A systematic review and meta-analysis of the effects of isoflavone formulations against estrogen-deficient bone resorption in peri- and postmenopausal women

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ABSTRACT

Background: Age-related estrogen deficiency leads to accelerated bone resorption. There is evidence that, through selective estrogen receptor modulation, isoflavones may exert beneficial effects against estrogen-deficient bone loss. Isoflavone aglycones show higher bioavailability than their glycosidic counterparts and thus may have greater potency.

Objective: To summarize evidence, we executed a systematic review and meta-analysis examining isoflavone therapies and bone mineral density (BMD) loss in peri- and postmenopausal women.

Design: We systematically searched EMBASE and PubMed for randomized controlled trials (RCTs) evaluating isoflavone therapies for treating BMD loss at the lumbar spine and femoral neck in estrogen-deficient women. Separate meta-analyses were carried out with the use of random-effects models for the lumbar spine and femoral neck for all studies providing isoflavones as aglycones.

Results: Twenty-six RCTs (n = 2652) were included in the meta-analysis. At the lumbar spine, isoflavone treatment was associated with a significantly ($P < 0.00001$) higher weighted mean difference (WMD) of BMD change of 0.01 (95% CI: 0.01, 0.02) than the control. For the femoral neck (18 RCTs, n = 1604), isoflavone treatment showed a significantly ($P < 0.01$) higher WMD of BMD change of 0.01 (95% CI: 0.00, 0.02) compared with the control. When isolating studies that provide isoflavone aglycones in their treatment arm, the average effect was further significantly increased at the spine (5 RCTs, n = 682) to 0.04 ($P < 0.00001$; 95% CI: 0.02, 0.05) and femoral neck (4 RCTs, n = 524) to 0.03 ($P < 0.05$; 95% CI: 0.00, 0.06) compared with the control. This protective effect against bone loss disappeared when only studies with formulations comprising predominantly isoflavone glycosides were included.

Conclusions: Isoflavone treatments exert a moderately beneficial effect against estrogen-deficient bone loss in women. The effect appears dependent on whether isoflavone treatments are in aglycone form; we conclude that beneficial effects against bone loss may be enhanced for isoflavone aglycones. *Am J Clin Nutr* 2017;106:801–11.

Keywords: isoflavone, bone, estrogen deficiency, osteopenia, osteoporosis

INTRODUCTION

A natural decline in endogenous estrogen synthesis during menopause reduces bone mineral density (BMD) and incurs negative changes to bone microarchitecture, increasing the risk of osteoporosis and, as a consequence, fracture risk in women (1, 2). Because of estrogen dysregulation and deficiency, menopausal women lose a mean of 2–5% BMD/y, and postmenopausal women lose a mean of 1–3% BMD/y (3, 4).

Randomized controlled trials (RCTs) support that estrogen therapies (ETs) are effective in the prevention and treatment of accelerated bone resorption in estrogen-deficient women (5, 6). These effects are largely caused by estrogen-mediated agonistic action through estrogen receptor (ER) α and ERβ (7). Increased cancer risk has been associated with ETs, likely due to excessive stimulation of ERα by ETs in sensitive tissues such as the breasts, ovaries, and endometrium, all of which retain high ERα expression (8). As such, there is medical value in identifying surrogate bioactive substances with minimized and/or eliminated side effects of hormone therapies (9, 10). Isoflavones are naturally present in certain dietary legumes and can selectively modulate ERs; in contrast, ET isoflavones have selective affinity for ERβ (11, 12). ERβ is expressed in tissues requiring certain stimulation by estrogen to function optimally (e.g., bone tissue, bone marrow, adipose, brain, kidney, endothelial cells, and liver) (13). Researchers have sought to harness this selective affinity for targeting tissues to more safely and effectively treat estrogen-deficient bone resorption and BMD loss in estrogen-deficient women (14). Previous meta-analyses have endeavored to compare the effects of isoflavone-rich soy preparations on

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Supplemental Figures 1 and 2 and Supplemental Table 1 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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Abbreviations used: BMD, bone mineral density; DXA, dual-energy X-ray absorptiometry; ER, estrogen receptor; ET, estrogen therapy; RCT, randomized controlled trial; WMD, weighted mean difference.

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estrogen-deficient bone loss. These analyses show inconsistent and often conflicting results, largely because of high heterogeneity between the study designs of the included RCTs and the majority of these studies do not use methodologies to determine isoflavone content and form (15–17). A key determinant of the efficacy of any bioactive substance is its bioavailability and metabolism in humans. Glycosides have higher molecular weights and hydrophobicity than their aglycone counterparts; hence, conversion to aglycones via deconjugation of glycoside residues by intestinal bacteria and epithelial cells is requisite for passive diffusion of isoflavones into intestinal cells (18). Isoflavones occur predominantly as glycosides in plants, and recent human trials demonstrated isoflavones improved bioavailability when provided as aglycones; hence, aglycones may have improved biological efficacy (19, 20). Food matrix effects may also influence the outcomes of isoflavone RCTs: a recent trial indicated that complex nondigestible dietary fibers fructooligosaccharides may reduce the absorption of dietary isoflavone components, which has implications for RCTs using isoflavone food matrices in treatment arms (21). To our knowledge, there is no systematic review or meta-analysis investigating the effects of isoflavones against estrogen-deficient BMD loss for RCTs providing isoflavone aglycones in treatment arms. This systematic review and meta-analysis was performed to provide an overview of the effects of isoflavone treatments against estrogen-deficient bone resorption in women and to inform researchers of new potential sources of bias to be addressed in future clinical trials.

METHODS

Study selection and search strategy

The meta-analysis was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines (22). PubMed and EMBASE were searched for RCTs up to December 2016. Key search terms were as follows: isoflavone, genistein, biochanin A, daidzein, formononetin, genistin, daidzin, sissotrin, ononin, bone, bone tissue, osteoporosis, fractures, bone density, bone mineral density, bone resorption, bone degeneration, bone loss, bone remodelling, bone turnover, osteogenesis, bone regeneration, physiologic ossification, bone formation, bone disease, bone metabolism, fracture, bone destruction, osteopenia, absorptiometry, photon absorptiometry, duel-energy X-ray absorptiometry scan, essential osteolysis, climacteric, menopause, pre-menopause, peri-menopause, post-menopause, menopausal, and estrogen deficiency. Dual-energy X-ray absorptiometry (DXA) is the most tested and used bone density assessment technology. It functions by emitting X-rays with differing energy levels, which are absorbed to varying degrees by human tissues, and BMD is determined by the absorption of the X-ray beams (23, 24). Each term was searched as free terms and in combinations, including the [MESH] terms in PubMed and [EMTREE] versions in EMBASE. Studies were considered eligible for inclusion in the meta-analysis if they met all of the following criteria: RCTs including menopausal or postmenopausal women treated with isoflavones for ≥3 mo, with the use of DXA as a measurement marker; BMD specified in grams per square centimeter with ≥1 measurement taken at the femoral neck or lumbar spine; a specified labeled or determined isoflavone dose as aglycones or aglycone equivalents or isoflavones; and reported mean change in BMD from baseline to the end of the study along with corresponding SDs, SEMs, or 95% CIs. Studies were excluded if participants had used hormone therapy or selective estrogen receptor modulator preparations during or ≤3 mo before initiation of the trial or estimated isoflavone intake was used (i.e., self-report or registry-based data). In cases where stratified data were reported together with total data from the same subject data pool, the largest sample and most detailed data were included in the meta-analysis. Two reviewers independently reviewed and evaluated all article titles and abstracts and thereafter performed independent full-text assessments. A third independent reviewer was consulted in cases where reviewers' evaluations were not in consensus.

Outcome measures

Outcomes of interest were changes in DXA-determined BMD at the lumbar spine and femoral neck; these were used to determine the effects of isoflavone treatment on bone health compared with control. Second, data from RCTs providing purified isoflavone aglycones to participants were further analyzed separately. As glycoside standards are difficult to acquire, most analytic techniques for determining isoflavone concentration in treatments include an enzymatic or acidic hydrolysis step to convert glycoside species to their respective aglycones. The isoflavone content data are often published as aglycone equivalents and only reflect the total isoflavone content and not actual glycoside or aglycone concentrations. All studies included in the meta-analysis providing soy protein isolates, powders, milk, or soy extracts that did not specify any processing or fermentation steps for their interventions (to convert glycosides to aglycones)

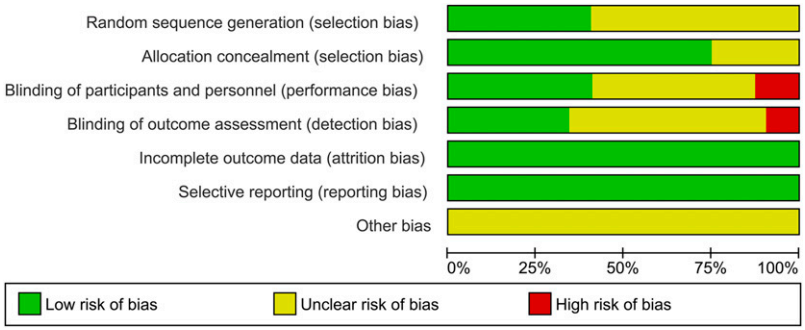

FIGURE 1 Risk of bias, including the assessment of risk of bias for each item by the authors. Data are shown as percentages for all studies.

were considered by reviewers to be isoflavone glycoside treatments, in line with previous research on soy-based products (25). RCTs that performed assessment of the isoflavone content and form of their interventions that indicated predominantly glycoside concentrations were also considered glycoside interventions (26–28).

Quality assessment

The quality of the included studies was assessed with the use of the risk of bias assessment tool outlined in the Cochrane Handbook for Systematic Reviews of Interventions (version 5.3.0) (29). This tool comprises selection bias (random sequence generation and allocation concealment), performance bias (blinding of participants and personnel), detection bias (blinding of outcome data), attribution bias (incomplete outcome data), reporting bias (selective reporting), and other sources of bias (**Figures 1** and **2**).

Statistics

Meta-analyses were performed, when appropriate, for each DXA site with the 26 included articles. These were then further stratified into RCTs providing isoflavone as aglycones and those treating with isoflavone glycosides. The Revman 5 (Cochrane Information Management System) was used when data were available and trials were considered of sufficient quality. For articles not specifying means or SDs (medians, ranges, or 95% CIs), the correlation coefficient of 0.5 was imputed for calculating the SD of mean change (30). For the articles with no useful data, letters and e-mails were sent to the authors requesting data; if data were still unobtainable, articles were then excluded. The random-effects model was used to calculate weighted mean difference (WMD) and 95% CI, and $P < 0.05$ was considered statistically significant. Outcomes were assessed by comparing the mean ± SD of the BMD change of the isoflavone treatment group with the control group. Heterogeneity between studies was determined with the use of I^2 and the Cochrane χ^2 statistic: I^2 values of ≤25% were considered low, >25% or <75% as moderate, and ≥75% as high heterogeneity (29). Funnel plots were used to evaluate publication bias (**Supplemental Figures 1** and **2**).

RESULTS

A total of 1544 articles were identified from the initial search in EMBASE and PubMed. Only the title and abstract were screened in the first selection round, where 124 articles were identified and passed on to the full-text assessment. Of the 124 articles, there were a total of 51 duplicates, 5 abstract-only articles (published at congresses and conferences), 3 articles that shared the same participants as other studies, and 39 others that did not meet all the inclusion criteria. All trials were RCTs. After removing duplicates and congress papers, 73 papers were eligible and systematically assessed for inclusion. Twenty-six RCTs met the inclusion criteria and were used in the meta-analysis (26–28, 31–53). A detailed overview of the selection process is outlined in **Figure 3**.

The included studies varied substantially in duration; 9 were 24 mo long, 6 were 6 mo long, 2 spanned 3 mo, and only 1 study was 15 mo in duration. Five studies included 2 treatment arms with different isoflavone doses; these arms were considered separately in the analysis (42, 43, 48–50). All 26 RCTs provided BMD data from DXA scans at the lumbar spine (26–28, 31–53). Fifteen of the studies also provided DXA data for femoral neck BMD (28, 31, 32, 34, 36–43, 48, 53). Of the 26 RCTs (26–28, 31–53) providing data at the lumbar spine, 6 studies (31, 40, 41, 44, 47, 53) found substantial improvements to BMD with

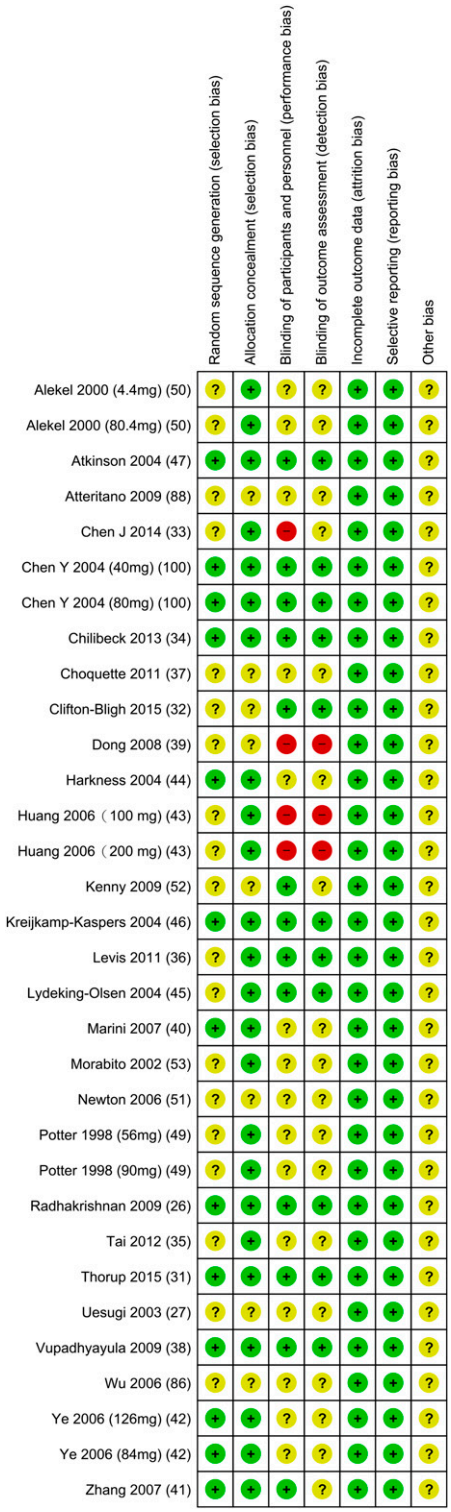

FIGURE 2 Risk of bias summary for each study as determined by the judgments of the authors.

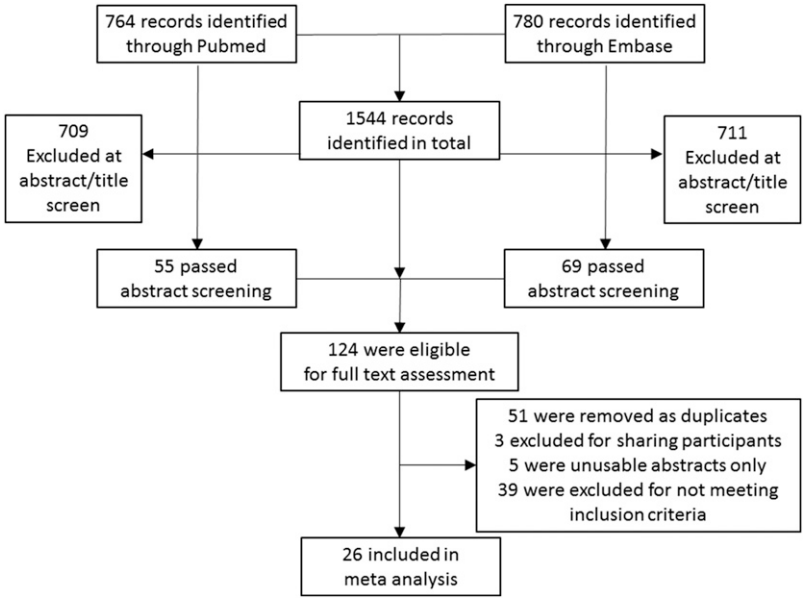

FIGURE 3 A flow diagram presenting the number of randomized controlled trials that were screened and assessed for eligibility, and the number included in the final meta-analysis.

isoflavone treatment. Four (31, 40, 41, 53) of the 15 RCTs (28, 31, 32, 34, 36–43, 48, 53) demonstrated substantial effects on BMD at the femoral neck.

Quality of the studies

None of the studies reported any relevant intergroup differences in the baseline characteristics of the intervention and control groups. Apart from 3 of the RCTs (33, 39, 43), all other included trials, to various degrees, described measures for blinding of participants and personnel taking part in the studies (33, 39, 43). Two of the aforementioned RCTs did not indicate any measures for blinding of outcome assessment (39, 43).

Participants

A total of 2652 participants were included in the final analysis from the 26 RCTs (**Table 1**). The majority of the RCTs included postmenopausal participants, which totaled 18 (26, 28, 33–35, 37–41, 43–46, 48, 49, 51, 52). Four of the studies included exclusively perimenopausal women (31, 32, 36, 50). Four studies mixed both participant types (27, 42, 47, 53). One study included both male and female participants, but only data from women included in this study was included in the meta-analysis (51).

Interventions

Two trials compared an extract rich in isoflavones against the control (27, 31). Two RCTs administered red clover–derived isoflavone tablets compared with placebo tablets (32, 47). Eight studies used isoflavones isolated from soy in the treatment arm compared with soy protein alone or placebo (35, 36, 39, 42–44, 46, 48). One trial tested isoflavone-enriched food matrices (particularly soymilk) comparing against isoflavone-free equivalents in the control arms (45). Lydeking-Olsen et al. (45) performed an analysis of the soy milk isoflavone contents specifying aglycones; however, the analytic procedure included an enzymatic hydrolysis step, and the treatment was therefore considered to be glycoside based. Five assessed soy protein isolate against control (26, 38, 49–51). Three administered isoflavone equivalents compared with placebo (33, 34, 52). Four RCTs compared intake of tablets with purified isoflavones (genistein, daidzein, and glycitein) against placebo tablets (37, 40, 41, 53). One study investigated the isoflavone conjugates (genistin, daidzin, daidzin, acetyldaidzin, genistin, and glycitin), comparing with isoflavone-free dextrin placebo capsules (28).

Meta-analysis

Lumbar spine BMD

The effects of isoflavone treatments against BMD loss at the lumbar spine in estrogen-deficient women are shown in **Figure 4**. Data from all 26 studies were pooled and analyzed, and results indicated a significant ($P < 0.00001$) improvement in estrogen-deficient BMD loss in participants given daily isoflavone treatment compared with controls, with a WMD favoring isoflavone of 0.01 g/cm^2 (95% CI: 0.01, 0.02 g/cm^2; I^2, 90%) (26–28, 31–53). Subgroup analysis results for studies used to assess isoflavone effects on lumbar BMD are shown in **Supplemental Table 1**.

Femoral neck BMD

The effects of isoflavone treatments for reducing estrogen-deficient BMD loss at the femoral neck in women for the 15 RCTs are displayed in **Figure 5**. Analysis of the femoral neck data comparing isoflavone and control showed a significant ($P < 0.01$) reduction of BMD loss in isoflavone groups in contrast to the control (WMD favoring isoflavone of 0.01 g/cm^2; 95% CI: 0.00, 0.02 g/cm^2; I^2, 90%). Subgroup analysis results for studies used to determine isoflavone effects on femoral neck BMD are presented Supplemental Table 1.

TABLE 1
Summary of included studies[1]

Study, year (ref)	Trial length	*n* (I/C)	Intervention	IS (dose, mg; form, Agl, Eq, Gly)	Age, y	Status	DXA site
Thorup et al., 2015 (31)	3 mo	31/29	I: 150 mL RC extract C: Placebo	37.1; Agl	40–65	Peri	LS, FN
Clifton-Bligh et al., 2015 (32)	24 mo	55/42	I: 2 RC tablets C: Placebo	50; Eq	50–58	Peri	LS, FN
Chilibeck et al., 2013 (34)	24 mo	76/73	I: Exercise placebo + IS C: Exercise placebo + IS placebo	165; Eq	Mean: 56	Post	LS, TH
Tai et al., 2012 (35)	24 mo	200/199	I: SI C: Placebo	300; Eq	45–65	Post	LS, TH
Levis et al., 2011 (36)	24 mo	97/80	I: SI tablets C: Placebo	200; Eq	45–60	Peri	LS, FN, TH
Vupadhyayula et al., 2009 (38)	24 mo	57/48	I: SP + IS C: SP isolate	90; Eq	>55	Post	LS, FN, TH
Dong et al., 2008 (39)	12 mo	26/26	I: Ca + SI C: Ca	100; Eq	45–65	Post	LS, FN
Marini et al., 2008 (54)	24 mo	150/154	I: GE C: Placebo	54; Agl	51–56	Post	LS, FN
Ye et al., 2006 (84 mg) (42)	6 mo	28/30	I: SI C: Placebo	84; Eq	45–60	Peri and post	LS, FN, TH
Ye et al., 2006 (126 mg) (42)	6 mo	26/30	I: SI C: Placebo	126; Eq	45–60	Peri and post	LS, FN, TH
Wu et al., 2006 (28)	6 mo	33/33	I: IS conjugate C: Placebo	75; Gly	45–60	Post	LS, FN, TH
Harkness et al., 2004 (44)	6 mo	10/9	I: SI C: Placebo	110; Eq	64–76	Post	LS, TH
Atkinson et al., 2004 (47)	12 mo	77/81	I: RC IS C: Placebo	43.5; Agl	49–65	Peri and post	LS
Chen et al., 2003 (40 mg) (48)	12 mo	57/53	I: SI C: Placebo	40; Eq	48–62	Post	LS, FN, TH
Chen et al., 2003 (80 mg) (48)	12 mo	50/53	I: SI C: Placebo	80; Eq	48–62	Post	LS, FN, TH
Kenny et al., 2009 (52)	12 mo	26/22	I: CP + IS tablets C: CP + placebo tablets	105; Eq	60–93	Post	LS, FN, TH
Newton et al., 2006 (51)	12 mo	9/7	I: SP + 83 mg IS C: SP + 3 mg IS	83; Eq	50–80	Post	LS, TH
Chen and Liu, 2014 (33)	12 mo	40/30	I: MP + IS C: Normal diet	70; Eq	50–70	Post	LS
Alekel et al., 2000 (80.4 mg) (50)	24 wk	24/21	I: SP + IS C: Whey	80.4; Eq	44.7–59.4	Peri	LS
Alekel et al., 2000 (4.4 mg) (50)	24 wk	24/21	I: SP + IS C: Whey	4.4; Eq	41.9–61.6	Peri	LS
Choquette et al., 2011 (37)	6 mo	23/22	I: DE + GE + glycitein C: Placebo	70; Eq	50–70	Post	LS, FN, TH
Zhang et al., 2007 (41)	24 mo	50/50	I: DE + GE + icariin C: Placebo	78; Agl	60–68	Post	LS, FN
Huang et al., 2006 (100 mg) (43)	12 mo	15/12	I: SI C: Placebo	100; Eq	45–67	Post	LS, FN
Huang et al., 2006 (200 mg) (43)	12 mo	15/12	I: SI C: Placebo	200; Eq	45–67	Post	LS, FN
Lydeking-Olsen et al., 2004 (45)	24 mo	23/22	I: IS-rich soymilk C: IS-poor soymilk	76; Eq	50–75	Post	LS
Kreijkamp-Kaspers et al., 2004 (46)	12 mo	88/87	I: IS-rich SP C: MP	99; Eq	60–75	Post	LS, TH
Uesugi et al., 2003 (27)	3 mo	11/10	I: IS extract C: Placebo	61.8; Gly	45–65	Peri and post	LS
Potter et al., 1998 (56 mg) (49)	6 mo	22/22	I: SP + IS C: Casein + nonfat dry milk	56; Eq	49–73	Post	LS

(Continued)

TABLE 1 (*Continued*)

Study, year (ref)	Trial length	*n* (I/C)	Intervention	IS (dose, mg; form, Agl, Eq, Gly)	Age, y	Status	DXA site
Potter et al., 1998 (90 mg) (49)	6 mo	22/22	I: SP + IS C: Casein + nonfat dry milk	90; Eq	39–83	Post	LS
Radhakrishnan et al., 2009 (26)	6 mo	44/41	I: SP + IS C: Casein protein	75; Gly	Mean: 49	Post	LS
Morabito et al., 2002 (53)	12 mo	30/30	I: GE C: Placebo	54; Agl	47–57	Peri and post	LS, FN

[1] Agl, aglycone; C, control group; CP, control protein; DE, daidzein; DXA, dual-energy X-ray absorptiometry; Eq, aglycone equivalents; FN, femoral neck; GE, genistein; Gly, glycoside; I, intervention group; IS, isoflavone; LS, lumbar spine; MP, milk protein; Peri, perioperative; Post, postoperative; RC, red clover; ref, reference; SI, soy isoflavone; SP, soy protein; TH, total hip.

Assessment of RCTs determining isoflavone content

An assessment of the 5 articles with a given isoflavone aglycone content of interventions compared with the control was performed. When isolating the results of these RCTs, a substantial beneficial effect was found for both the BMD change outcomes for the lumbar spine (**Figure 6**) and femoral neck (**Figure 7**) in favor of the isoflavone treatment compared with the control. The 5 studies in the pool providing data of the lumbar spine yielded a significant ($P < 0.00001$) effect of isoflavone intake against BMD loss compared with the control, showing the following WMD of BMD change favoring isoflavone: 0.04 g/cm^2 (95% CI: 0.02, 0.05 g/cm^2; I^2, 98%) (31, 40, 41, 47, 53). For the femoral neck, a significant ($P < 0.05$) beneficial effect of isoflavone treatment was found in the 4 studies providing BMD data at the femoral neck and assessing isoflavone content, with the following WMD of BMD change favoring isoflavone: 0.03 g/cm^2 (95% CI: 0.00, 0.06 g/cm^2; I^2, 98%) (31, 40, 41, 53).

Studies excluded from the meta-analysis

A total of 47 RCTs were excluded from the meta-analysis during the full-text assessment. Fifteen original articles were excluded for not having an isoflavone intervention arm (there was prevalent use of self-report methods for estimating isoflavone

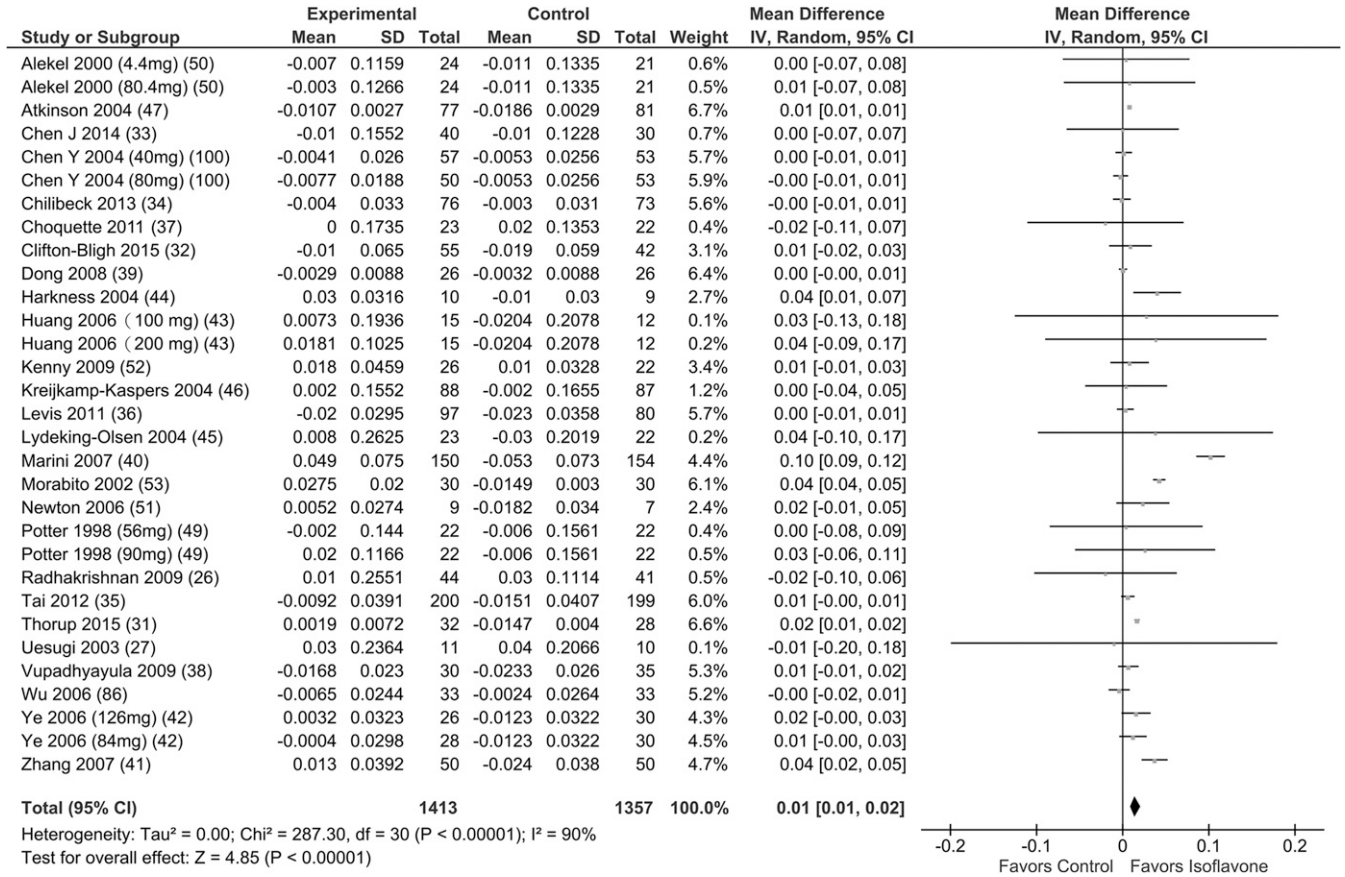

FIGURE 4 Forest plot showing the difference in bone mineral density change at the lumbar spine in all trials ($n = 26$) between isoflavone-administered and control groups. Data calculated from the random-effects model are presented as weighted mean difference and 95% CI. IV, inverse varience.

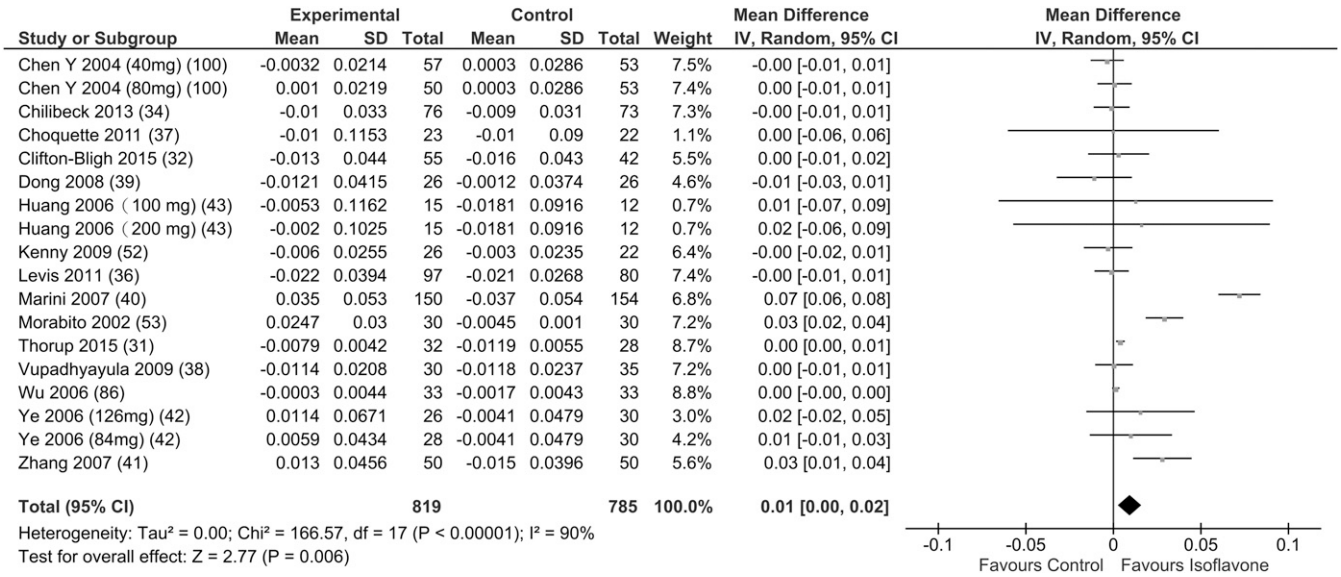

FIGURE 5 Forest plot showing the difference in bone mineral density change at the femoral neck in all trials between isoflavone-administered and control groups (n = 15). Data calculated from the random-effects model are presented as weighted mean difference and 95% CI. IV, inverse varience.

intake) (55–69). Twelve studies were excluded because their study designs were not in line with other aspects of the inclusion criteria (70–81). Twelve RCTs did not provide usable data (i.e., publishing BMD as a percentage, showing BMD changes only graphically, only showing start and end BMD, or taking BMD measurements at other skeletal sites or not with the use of only bone turnover biomarkers) (82–93). Five of the articles were published as congress or conference abstracts without full texts (94–98). Three trials were substudies of other trials already included in the meta-analysis (54, 99, 100).

DISCUSSION

This review provides evidence that isoflavone supplementation can blunt estrogen-deficient BMD resorption on peri- and postmenopausal women. Overall, an equivalent attenuative effect was found for isoflavone treatment on BMD loss at the lumbar spine (0.01 g/cm^2) compared with the femoral neck (WMD: 0.01 g/cm^2). The higher efficacy of isoflavone treatment at the lumber spine could be attributed to a number of factors. It may be caused by differences in bone tissue composition between these sites, with trabecular (spongy) bone comprising a greater proportion of the bone tissue at the lumber spine in contrast to the femoral neck, which incorporates a higher proportion of dense cortical bone tissue (101). Trabecular bone offers a larger surface area, allowing for greater accessibility for estrogenic agents. The affinity of isoflavones for ERβ could also contribute to the enhanced effects at trabecular bone sites, as trabecular and cortical bone retain differential expression profiles of ER isoforms, where trabecular bone retains a higher expression of ERβ:ERα than cortical-rich bone sites that have expressed ERα to a greater extent (102).

The outcomes of other meta-analyses investigating the effects of isoflavones on bone remain inconsistent and controversial. This is most likely due to the high heterogeneity of RCTs in this field of research. One of the most prevalent sources of bias for this group of papers was differences in formulations and the apparent lack of RCTs that use methodologies for validation and standardization of isoflavone content and isoflavone form (methylated forms, glycosides, and aglycones). Research has consistently shown that commercial isoflavone preparations have highly variable isoflavone contents and often do not contain the corresponding isoflavone concentrations or forms claimed on the label (103–105). This represents a key source of bias when all 26 articles are pooled. The present meta-analysis found that RCTS using interventions with a well-controlled isoflavone content and providing isoflavones as aglycones tended to attain enhanced beneficial effect sizes against estrogen-deficient BMD resorption

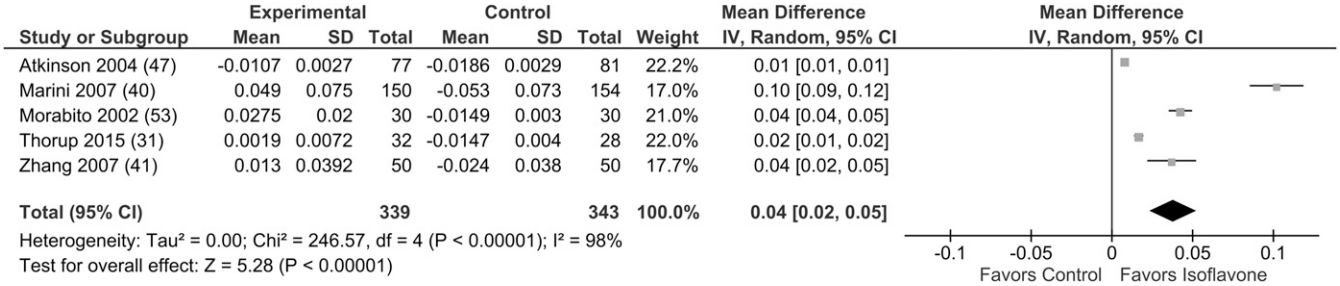

FIGURE 6 Forest plot showing the difference in bone mineral density change at the lumbar spine in studies providing isoflavone aglycones (n = 5) to treatment compared with control. Data calculated from the random-effects model are presented as weighted mean difference and 95% CI. IV, inverse varience.

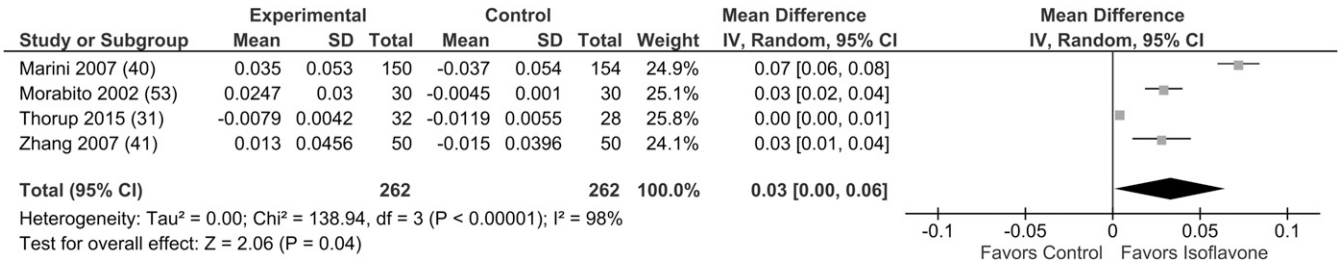

FIGURE 7 Forest plot showing the difference in bone mineral density change at the femoral neck in studies providing isoflavone aglycones (n = 4) to treatment compared with control. Data calculated from the random-effects model are presented as weighted mean difference and 95% CI. IV, inverse varience.

at both the femoral neck (mean difference: 0.04 g/cm^2) and lumbar spine (mean difference: 0.03 g/cm^2) in women compared with RCTs that did not. This both supports and underlines the importance for future RCTs investigating isoflavone and bone health to use higher-quality methods to analyze the contents of test preparations before initiating studies. It is important to consider that there were a limited number of RCTs that actually provided isoflavone aglycones (5); if a greater number of RCTs with determined aglycone content were available, it would go further to confirm these findings. Intestinal isoflavone uptake is dependent on passive diffusion, and isoflavone glycosides require hydrolytic conversion to aglycones to be absorbed; hence, aglycones may have improved bioavailability and thereby enhanced biological efficacy (106). A meta-analysis by Dören (107) found that 2-y treatment with various estrogen therapies exerted equivalent mean improvements to BMD at the spine relative to placebo of ~6.71%. Considering that the mean BMD for women across the menopausal transition has been shown to be ~1.07 g/cm^2 for the lumbar spine, this represents a BMD improvement relative to placebo in the average range of ~0.072 g/cm^2 (3). A reduction of 1 SD in femoral neck BMD has been associated with an increased fracture risk ratio of 2.88 in elderly women (≥65 y). Elderly women have also been demonstrated to retain a mean ± SD femoral neck BMD of 0.72 ± 0.11 (108, 109). As such, the relative BMD increases found in the present meta-analysis of 0.04 g/cm^2 at the spine and 0.03 g/cm^2 at the femoral neck can be considered clinically relevant.

The present meta-analysis supports that isoflavone form appears to be an often-unaccounted for confounding factor that affects many of the current RCTs investigating isoflavones and bone health in women. This may also contribute to the conflicting and inconsistent results of previous meta-analyses (15–17).

There was a high heterogeneity in all of the data pools, with I^2 values ranging from 90% to 98%, leading to the choice of the random-effects model in this analysis. The high I^2 values indicate that the studies substantially differed. Indeed, the findings from the present meta-analysis must be critically interpreted because of the limited number of studies and the great discrepancies in their design. There were substantial confounders across all the RCTs within this meta-analysis. Interventions varied substantially in terms of dose (4.4–300 mg) and the reported molecular form of isoflavones given; these ranged from aglycone equivalents, reporting concentrations for specific isoflavones, to only specifying isoflavone concentration without stating which isoflavone species were included. In some cases, interventions were combined with other components that may influence bone health such as exercise, micronutrient supplementation, and soy and milk proteins. The duration of all studies ranged from 3 to 15 mo; therefore, considering that the bone remodeling cycle lasts ~4–8 mo, longer study durations would be more relevant for determining BMD changes (110). The characterization of participants in the RCTs may also introduce confounding to the current meta-analysis, as peri- and postmenopausal women have differing rates of estrogen-deficient bone loss and may respond differently to estrogenic therapies, with the former losing 2–5% BMD/y and the latter losing 1–2% BMD/y (3, 4). A few RCTs within the analysis did not report blinding of participants or blinding of outcome assessor. All of these factors contribute to the high heterogeneity and confounding. Other differences between the studies were menopause status (definition and determination of peri- and postmenopause); including either peri- or postmenopausal participants alone or mixed; the number of participants (ranging from 16 to 399); and dosing strategies (with or without meal, single dose in the morning or twice daily in the morning and evening). Oral isoflavone supplements have been shown to retain a plasma half-life of 4–8 h, and a twice-daily dose with a meal may have improved efficacy that a single daily dose (103).

This meta-analysis supports the statement that a greater number of longer-duration, high-quality RCTs that use analytic methods to ensure isoflavone concentration with defined molecular forms are required to further elucidate the effects of isoflavone treatments on BMD in women, particularly with regard to isoflavone aglycones. Moreover, the evidence provided by this meta-analysis would be strengthened by RCTs that have more standardized and rigorous study designs. The systematic review identified 10 otherwise useful RCTs that were unable to be included as they opted to publish their BMD results in an unsuitable fashion, often as percentiles, ranges, or medians (rather than mean differences); by only presenting certain data graphically; or by providing only start and end values without specifying the absolute change. These inconsistencies in the publishing of data present a challenge to authors seeking to include RCTs in meta-analyses and inevitably results in smaller data pools and data loss.

In conclusion, our systematic review of existing literature of isoflavone treatments against estrogen-deficient bone loss in women suggests that isoflavone treatments can be effective in preserving BMD and attenuating accelerated bone resorption. In particular, our review emphasizes that RCTs using isoflavone aglycones with well-controlled, standardized, and defined isoflavone interventions appear to achieve greater efficacy when used to treat BMD loss in estrogen-deficient women compared with glycosides and less well-defined isoflavone formulations. Only isoflavone aglycones showed potency against BMD loss; as such,

aglycone content may represent a key factor influencing the outcomes of many RCTs focused on treating bone health in estrogen deficiency.

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