno *et al.*, 2003; Power

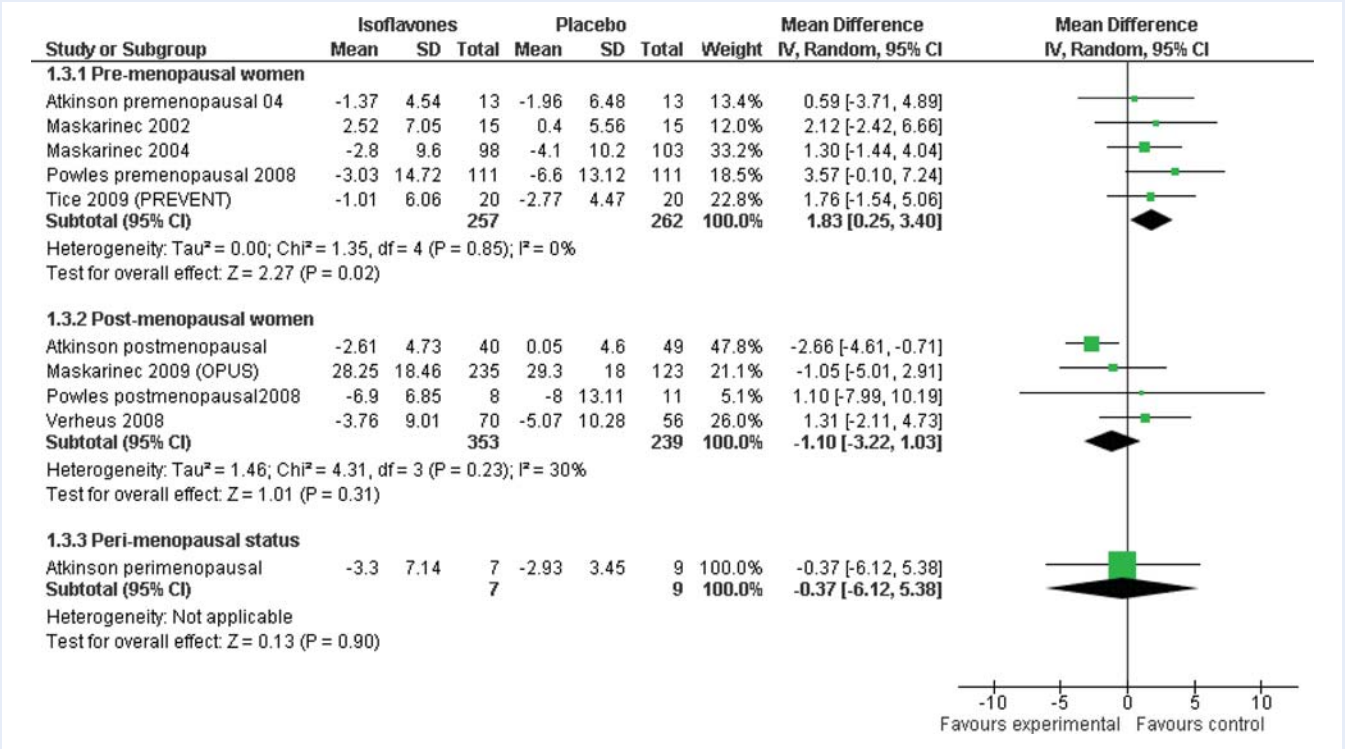

Figure 3 Subgrouping by menopausal status, using only percentage breast density data (mean difference analysis).

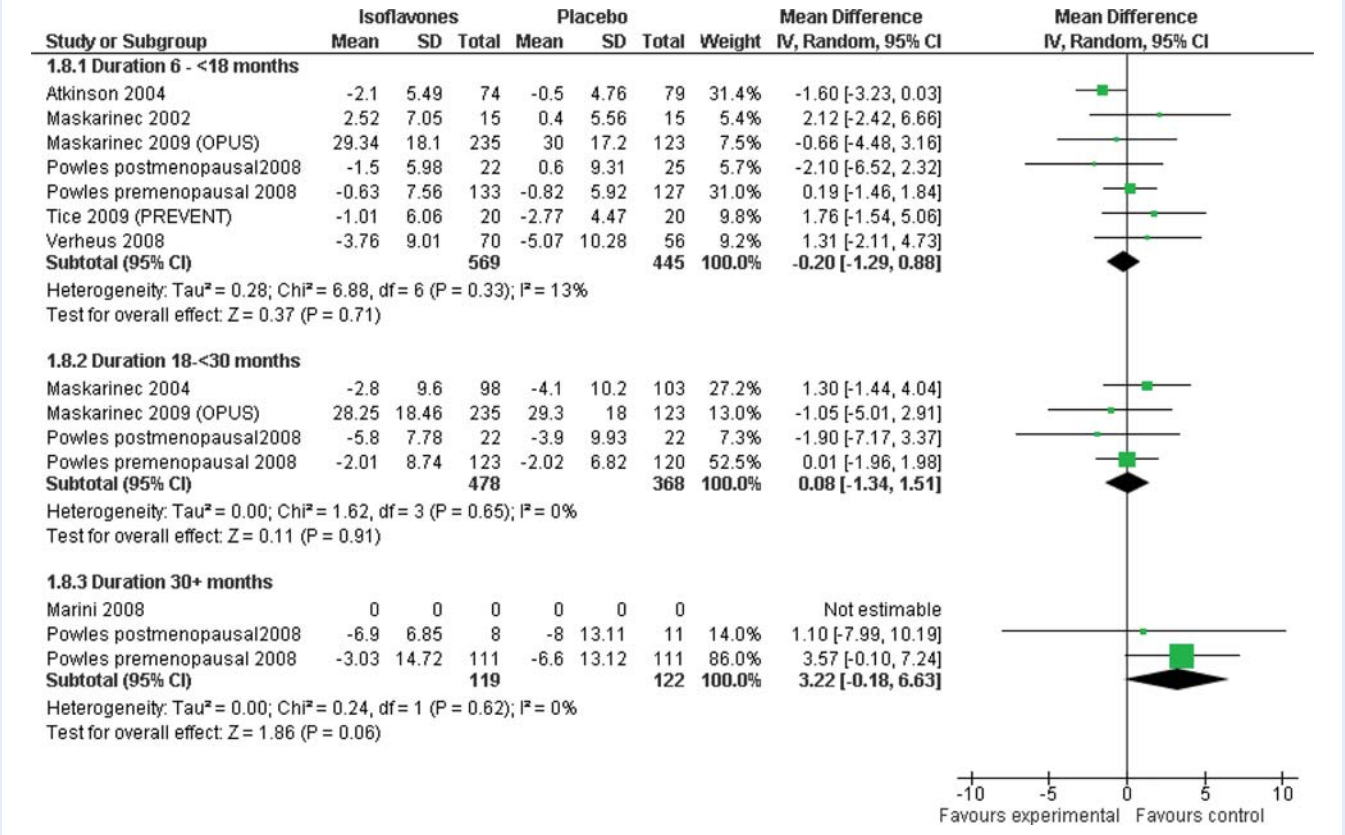

Figure 4 Subgrouping by study duration, only percentage breast density data (mean difference analysis).

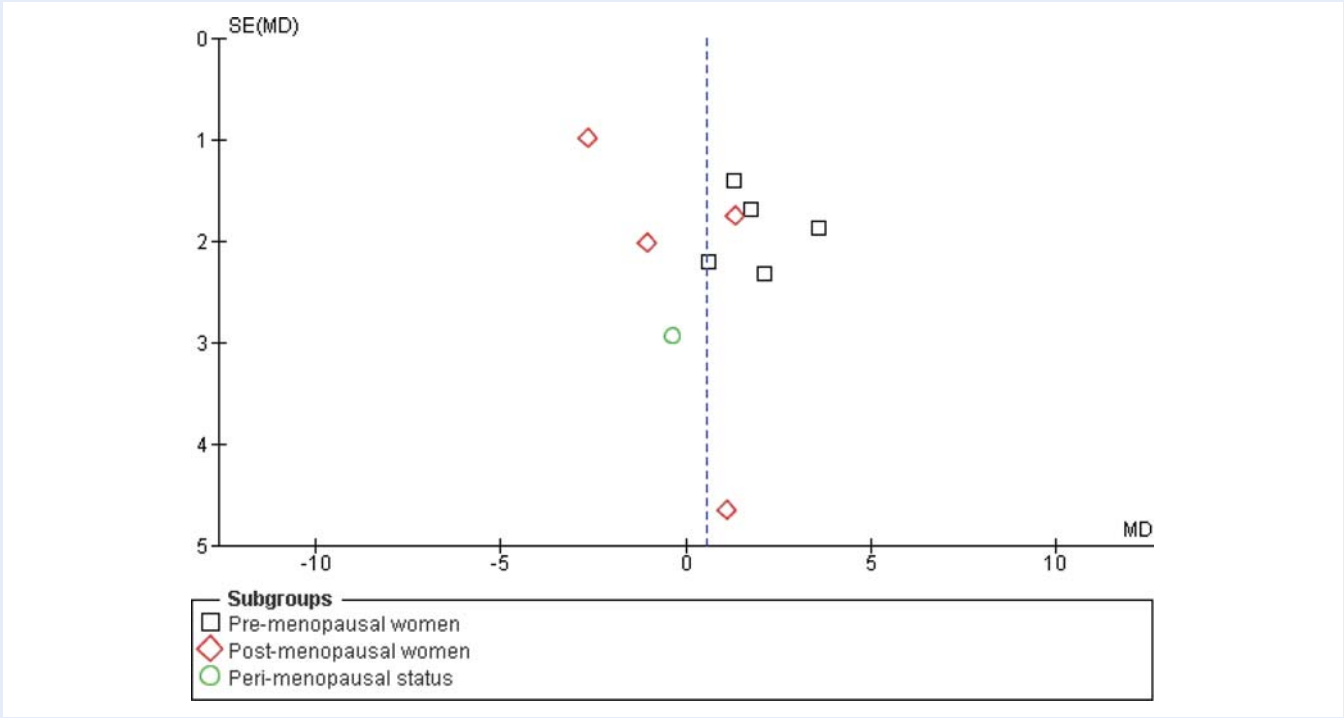

Figure 5 Funnel plot assessing risk of publication bias (plotting mean difference in breast density vs. the standard error of the mean difference).

Table IV Breast cancer diagnoses, mortality and side effects in included studies.

Study	Diagnoses of breast cancer during study		Deaths from any cause during the study		Reported side effects	
	Isoflavone arm(s)	Placebo arm	Isoflavone arm(s)	Placebo arm	Isoflavone arm(s)	Placebo arm
Atkinson 2004	1/102	0/103	NR	NR	NR	NR
Marini 2008	0	0	0	0	37/150 GI complaint	15/154 GI complaint
Maskarinec 2002	NR	NR	NR	NR	NR	NR
Maskarinec 2004	NR	NR	NR	NR	0/108 adverse effects	0/110 adverse effects
Maskarinec 2009 OPUS	NR	NR	NR	NR	Several dropped out due to GI complaints in both arms, but numbers unclear	
Powles 2008	3/199	5/202	NR	NR	9/111 GI complaint, 7/111 weight gain	6/111 GI complaint, 13/111 weight gain
Tice 2009 PREVENT	0/24	0/23	0/24	0/23	3/20 upset stomach, 3/20 constipation, 2/20 heartburn, 0/20 hot flashes, 1/20 diarrhoea	4/20 upset stomach, 3/20 constipation, 1/20 heartburn, 2/20 hot flashes, 1/20 diarrhoea,
Verheus 2008 (Finesse)	0	0	0	0	7/70 GI complaint	8/56 GI complaint

GI, gastrointestinal; NR, not reported.

et al., 2006a, b, 2007; Seo *et al.*, 2006; Cline and Wood, 2009; Messina *et al.*, 2009; Messina and Wu, 2009; Saarinen *et al.*, 2009). Our findings from the available RCTs support the epidemiological data observed for post-menopausal women but not for premenopausal women as we observed a small increase in breast density following isoflavone consumption in the younger women.

Relationships exist between physical activity, body weight or BMI and breast density (Masala *et al*., 2009; Reeves *et al*., 2009) which might confound the relationship between isoflavones and breast density in epidemiological studies. However, there was no evidence of weight or BMI being consistently different in intervention and control groups in a way that could confound the relationship between isoflavone supplementation and breast density in our analyses of these dietary intervention trials. There was little information provided on physical activity, and although it is possible that individual relatively small studies may have been unbalanced with regards to physical activity, this is unlikely across the whole set of studies unless there was a specific bias in place. Despite the limitations of the included studies, their randomized controlled design will have controlled for any confounding by socioeconomic status, BMI, differential weight change, other health behaviours such as physical activity or interest in healthy eating for example, that may be manifest in epidemiological studies.

A further factor that may affect breast density readings is the timing of a mammogram within the menstrual cycle in premenopausal women. A review (Martin and Boyd, 2008) found little evidence of effects of steroid sex hormones on premenopausal mammographic density, whereas two more recent studies have found relationships between levels of endogenous hormones and breast density in premenopausal women (Walker *et al*., 2009; Yong *et al*., 2009). The effect of the menstrual cycle on breast density has also been studied directly. Several studies showed that variations in density during the cycle are small and not clinically important (Buist *et al*., 2006; Hovhannisyan *et al*., 2009), whereas Ursin *et al*. (2001) found that while changes were generally small, in some women they were more significant, suggesting that mammograms should be collected in the follicular phase of the cycle. This study echoed the findings of an earlier study (White *et al*., 1998) which was specific to women in their 40s. In our systematic review, of the six studies in premenopausal women menstrual cycle phase was mentioned only in one (Tice, 2006), in which the final report stated that mammograms were collected during Days 7–13 of the cycle. In three studies it was unlikely that phase had been taken into account as mammograms were taken as part of normal screening activities (Atkinson *et al*., 2004; Maskarinec *et al*., 2002, 2004a, b), and no details of timing were provided in the others. If phase of the menstrual cycle is important in breast density then lack of timing of mammograms is likely to make it more difficult to see any changes in breast density that may occur differentially over time.

Overall, the studies included were of moderate risk of bias. Whereas masking, assessment of compliance and reporting of withdrawals were generally carried out appropriately and well reported, allocation concealment was unclear in all but two trials, and industry at least partly funded almost all of the studies. Allocation concealment has been associated with bias in a pooled analysis of methodological studies, suggesting that inadequate or unclear reporting of allocation concealment treatments were 18% more beneficial than in studies with adequate allocation concealment (Pildal *et al*., 2007). However, this bias appears to be manifest in studies with subjective outcomes, and not objective ones, suggesting that the masking of outcome assessors to allocation, and method of assessment of breast density may be important in study outcomes (Wood *et al*., 2008). The predominance of industry funding for the studies may be expected to potentially lead to exaggerated suggestions of effectiveness, minimized suggestions of risk or publication bias (Lesser *et al*., 2007). Data from the Powles study were difficult to interpret as the 95% CIs presented did not correspond with the *P*-values presented for the same effect, and we were unable to elicit any discussion on this with the authors (Powles *et al*., 2008). As a result we ran the analysis first using the 95% CIs (as these were the most accessible), then ran a sensitivity analysis using the data from the *P*-values. The observed effect in premenopausal women was attenuated when the data from *P*-values, rather than 95% CIs, were used in analysis, but the pooled effect was still apparent (Table III, the effect in premenopausal women became a mean difference of 1.63% (95% CI −0.03 to 3.30, $P = 0.05$)).

Conclusions

Implications for practice

Isoflavones from different sources had no effect on breast density in post-menopausal women and a small effect in premenopausal women. The average 5 year absolute risk for breast cancer for a 50-year-old woman in the USA is 1.3%. The relative risk of breast cancer for a women with a breast density of 5–24% (~15%), compared with a woman with a breast density of <5% (~3%), is 1.79 (95% CI 1.48–2.16; McCormack and dos Santos Silva, 2006). As there appears to be an approximately linear relationship between RR and breast density moving from 3% density to 15% density is associated with a RR of 1.79, and increasing breast density by 1.83% would equate with a RR of 1.12. The 5 year risk for the average woman would therefore move from 1.3 to 1.46%. This does not seem to be of sufficient magnitude to justify any recommendation regarding isoflavone foods or supplements. Although the clinical relevance of this potential relationship merits further investigation there are no immediate implications for practice.

Implications for research

In the absence of sufficient data on the effects of isoflavones on breast cancer diagnoses it would be helpful to assess effects of isoflavones on breast density in premenopausal women in a large and high quality RCT of at least 4 years duration and which assesses breast density at a consistent point in the menstrual cycle.

Authors' roles

The protocol was drafted by G.M., L.H. and A.C., and all authors contributed to, and agreed, the final protocol. G.M. and L.H. ran the searches, assessed titles and abstracts for collection, collected relevant full text papers, assessed these for inclusion, merged papers into studies, extracted the data, assessed validity, wrote to authors for further information, collated data into tables and ran the analyses in RevMan. L.H. and A.C. prepared the first draft of the paper, and all authors contributed significantly to data interpretation, and preparation of the second draft of the manuscript. All authors agreed the final draft. L.H. is the guarantor of the study.

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