Effect of oral phytoestrogens on endometrial thickness and breast density of perimenopausal and postmenopausal women: a systematic review and meta-analysis

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Highlights

- Phytoestrogens do not affect endometrial thickness compared with placebo.
- Phytoestrogens do not affect endometrial thickness compared with menopausal hormone therapy.
- Compared with placebo, phytoestrogens do not affect breast density.
• Body mass index (BMI) was identified as a confounder (it has a significant inverse association with increased endometrial thickness, \( p=0.046 \)); in contrast, no such correlation was found for age and study duration \( (p=0.243 \) and \( p=0.439 \), respectively).

Highlights

• Phytoestrogens did not affect endometrial thickness compared with placebo.
• Phytoestrogens did not affect endometrial thickness compared with MHT.
• Compared with placebo, phytoestrogens did not affect breast density.
• BMI was identified as a confounder (inverse association between BMI and increase of the endometrial thickness, \( p=0.046 \)); in contrast, no such correlation was found for age and study duration \( (p=0.243 \) and \( p=0.439 \), respectively).

Abstract

Background: Phytoestrogens constitute an alternative, non-pharmacologic approach for the management of menopausal symptoms. However, few studies have focused on their safety, specifically in relation to endometrial thickness and breast density.

Aim: To systematically search for and quantitatively synthesize the evidence regarding the effect of phytoestrogens on endometrial thickness and breast density in perimenopausal and postmenopausal women.

Methods: Randomized controlled trials (RCTs) examining the effect of phytoestrogens compared with placebo or menopausal hormone therapy (MHT) on endometrial thickness and/or breast density in perimenopausal or postmenopausal...
women were searched for in the MEDLINE, CENTRAL and Scopus databases as well as “gray literature” sources until October 31, 2018. Main outcomes were the change from baseline in endometrial thickness and breast density. Statistical analysis was performed with RevMan 5.3, using R language and Open Meta- Analyst software.

**Results:** The meta-analysis for endometrial thickness included 30 RCTs (with a total of 3,497 women), and that for breast density four RCTs (with a total of 674 women). Phytoestrogens did not affect endometrial thickness compared with placebo [weighted mean difference (WMD) -0.04 mm, 95% confidence interval (CI) -0.18 to 0.11, $I^2$ 66%] or MHT (WMD -1.40 mm, 95% CI -2.98 to 0.18, $I^2$ 84%). In addition, phytoestrogens did not affect breast density compared with placebo [standardized mean difference (SMD) -0.76, 95% CI -1.54 to 0.2, $I^2$ 95%].

**Conclusion:** Phytoestrogens have no effect on endometrial thickness or breast density, when administered at various doses and for various durations, in perimenopausal and postmenopausal women. However, the high heterogeneity of the studies makes it necessary to conduct RCTs with less risk of systematic error.

**Keywords:** phytoestrogens; endometrial thickness; breast density; perimenopause; menopause; systematic review.

**Introduction**
Given the increase in life expectancy, women have to spend 30 years approximately at postmenopausal status [1]. The lack of estrogens leads, among other consequences, to vasomotor (mainly hot flashes and night sweats) and vulvovaginal symptoms (mainly vaginal atrophy and dyspareunia), affecting woman's daily routine and having a negative impact on her quality of life [2]. Menopausal Hormone Therapy (MHT) is the treatment of choice for vasomotor and vulvovaginal symptoms [3]. However, some women cannot receive MHT, because of relative or absolute contraindications or the fear for possible adverse effects on endometrium and breast. Phytoestrogens constitute an alternative non-pharmacological option for the management of menopausal symptoms [4]. Furthermore, they may improve bone mineral density during menopause [5]. However, few studies only focused on their effect on endometrial thickness and breast density [6,7].

The aim of this study was to conduct a systematic review and meta-analysis of studies which refer to the effect of phytoestrogens on endometrial thickness and breast density, in perimenopausal and postmenopausal women who received them for alleviation of menopausal symptoms. By documenting the safety of phytoestrogens in endometrium and breast, more women could use them, especially if they have a contra-indication for MHT.

Materials and Methods

Literature search. Search for studies fulfilling the admission criteria was conducted on the MEDLINE, CENTRAL and Scopus electronic databases from conception until October 31, 2018, without language restrictions, using relevant keywords in both free text and Medical Subject Headings (MeSH terms) format. Search strategy in PubMed
has been combined with an enhanced sensitivity and accuracy filter for randomized control trials (RCTs), designed by the Cochrane Collaboration. The search for grey literature was conducted in repositories, catalogues (EThOS) and websites (OpenGray, GetNet International) as well as conference proceedings of major international congresses. A detailed search strategy is presented in Supplementary Table 1.

**Study selection.** Selection criteria consisted of RCTs (of parallel or cross-over design) with any duration of treatment that compared any dose of phytoestrogens with placebo or MHT. Studies should report at least one of the predetermined outcomes: endometrial thickness and/or breast density before and after intervention.

**Data extraction and quality assessment.** Two researchers (EM and CA) independently extracted data from studies, using Microsoft Excel panels. Any disagreement between them have been resolved in consensus with a third researcher (DGG). Data were derived from all studies, in which perimenopausal or postmenopausal women were given any dose of phytoestrogens compared with placebo for any duration and in which endometrial thickness and/or breast density were measured before and after the intervention. The outcomes of the systematic review and meta-analysis were the change from baseline in endometrial thickness (in mm), measured by transvaginal ultrasound and the change in breast density measured by mammography [as a percentage (in two studies) or as BIRADS (Breast Imaging Reporting and Data System) assessment units of the American College of Radiologists (in two studies)].
The revised Rob2.0 Cochrane Risk Assessment Tool for Randomized Controlled Trials was used to evaluate the quality of clinical trials included in the meta-analysis [8]. Two researchers (EM and CA) independently evaluated the risk of a systematic error for both outcomes and any disagreements were resolved in consensus with a third researcher (DGG). The overall risk was considered to be “high” in the presence of high risk in any rating area, “low” if all sectors had a low risk of systematic error and “intermediate” in any other case. Finally, the presence of publication bias for the two outcomes was evaluated by funnel plots.

Data synthesis and analysis. For continuous variables, mean differences (MD) and 95% confidence intervals (CI) were calculated using the inverse-variance random effects model. Since breast density measurements were performed on two different scales, standardized mean differences (SMD) and 95% CIs were calculated using the inverse-variance random effect model. In case the mean differences and the corresponding standard deviations (SD) were not available, e-mails were sent to the authors of the original publications. The procedures suggested by the Cochrane Handbook for Systematic Reviews of Interventions (version 5.1.0), were always followed [9]. The heterogeneity among the included studies was evaluated with the I^2 index, considering values >60% as indicative of high heterogeneity. The analyses were performed using the RevMan 5.3 software (Nordic Cochrane Center, Copenhagen, Denmark). Meta-regression analyses were performed using the Open Meta-Analyst software [10]. Egger’s test for publication bias was performed using R language.
Results

Search results and study characteristics. The systematic review and the meta-analysis included 33 RCTs with a total of 4047 patients. Endometrial thickness analysis included 30 RCTs with 3,497 patients and breast density analysis four RCTs with 674 patients. One RCT included both endpoints. The flow diagram of the study selection process is shown in Figure 1.

Characteristics of the included studies and baseline characteristics of the women are summarized in Tables 1-2. From the 33 RCTs, 30 had parallel design and three were cross-over studies. In the latter, only the first part of the study was used. From 30 RCTs relevant to endometrial thickness, 25 compared phytoestrogens with placebo, two compared phytoestrogens with MHT and three compared phytoestrogens with both placebo and MHT. The dose of phytoestrogens ranged from 10 mg (equol) to 270 mg (lignans). The duration of the intervention ranged from eight to 156 weeks. Five RCTs included both premenopausal and postmenopausal women, while the remaining included only postmenopausal women. In three studies, the phytoestrogen metabolite equol was administered as a phytoestrogen source, in one study the Pueraria mirifica plant, in three studies the red clover plant, two studies used lignans and the other isoflavones. Participants were advised to maintain their usual eating habits. Endometrial thickness was assessed by transvaginal ultrasound and breast density by mammography.

Effect of phytoestrogens on endometrial thickness. The endometrial thickness meta-analysis included 30 RCTs, of which three compared two different doses of
phytoestrogens with placebo. The two comparisons were treated as two different studies without changing the final number of participants. In two RCTs, the control group received MHT rather than placebo. These studies were included in the meta-analysis that compared MHT with phytoestrogens. The overall effect of phytoestrogens compared with placebo on endometrial thickness is shown in Figure 2. Phytoestrogens did not affect endometrial thickness (WMD -0.04 mm, 95% CI -0.18 to 0.11, $I^2$ 66%). In one study [11], phytoestrogens increased endometrial thickness compared with placebo, in three studies [12–14] phytoestrogens reduced endometrial thickness compared with placebo, and in the other studies no difference was observed. A sensitivity analysis, excluding studies with a daily dose $\leq$ 54 mg, resulted in no effect on endometrial thickness (WMD -0.08 mm, 95% CI -0.28 to 0.12, $I^2$ 75%). A sensitivity analysis, excluding studies with a mean age of $>60$ years, resulted in no effect on endometrial thickness (WMD 0.00 mm, 95% CI -0.14 to 0.13, $I^2$ 46%). In a similar way, a sensitivity analysis excluding studies with a duration $\leq$ 12 weeks, resulted in no effect on endometrial thickness (WMD -0.06 mm, 95% CI -0.26 to 0.13, $I^2$ 75%).

The overall effect of phytoestrogens compared with MHT on endometrial thickness is shown in Figure 3. Five RCTs were included in this meta-analysis. Phytoestrogens did not affect endometrial thickness compared with MHT (WMD -1.40 mm, 95% CI -2.98 to 0.18, $I^2$ 84%).

Effect of phytoestrogens on breast density. Four RCTs were included in the breast density meta-analysis. One of these [15] had two parts (comparison of two doses of phytoestrogen with placebo). The two comparisons were treated as two different
studies without changing the final number of participants. Compared with placebo, phytoestrogens did not affect breast density (SMD -0.25, 95% CI -0.60 to 0.11, I² 80%) (Figure 4).

**Meta-regression analysis.** Meta-regression analysis was performed to investigate for possible confounders of the effect of phytoestrogens on endometrial thickness, such as BMI, age and study duration. BMI was identified as a confounder (inverse association between BMI and increase of the endometrial thickness, p=0.046); in contrast, no such correlation was found for age and study duration (p=0.243 and p=0.439, respectively) (Supplementary Figures 1-3).

**Assessment of publication and systematic bias.** Many studies were considered as “high risk”. This was due to allocation concealment violations and attrition bias. A detailed presentation of the systematic error risk assessment of all included studies is given in Supplementary Tables 2 (endometrial thickness) and 3 (breast density). The funnel plot for the endometrial thickness outcome (Figure 5) does not provide evidence for publication bias. Egger’s test (p=0.151) also detected no publication bias.

**Discussion**

The aim of this systematic review and meta-analysis was to investigate the effect of phytoestrogens on endometrial thickness and breast density in perimenopausal and postmenopausal women with vasomotor and other menopausal symptoms. The study provided evidence that phytoestrogens [isoflavones (genistein, daidzein), lignans, equol, S-equol] at different doses, ranging from 10 mg (equol) to 270 mg (lignans), over a period of 8 to 156 weeks do not affect either endometrial thickness or breast
density. In addition, phytoestrogens have no effect on endometrial thickness compared with MHT.

This study provides the most up-to-date and complete data synthesis on the effect of phytoestrogens on endometrial thickness and breast density in perimenopausal and postmenopausal women. The results of the meta-analysis are reassuring about the safety of phytoestrogens on endometrial thickness and breast density up to three years of use and in agreement with the other studies. In the meta-analysis by Liu et al. [16], 23 RCTs were included for a total of 2305 women; no effect of phytoestrogens was shown on endometrial thickness as compared with placebo. However, in a subgroup analysis, the administration of phytoestrogens at a daily dose >54 mg reduced the endometrial thickness in postmenopausal women by SMD -0.26 mm, 95% CI -0.45 to -0.07. In the present meta-analysis there was no effect on endometrial thickness, when only the studies with a dosage of >54 mg were included. This may be due to new studies added and a different classification of studies. Specifically, in the present meta-analysis, Murray et al. study [17] was used in the comparison of phytoestrogen with MHT, as it would be wrong to classify it in the comparison of phytoestrogens with placebo. Also, in the meta-analysis by Liu et al., the Penotti et al. study was misclassified in the group “<54 mg”, while the daily dose of phytoestrogens was 72 mg of isoflavone. When only the studies with a mean age of ≤60 years were included in the sensitivity analysis, there was no effect on endometrial thickness (WMD -0.00 mm, 95% CI -0.14 to 0.13, I^2 46%).

The present meta-analysis further evaluated the effect of phytoestrogens compared with MHT on endometrial thickness in perimenopausal and postmenopausal women.
In this comparison, five RCTs were included with 226 patients, who received either phytoestrogens or MHT. The meta-analysis did not reveal a significant effect. Therefore, it is concluded that phytoestrogens are a safe choice, with regard to endometrial thickness, for the treatment of climacteric symptoms in perimenopausal and postmenopausal women.

Four RCTs, with 674 patients, were included in the analysis of breast density. Phytoestrogens did not affect the breast density over a period of three years. It is, therefore, concluded that they can be used safely with regard to breast density. This conclusion is consistent with the study by Hooper et al. [18], which studied the effect of isoflavones on breast density of premenopausal and postmenopausal women. This study showed that isoflavones do not affect the breast density, which is one of the most important independent risk factors for developing breast cancer.

The meta-regression analysis showed that the age of the participants and the study duration are not related to the effect of phytoestrogens on endometrial thickness. However, the lower the BMI, the higher the increase of endometrial thickness in the phytoestrogens group. The Kenny et al. study [13] was removed from the meta-regression analysis of age, because its average age (73.1 years) greatly increased the heterogeneity of included studies. A meta-regression analysis for possible confounders with regard to breast density was not performed because of the small number of included studies (less than ten).

It is important to recognize some limitations of this meta-analysis. Although there is a large number of clinical trials examining the efficacy and potential adverse effects of
Phytoestrogens in perimenopausal and postmenopausal women, there are only few clinical trials examining the effect on endometrial thickness and breast density. Additionally, the included studies are characterized by great heterogeneity in the daily dose of phytoestrogens, the formulation administered and the duration of the studies. These factors result in high heterogeneity in statistical analysis, which gives rise to reservations about the outcome of the study. Another limitation is the high rate of missing data in studies. The high rate of missing data, especially in mean difference values and standard deviations of difference values led, to the use of the final means and final standard deviations, according to the Cochrane Handbook's guidelines for missing data. In some RCTs, final standard deviations were not reported. These values were added by imputation methods from the adjacent studies without affecting the result of the meta-analysis. The small number of studies in the comparison of MHT versus phytoestrogens for endometrial thickness and the comparison of placebo versus phytoestrogens for breast density also limits the strength of the result. With regard to breast density, the existence of two different measurement scales made it necessary to use the standardized mean difference value in the analysis. It is noteworthy, that while Delmanto et al. study [19] for breast density did not find statistically significant effect, in our analysis it was found. The risk of systematic error was found to be high in most studies because they did not clearly describe the process of randomization and allocation concealment. In addition, there are high dropout rates and missing data.

In conclusion, phytoestrogens in various types and doses did not affect endometrial thickness or breast density over a maximum use of three years. The high heterogeneity of the studies, mainly regarding types and dose of phytoestrogens,
underlines the necessity of further clinical trials to assess the safety of phytoestrogens on the long term.

Contributors
All authors (Evangelia Mareti, Christina Ampratzi, Dimitrios Vavilis, Irene Lambrinoudaki, Dimitrios Goulis) were involved in the search of the literature for appropriate studies, data extraction, statistical analysis and preparation of the manuscript.

Conflict of interest
The authors declare that they have no conflict of interest.

Funding
No funding was received for the preparation of this review.

Provenance and peer review
Peer review was directed by Professor Yvonne van der Schouw independently of Irene Lambrinoudaki, an author and Maturitas editor, who was blinded to the process.

Conflict of interest
The authors whose names are listed immediately below (Evangelia Mareti, Christina Ampratzi, Dimitrios Vavilis, Irene Lambrinoudaki, Dimitrios G. Goulis) certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers’ bureaus; membership,
employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

Author's contribution-funding

All authors (Evangelia Mareti, Christina Ampratzi, Dimitrios Vavilis, Irene Lambrinoudaki, Dimitrios G.Goulis ) were involved in search of the literature for appropriate studies, data extraction, statistical analysis and preparation of the manuscript.

There were no financial and personal connections with other people or organizations that could inappropriately influence (bias) the current study. There was no financial support for this research and preparation of the article.
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[38] M.C. Colli, A. Bracht, A.A. Soares, A.L. de Oliveira, C.G. Boer, C.G.M. de


Table 1. Baseline characteristics of studies (endometrial thickness).

<table>
<thead>
<tr>
<th>Id</th>
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<th>Year</th>
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<th>Control</th>
<th>Placebo</th>
<th>Source</th>
<th>Active substance and dose (mg/d)</th>
<th>Control</th>
<th>Phytoestrogens</th>
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<td>Vasomotor (hot flushes)</td>
<td>Placebo</td>
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<td>3.2 ± 1.5</td>
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<td>Lipids</td>
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<td>-</td>
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<td>20</td>
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<td>India</td>
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<td>Vasomotor (hot flushes), Lipids, BMD</td>
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<td>Powder sach</td>
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<td>21</td>
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<td>2010</td>
<td>Brazil</td>
<td>Peri- and Post-menopausal</td>
<td>38</td>
<td>Vasomotor (hot flushes)</td>
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<td>Bread (flaxseed)</td>
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<td>3.0 ± 3.0</td>
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<td>Canada</td>
<td>Peri- and Post-menopausal</td>
<td>82</td>
<td>Vasomotor (hot flushes)</td>
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<td>Oral caps</td>
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<td>23a</td>
<td>Steinberg et al.</td>
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<td>104</td>
<td>2.0 ± 1.2</td>
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<tr>
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<td>2011</td>
<td>USA</td>
<td>Post-menopausal</td>
<td>180</td>
<td>Clinical outcomes</td>
<td>Placebo 120 mg isoflavone</td>
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<td>2.0 ± 1.2</td>
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<tr>
<td>24a</td>
<td>Colli et al.</td>
<td>2012</td>
<td>Brazil</td>
<td>Post-menopausal</td>
<td>41</td>
<td>Vasomotor (hot flushes)</td>
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<td>-</td>
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<td></td>
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<td>Year</td>
<td>Country</td>
<td>Status</td>
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<td>BMD</td>
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<td>Brazil</td>
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<td>25a</td>
<td>Oyama et al. [39]</td>
<td>2012</td>
<td>Japan</td>
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<td>Skin aging</td>
<td>Placebo</td>
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<td>Oral tabs (soy germ)</td>
<td>55.2</td>
<td>21.6</td>
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<td>2012</td>
<td>Japan</td>
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<td>Post-menopausal</td>
<td>124</td>
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<td>26.4</td>
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<td>28</td>
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<td>2015</td>
<td>Brazil</td>
<td>Post-menopausal</td>
<td>60</td>
<td>Urogenital</td>
<td>Placebo, MHT</td>
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<td>Villa et al. [43]</td>
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<td>Italy</td>
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<td>75</td>
<td>Quality of life</td>
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<td>2018</td>
<td>Iran</td>
<td>Post-menopausal</td>
<td>204</td>
<td>Vasomotor (hot flushes)</td>
<td>Placebo</td>
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<td>Oral tabs</td>
<td>51.7</td>
<td>24.9</td>
<td>12</td>
</tr>
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</table>

Data are given as mean ± SD. BMD: bone mineral density; BMI: body mass index; HRT: hormone replacement therapy SD: standard deviation.
### Table 2. Baseline characteristics of studies (breast density).

<table>
<thead>
<tr>
<th>Id</th>
<th>Study</th>
<th>Year</th>
<th>Country</th>
<th>Population</th>
<th>n</th>
<th>Aim</th>
<th>Control</th>
<th>Phytoestrogens</th>
<th>Active substance and dose (mg/d)</th>
<th>Source</th>
<th>Age (y)</th>
<th>BMI (kg/m²)</th>
<th>Duration (w)</th>
<th>Baseline BD Control</th>
<th>Baseline BD Phytoestrogens</th>
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<tbody>
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<td>1</td>
<td>Verheus et al. [45]</td>
<td>2008</td>
<td>Netherlands</td>
<td>Post-menopausal</td>
<td>126</td>
<td>Mammographic density</td>
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<td>26.0</td>
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<td>15.4%</td>
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<td>2009</td>
<td>USA</td>
<td>Post-menopausal</td>
<td>177</td>
<td>Osteoporosis</td>
<td>Placebo</td>
<td>80 mg isoflavone</td>
<td>Oral tabs</td>
<td>54.9</td>
<td>25.1</td>
<td>104</td>
<td>32.0 ± 17.5%</td>
<td>28.9 ± 17.4%</td>
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<tr>
<td>2b</td>
<td>Maskarinec et al. [15]</td>
<td>2009</td>
<td>USA</td>
<td>Post-menopausal</td>
<td>181</td>
<td>Osteoporosis</td>
<td>Placebo</td>
<td>120 mg isoflavone</td>
<td>Oral tabs</td>
<td>54.9</td>
<td>25.1</td>
<td>104</td>
<td>32.0 ± 17.5%</td>
<td>32.3 ± 19.0%</td>
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<tr>
<td>3</td>
<td>Colacurci et al. [40]</td>
<td>2013</td>
<td>Italy</td>
<td>Post-menopausal</td>
<td>124</td>
<td>Safety</td>
<td>Placebo</td>
<td>60 mg isoflavone</td>
<td>Oral tabs</td>
<td>55.7</td>
<td>25.0</td>
<td>52</td>
<td>1.8 ± 0.9</td>
<td>1.9 ± 1.0</td>
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<td>4</td>
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<td>2013</td>
<td>Brazil</td>
<td>Post-menopausal</td>
<td>66</td>
<td>Mammographic density</td>
<td>Placebo</td>
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<td>Oral tabs</td>
<td>55.7</td>
<td>29.1</td>
<td>40</td>
<td>2.0</td>
<td>1.5</td>
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</tr>
</tbody>
</table>

Data are given as mean ± SD. BD: breast density; BMI: body mass index; SD: standard deviation; tabs: tablets.
Figure 1. PRISMA study flow diagram.

Records identified through database searching (n = 1070)

Records after duplicates removed (n = 543)

Records screened (n = 543) → Records excluded (n = 358)

Full-text articles assessed for eligibility (n = 185) → Full-text articles excluded, with reasons (n = 151)

Studies included in qualitative synthesis (n = 33)

Studies included in quantitative synthesis (meta-analysis) (n = 33)
Figure 2. Meta-analysis of the effect of phytoestrogen supplementation on endometrial thickness compared with placebo.
PE: phytoestrogens.

Figure 3. Meta-analysis of the effect of phytoestrogens supplementation on endometrial thickness compared with MHT.

HRT: hormone replacement therapy; PE: phytoestrogens.
**Figure 4.** Meta-analysis of the effect of phytoestrogens supplementation on breast density compared with placebo.

PE: phytoestrogens.
Figure 5. Funnel plot of studies from the endometrial thickness meta-analysis.