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REVIEW

Soy isoflavones prevent bone resorption and loss, a systematic review and meta-analysis of randomized controlled trials

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ABSTRACT

Background: Osteoporosis is a common bone disease characterized by reduced bone mass resulting from continuous bone resorption.

Methods: PubMed, Scopus, and Embase were searched to find published trials on the effect of soy isoflavones on bone mineral density (BMD) and bone turnover markers (bone-specific alkaline phosphatase, osteocalcin, osteoprotegerin, pyridinoline, deoxypyridinoline, C-telopeptide, and N-telopeptide). Random-effects inverse-variance model was used to calculate the pooled effects.

Results: A total of 5313 articles were found, screened, and assessed for eligibility, and finally 52 trials were included in the meta-analysis. Consumption of soy isoflavones caused significant improvement in BMD of lumbar spine (mean difference (MD) = 0.76%; 95% CI: 0.09, 1.42%; $p = 0.03$), hip (MD = 0.22%; 95% CI: 0.02, 0.42%; $p = 0.04$), and femoral neck (MD = 2.27%; 95% CI: 1.22, 3.31%; $p < 0.001$). Subgroup analysis showed that in all 3 sites, the improvement was significant in normal weight subjects and interventions longer than a year, although trial location and dosage were also factors influencing isoflavones' impact on BMD. Among markers of bone turnover, osteoprotegerin (MD = 5.79; 95% CI: 3.08, 8.51 pg/ml; $p < 0.001$), pyridinoline (MD = −5.13; 95% CI: −7.76, −2.50 nmol/mmol; $p < 0.001$), and C-telopeptides (MD = −0.08; 95% CI: −0.16, −0.00 ng/ml; $p = 0.04$) were favorably affected by isoflavones while osteocalcin and bone alkaline phosphatase did not change. Subgroup analysis of bone markers showed that in overweight/obese individuals and dosages <90 mg/day, isoflavones are more effective.

Conclusions: Soy isoflavones prevent osteoporosis-related bone loss in any weight status or treatment duration. They increase BMD in normal weight subjects and diminish bone resorption in overweight/obese individuals. Although bone resorption may be decelerated over short-term isoflavone consumption, periods longer than a year are probably needed to affect BMD. Isoflavones also appear benefits on bone in any dose or subjects' ethnicity.

KEYWORDS
Isoflavones; bone; bone mineral density; BMD; bone turnover markers; bone resorption

Introduction

Bone is dynamic tissue with continual remodeling throughout the life (Shetty et al. 2016). Bone remodeling or bone turnover includes two phenomena: bone formation and bone resorption (Eastell and Szulc 2017). During growth, the rate of bone formation exceeds that of bone resorption, but at middle and old ages (>40 years) the opposite occurs. Accelerated and continuous bone resorption in old ages leads to osteoporosis and increased risk of fracture which is prevalent and complicated among elderly.

Osteoporosis is characterized by reduced bone mass and highly porous structure associated with increased bone fragility and susceptibility to fracture (Shetty et al. 2016). What makes osteoporosis important is that it is a silent disease that progresses without manifestation of any symptom for years; in the majority of cases, fractures are the first announcement of the disease. Bone mineral density (BMD) measured by dual-energy x-ray absorptiometry has been accepted as the gold standard tool for diagnosis of osteoporosis (Carey and Buehring 2018; Lorentzon and Cummings 2015). However, BMD assessment does not provide detailed information about bone turnover (Vervloet and Brandenburg 2017).

Bone turnover markers are metabolites liberated from osteoblasts and osteoclasts during bone remodeling in the amount relative to the rate of bone turnover (Greenblatt, Tsai, and Wein 2017). These markers are useful for diagnosis of bone metabolic disorders and evaluating the efficiency of anti-osteoporotic treatments (Eastell and Szulc 2017). Most commonly measured bone turnover markers are osteocalcin and bone-specific alkaline phosphatase as markers of bone formation, and pyridinoline, deoxypyridinoline, C-terminal cross-linked type I collagen telopeptide (C-telopeptide), and N-terminal cross-linked type I collagen telopeptide (N-telopeptide) as markers of bone resorption.

Based on cell culture and animal studies, isoflavones, a group of phytochemicals with extensive biological activity,

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stimulate osteoblastic bone formation and inhibit osteoclastic bone resorption (Castelo-Branco and Cancelo Hidalgo 2011; Zheng, Lee, and Chun 2016). Due to structural similarity with estrogen, isoflavones are classified as phytoestrogens, plant compounds that are able to bind to estrogen receptors and imitate estrogen activity (Taku et al. 2011). Soy is the major source of isoflavones. Soy and soy isoflavones have been known as protective compounds for bone against estrogen-deficient states such as postmenopausal bone loss (Taku et al. 2011; Zheng, Lee, and Chun 2016).

Studies on estrogen-deficient animals have documented the ability of soy isoflavones to increase serum concentration of osteocalcin and alkaline phosphatase. In cultured osteoblasts, daidzein demonstrated anti-resorptive activity via stimulation of osteoblast differentiation, increased estrogen receptor β expression, and inhibition of osteoclastogenesis, the last one through decreasing receptor activator of nuclear factor-κB ligand (RANK-L)/osteoprotegerin ratio (De Wilde et al. 2004). In cultured bone marrow cells, genistein has decreased formation or viability of osteoclasts (Sliwiński et al. 2005). In addition, genistein has shown the ability to regulate dozens of bone-related genes (Pie et al. 2006). Soy isoflavones may also improve intestinal calcium absorption and thus reduce bone turnover and loss (Zafar et al. 2004).

A number of past meta-analyses have investigated the effect of soy isoflavones on BMD or bone turnover markers (Ma et al. 2008a; Ma et al. 2008b; Liu et al. 2009; Taku et al. 2010; Ricci et al. 2010; Lambert, Hu, and Jeppesen 2017). However, all but one of these meta-analyses were conducted almost a decade ago. On the other hand, no meta-analysis has yet investigated the effect of soy isoflavones on both BMD and bone turnover markers using various subgroup analysis. This meta-analysis is an update on previous meta-analyses and gives a rather comprehensive insight on various bone turnover markers and BMD sites.

Methods

Search

PubMed, Scopus, and Embase were searched to find published articles on the effect of soy isoflavones on bone turnover and bone density up to December 2018. No limitation on date, language, etc. was made in the search. Main search words included: isoflavone, phytoestrogen, genistein, daidzein, glycitin, soy, soya, soybean, soymilk, bone, bone mineral density, bone mass, bone turnover, bone remodeling. Search strategies are available as supplementary file. Two independent investigators searched the literature, screened titles and abstracts, and assessed articles for eligibility. Disagreements were resolved through discussion with a third investigator.

Eligibility criteria

The articles were screened, evaluated, and selected based on predetermined eligibility criteria. We included controlled trials examining the effect of soy isoflavones on BMD or bone turnover markers. Studies were excluded in case of the followings: 1) participants younger than 18 years; 2) participants with diseases affecting bone health such as ovarian failure; 3) lack of control group; 4) unsuitable control, for instance, administration of calcium/vitamin D supplements to the control without giving them to the treatment group; 5) estrogen therapy in either treatment or control group during the study period; 6) administration of indefinite amounts of isoflavones in the treatment group; 7) considerable difference in the amounts of nutrients administered to treatment and control groups, for instance when unequal quantities of calcium, vitamin D, vitamin K, or other effective compounds were administered; 8) treatments with isoflavones from origins other than soy, such as red clover or synthetic isoflavones; 9) isoflavone treatments combined with other bone-effective compounds such as milk; 10) repeated publications; and 11) reports with insufficient information on mean and standard deviation (SD) or standard error.

Outcomes

Selected locations for BMD were lumbar spine, femoral neck, and total hip because these were the most assessed sites. Less reported sites included trochanter ($n = 9$), Ward's triangle ($n = 6$), intertrochanter ($n = 4$), total femur ($n = 2$), proximal femur ($n = 2$), greater trochanter ($n = 1$), and radius ($n = 1$). The most reported markers of bone remodeling were bone-specific alkaline phosphatase, osteocalcin, pyridinoline, deoxypyridinoline, osteoprotegerin, C-telopeptide, and N-telopeptide. There were also reports on procollagen 1 N-terminal (P1NP) that due to low number of trials ($n = 3$) were not included in this meta-analysis.

Data extraction

In addition to the mean and SD of the outcomes, following information was extracted from selected articles and gathered in an excel sheet: the first and corresponding authors' name, Journal's name, publication year, participants' description (e.g. healthy or osteoporotic, pre- or post-menopausal), participants' age and body mass index, study location and design (parallel or crossover), intervention duration, blinding, randomization, number of participants in each group, the form of treatment (e.g. isoflavones, soy, or soy milk), the dosage of isoflavones, and the substance used as placebo. Data presented as graphs was enumerated by Plot Digitizer software version 2.6.6 (Free Software Foundation Inc., USA).

Risk of bias assessment

The quality of trials was assessed by two investigators according to Cochrane's instructions for risk of bias assessment (Higgins and Green 2011). The criteria used by this tool include: random sequence generation (selection bias), allocation sequence concealment (selection bias), blinding of participants and personnel (performance bias), blinding of outcome assessment (detection bias), incomplete outcome data (attrition bias), selective outcome reporting (reporting bias), and other potential sources of bias.

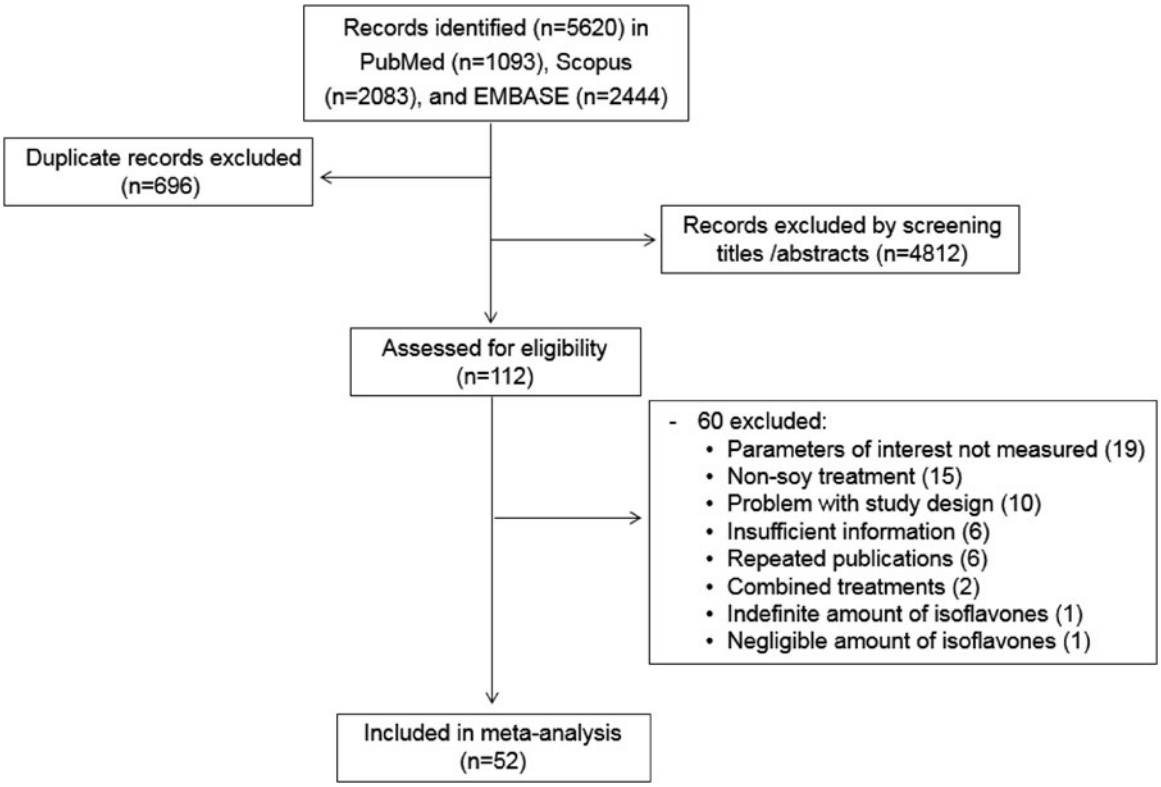

Figure 1. Summary of the screening and selection process of the trials.

Statistical analysis

Mean and SD of the difference between pre- and post-intervention data for control and treatment groups were used to calculate pooled effects. Since some studies had expressed net BMD data and some had presented them as percent change, calculations were performed to convert net data to percentage change. So, BMD data used in the analysis was percentage change. The random-effects inverse-variance model was used to calculate weighted mean difference and 95% confidence interval (CI). Between-study heterogeneity was evaluated using Cochrane χ^2 test and I^2. Publication bias was determined by visual evaluation of funnel plots and Egger's and Begg's tests (Sedgwick 2015). Subgroup analysis was performed based on trial region (Asia *vs.* West), body mass index (BMI) ($<25\,kg/m^2$ *vs.* $\geq 25\,kg/m^2$), isoflavone dose (<90 mg/day *vs.* ≥ 90 mg/day) (Ricci et al. 2010), and intervention length (<1 year *vs.* ≥ 1 year). STATA software version 12.0 (StataCorp, USA) was used for data analysis. $p < 0.05$ was considered statistically significant.

Results

After searching the literature, titles and abstracts were carefully screened to find reports on the effect of soy isoflavones on BMD or bone turnover markers. One hundred and eleven articles passed the screening process and then were more carefully explored through reading their full text. At last, 52 citations were found eligible to be included in the meta-analysis according to the criteria described in the Methods (Figure 1). Ten of the articles were cited more than once because of reports on multiple doses (Alekel et al. 2010; Chen et al. 2003; Cheong et al. 2007; Gallagher et al. 2004; Huang et al. 2006; Potter et al. 1998; Wangen et al. 2000; Ye et al. 2006), study locations (Brink et al. 2008), and participants' bone mass (Arcoraci et al. 2017).

Overall, data of 5313 participants was used in the meta-analysis. All but 16 participants (Newton et al. 2006) were females and mostly postmenopausal. Intervention duration was various, ranging from 1 month to 3 years. The dosages ranged from 36 mg/day to 200 mg/day. Isoflavones were administered in the supplement form, soy protein, or soy foods. More details of the included trials are provided in Table 1.

Effect of soy isoflavones on BMD

Isoflavones caused significant improvement in BMD of lumbar spine (mean difference (MD) of BMD percentage change in isoflavones minus control group = 0.76%; 95% CI: 0.09, 1.42%; $p = 0.03$), hip (MD = 0.22%; 95% CI: 0.02, 0.42%; $p = 0.04$), and femoral neck (MD = 2.27%; 95% CI: 1.22, 3.31%; $p < 0.001$) (Table 2). Subgroup analysis based on trial region (Asia vs. West) revealed that isoflavones increased lumbar spine BMD of Western and hip BMD of Asian populations but femoral neck BMD was improved in both populations. In subgroup analysis based on BMI, normal weight participants benefited from isoflavones for BMD of all sites but overweight/obese subjects only benefited for BMD of femoral neck. In isoflavone dose categorization, doses ≥ 90 mg/day were effective on lumbar spine and hip BMD but doses <90 mg/day were beneficial on femoral neck BMD. Subgroup analysis based on intervention length showed that interventions ≥ 1 year caused significant

Table 1. Characteristics of the trials of the meta-analysis of the effect of isoflavones on bone mineral density and bone turnover markers.

First author (year)	Location	*n*	Participants	Age	BMI (kg/m^2)	Trial length	Dose (mg)	Outcomes
Albertazzi et al. (2005)	Italy	99	Postmenopausal	53.5 ± 3	27 ± 5	6 weeks	90 mg	OC, CTX
Alekel et al. (2000)	USA	45	Perimenopausal	49.8 ± 5.3	24.0 ± 3.3	6 months	80 mg	LS
Alekel et al. (2010) - 80 mg	USA	170	Postmenopausal	54.3	24.6	3 years	80 mg	LS, FN
Alekel et al. (2010) - 120 mg	USA	168	Postmenopausal	54.5	24.5	3 years	120 mg	LS, FN
Anderson et al. (2002)	USA	28	Healthy	23.9 ± 1.1	21.3 ± 3.4	1 year	90 mg	LS, FN
Arcoraci et al. (2017) -Osteoporotic	Italy	121	Osteoporotic postmenopausal	54.4 ± 2.7	25.4 ± 2.9	2 years	54 mg	FN
Arcoraci et al. (2017) -Osteopenic	Italy	268	Osteopenic postmenopausal	54.7 ± 2.5	25.1 ± 3.8	2 years	54 mg	FN
Arjmandi et al. (2003)	USA	42	Postmenopausal	62.1 ± 2.4	32.2 ± 1.8	3 months	40 g	AP, DPyr
Arjmandi et al. (2005)	USA	62	Postmenopausal	54.5 ± 5.5	28.0 ± 1.0	1 year	60 mg	OC, AP, DPyr, LS, H
Atteritano et al. (2007)	Italy	389	Postmenopausal	54.5 ± 0.22	25.1 ± 0.3	2 years	54 mg	OPG
Brink et al. (2008) - Netherland	Netherland	91	Postmenopausal	53 ± 3.5	24.7 ± 1.9	1 year	110 mg	AP, DPyr, Pyr
Brink et al. (2008) - Italy	Italy	78	Postmenopausal	53 ± 3	24.9 ± 2.0	1 year	110 mg	AP, DPyr, Pyr
Brink et al. (2008) - France	France	67	Postmenopausal	54 ± 3	24.1 ± 2.2	1 year	110 mg	AP, DPyr, Pyr
Brink et al. (2008) - Multicenter	Multicenter	237	Postmenopausal	53 ± 3	24.5 ± 2.1	1 year	110 mg	LS
Brooks et al. (2004)	Canada	29	Postmenopausal	53.4 ± 0.8	27.4 ± 1.4	4 months	42 mg	AP, DPyr
Chen et al. (2003) - 40 mg	China	120	Postmenopausal	54.1 ± 3.1	23.9 ± 3.5	1 year	40 mg	LS, FN, H
Chen et al. (2003) - 80 mg	China	113	Postmenopausal	54.3 ± 3.2	24.2 ± 3.6	1 years	80 mg	LS, FN, H
Cheong et al. (2007) - 97.5 mg	USA	13	Postmenopausal	62.2 ± 3.6	31.6 ± 4.4	7 weeks	97 mg	OC, AP, NTX
Cheong et al. (2007) - 135.5 mg	USA	13	Postmenopausal	62.2 ± 3.6	31.6 ± 4.4	7 weeks	135 mg	OC, AP, NTX
Chilibeck et al. (2013)	Canada	149	Postmenopausal	56.6 ± 6.8	27.1	2 years	105 mg	LS, FN, H
Choquette et al. (2011)	Canada	45	Postmenopausal	59 ± 5	29.9 ± 3.2	6 months	70 mg	LS, FN, H
Dalais et al. (2003)	Australia	78	Postmenopausal	60 ± 1	25.4 ± 0.8	3 months	69 mg	Pyr, DPyr
Evans et al. (2007)	USA	22	Postmenopausal	63.2 ± 5.1	26.8	9 months	91 mg	AP, CTX
Gallagher et al. (2004) - 52 mg	USA	31	Postmenopausal	55.9 ± 1.3	26.6	9 months	52 mg	OC, NTX, LS, FN
Gallagher et al. (2004) - 96 mg	USA	33	Postmenopausal	55.2 ± 1.2	26.3	9 months	96 mg	OC, NTX, LS, FN
García-Martín et al. (2012)	Spain	94	Postmenopausal	55.8 ± 6.9	28.4 ± 4.7	1 year	50 mg	OC, AP, OPG, CTX
Harkness et al. (2004)	USA	19	Postmenopausal	70.6 ± 6.3	25.8	6 months	110 mg	OC, AP
Huang et al. (2006) - 100 mg	Taiwan	27	Postmenopausal	52.6 ± 3	23.4 ± 0.7	1 year	100 mg	DPyr, FN
Huang et al. (2006) - 200 mg	Taiwan	27	Postmenopausal	51.6 ± 2.9	23.9 ± 0.8	1 year	200 mg	DPyr, FN
Kenny et al. (2009)	USA	48	Postmenopausal	72.6 ± 5.9	28.1 ± 5.1	1 year	105 mg	AP, NTX, LS, FN
Knight et al. (2001)	Australia	20	Postmenopausal	53.1 ± 4.6	—	3 months	77 mg	OC, AP
Kreijkamp-Kaspers et al. (2004)	Netherland	175	Postmenopausal	66.6 ± 4.8	26.2 ± 3.8	1 year	99 mg	AP, LS, FN, H
Kwak et al. (2009)	Korea	53	Perimenopausal	37.2 ± 4.8	21.7 ± 2.6	3 months	120 mg	OC, DPyr
Lee et al. (2017)	Korea	84	Postmenopausal	53.6 ± 3.4	19–30	3 months	70 mg	OC, AP, DPyr, CTX, NTX
Levis et al. (2011)	USA	177	Postmenopausal	53 ± 3.3	26.3 ± 3.3	2 years	200 mg	NTX, LS, FN, H
Lydeking-Olsen et al. (2004)	Denmark	45	Postmenopausal	57.1 ± 7.6	23.9 ± 3.9	2 years	76 mg	LS
Marini et al. (2008)	Italy	138	Postmenopausal	53.7 ± 2.5	25.1 ± 3.8	2 years	54 mg	AP, OPG, Pyr, DPyr, CTX, LS, FN
Morabito et al. (2002)	Italy	60	Postmenopausal	51.5 ± 3.5	24 ± 2.5	1 year	54 mg	OC, AP, Pyr, DPyr, LS, FN
Mori et al. (2004)	Japan	43	Perimenopausal	40–63	22.3 ± 2.3	1 month	40 mg	OC, DPyr
Newton et al. (2006)	USA	115	Old subjects	65.7 ± 8.3	29.0 ± 4.8	1 year	83 mg	LS, H
Nikander et al. (2004)	Finland	56	Postmenopausal	55 ± 6	26.3 ± 3.3	3 months	114 mg	AP, Pyr, DPyr, NTX
Orsatti et al. (2013)	Brazil	38	Postmenopausal	55.7 ± 6.9	30.3 ± 5.0	9 months	100 mg	OC, CTX, LS, FN
Pérez-Alonso et al. (2017)	Spain	102	Postmenopausal	55 ± 4	25 ± 4	3 months	90 mg	CTX
Potter et al. (1998) - 56 mg	USA	44	Postmenopausal	60.6 ± 7.7	28.7 ± 5.6	6 months	56 mg	LS
Potter et al. (1998) - 90 mg	USA	44	Postmenopausal	61.3 ± 8.3	27.7 ± 4.9	6 months	90 mg	LS
Radhakrishnan, Agarwal, and Vaid (2009)	India	85	Postmenopausal	48.9 ± 6.3	25.5 ± 4.5	6 months	75 mg	LS, FN
Roughead et al. (2005)	USA	13	Postmenopausal	59.9 ± 5.0	26.0 ± 5.0	7 weeks	57 mg	AP
Sathyapalan et al. (2017)	UK	120	Postmenopausal	52.0	26.9 ± 5.8	6 months	66 mg	CTX
Spence et al. (2005)	USA	15	Postmenopausal	57 ± 6	29 ± 7	1 month	65 mg	OC, AP, NTX
Tai et al. (2012)	Taiwan	399	Postmenopausal	55.9 ± 3.8	22.9 ± 2.6	2 years	300 mg	LS, FN
Tit et al. (2018)	Romania	230	Postmenopausal	—	—	1 year	40 mg	DPyr
Turhan et al. (2008)	Turkey	80	Postmenopausal	51.5 ± 5.18	27.0 ± 3.14	6 months	40 mg	OC, CTX
Uesugi et al. (2002)	Japan	23	Postmenopausal	51.4 ± 1.8	22.7 ± 0.9	1 month	62 mg	OC, Pyr, DPyr

Uesugi et al. (2003)	Japan	21	Postmenopausal	53.7 ± 6.9	22.6 ± 2.8	3 months	61.8 mg	LS
Vupadhyayula et al. (2009)	USA	109	Postmenopausal	63.6 ± 0.6	26.1 ± 0.5	2 years	90 mg	NTX, LS, FN
Wangen et al. (2000) - 57 mg	USA	17	Postmenopausal	41.8 ± 5.2	24 ± 2.8	3 months	57 mg	OC, AP, DPyr, CTX
Wangen et al. (2000) - 122 mg	USA	17	Postmenopausal	41.8 ± 5.2	24 ± 2.8	3 months	122 mg	OC, AP, DPyr, CTX
Wu et al. (2006)	Japan	66	Postmenopausal	54.4 ± 2.9	21.1 ± 2.4	6 months	47 mg	LS, FN, H
Wu et al., (2006)	Japan	66	Postmenopausal	54.4 ± 2.9	21.1 ± 2.4	1 year	47 mg	OC, AP, DPyr
Yamori et al. (2002)	Brazil	40	Postmenopausal	53.2 ± 3.5	25.8 ± 3.7	10 weeks	37 mg	Pyr, DPyr
Ye et al. (2006) - 84 mg	China	58	Postmenopausal	52.6 ± 3.4	22.8 ± 2.5	6 months	84 mg	OC, AP, DPyr, LS, FN, H
Ye et al. (2006) - 126 mg	China	56	Postmenopausal	52.2 ± 3.5	22.8 ± 2.3	6 months	126 mg	OC, AP, DPyr, LS, FN, H
Zhou et al. (2011)	USA	63	Premenopausal	22 ± 2.3	22.7 ± 3.9	10 weeks	36 mg	AP, CTX
Zittermann et al. (2004)	Germany	14	Young women	24.0 ± 0.9	24.0 ± 0.9	1 month	52 mg	OC, CTX

OC, osteocalcin; BAP, bone-specific alkaline phosphatase; OPG, osteoprotegerin; Pyr, pyridinoline; DPyr, deoxypyridinoline; CTX, C-terminal cross-linked type I collagen telopeptide; NTX, N-terminal cross-linked type I collagen teleopeptide; FN, femoral neck bone mineral density; H, hip bone mineral density; LS, lumbar spine bone mineral density.

increase in BMD of all sites but shorter interventions were not effective. There was a high between-study heterogeneity in the results, and subgrouping did not resolve this heterogeneity (I^2 = 76.4%–99.9%).

Effect of soy isoflavones on biomarkers of bone turnover

Osteocalcin, one of the most important biomarkers of bone formation, was not significantly changed following isoflavone treatment (MD = 0.08 ng/ml; 95% CI: −0.72, 0.88 ng/ml; $p = 0.85$) (Figure 2). Similarly, bone-specific alkaline phosphatase showed no significant change over isoflavone treatment (MD = 0.14 μg/L; 95% CI: −0.87, 1.14 μg/L; $p = 0.79$) (Figure 3). Only 3 studies measured osteoprotegerin; they showed a significant elevation (MD = 5.79 pg/ml; 95% CI: 3.08, 8.51 pg/ml; $p < 0.001$) (Figure 4). Contrariwise, pyridinoline demonstrated a significant reduction (MD = −5.13 nmol/mmol; 95% CI: −7.76, −2.50 nmol/mmol; $p < 0.001$) (Figure 5) and deoxypyridinoline demonstrated a trend towards reduction upon isoflavone supplementation (MD = −0.54 nmol/mmol; 95% CI: −1.19, 0.11 nmol/mmol; $p = 0.10$) (Figure 6). C-telopeptide (MD = −0.08 ng/ml; 95% CI: −0.16, −0.00 ng/ml; $p = 0.04$) (Figure 7) and N-telopeptide (MD = −2.27 nmol/mmol; 95% CI: 6.80, 2.27 nmol/mmol; $p = 0.33$) (Figure 8) also showed reductions although only the change in C-telopeptide was statistically significant. Similar to BMD, results on bone markers had high between-study heterogeneity except for osteoprotegerin which showed a moderate heterogeneity.

Subgroup analysis was performed for 3 of the markers which had responded to isoflavones, i.e. pyridinoline, deoxypyridinoline, and C-telopeptide (Table 3). Subgroups of participants with BMI $\geq$ 25 kg/m^2 and isoflavone doses <90 mg showed reduction in all 3 markers. Also, subgroups of Western populations and intervention durations <1 year demonstrated decreases in pyridinoline and deoxypyridinoline. Again, high heterogeneity was observed between studies.

Quality of the trials

Risk of bias was assessed according to the criteria described in the Methods section (Supplemental Table 1 and Figure 1). Twenty five percent of the trials had risk of bias due to high attrition which is likely due to long duration of the interventions for bone protection against osteoporosis.

Publication bias

Except for femoral neck BMD (Egger's test $p = 0.039$), no publication bias was detected in the investigated outcomes. Funnel plots are available in supplementary materials (Supplemental Figures 2A–C and 3A–G).

Discussion

Results of this meta-analysis showed that consumption of soy isoflavones caused significant improvement in BMD of

femoral neck, lumbar spine, and hip. The improvement was more pronounced in normal weight subjects and interventions longer than one year although trial location and dosage were also factors influencing isoflavones' impact on BMD. Among markers of bone turnover, osteoprotegerin, pyridinoline, and C-telopeptides were affected by isoflavones while osteocalcin and bone alkaline phosphatase did not change. Subgroups of overweight/obese individuals and dosages <90 mg/day benefited more from the effect of isoflavones on bone biomarkers.

Participants' weight

Results of subgroup analysis of this meta-analysis showed that although isoflavones benefited BMD of both normal and overweight subjects, the effect was significant in normal weight individuals, indicating that isoflavones may have potential to compensate, in normal weight subjects, the lack of protection which could be provided by extra weight. Weight is generally thought to have a protective effect on bone mass, and overweight and obese individuals are assumed to be at lower risk of bone loss (Shapses and

Table 2. Subgroup analysis for the effect of soy isoflavones on bone mineral density of lumbar spine, hip, and femoral neck.

	Lumbar spine				Total hip				Femoral neck			
	n	Mean difference (95% CI)	*p*	I^2	*n*	Mean difference (95% CI)	*p*	I^2	*n*	Mean difference (95% CI)	*p*	I^2
All studies	30	0.76 (0.09, 1.42)	0.03	99.6%	11	0.22 (0.02, 0.42)	0.04	91.7%	22	2.27 (1.22, 3.31)	<0.001	99.7%
Subgroups												
Trial region												
Asia	8	−0.3 (−0.73, 0.14)	0.18	96.6%	5	0.20 (0.16, 0.25)	<0.001	0	6	0.68 (0.23, 1.14)	0.003	93.7%
West	22	1.07 (0.01, 2.14)	0.048	99.7%	6	0.22 (−0.32, 0.75)	0.43	95.4%	16	2.61 (0.70, 4.52)	0.007	99.8%
Participants' BMI												
<25 kg/m^2	14	0.61 (0.15, 1.07)	0.009	97.7%	5	0.20 (0.16, 0.25)	<0.001	0	8	0.67 (0.11, 1.24)	0.02	97.4%
≥25 kg/m^2	16	0.86 (−0.44, 2.15)	0.20	99.8%	6	0.22 (−0.32, 0.75)	0.43	95.4%	14	2.99 (0.86, 5.11)	0.006	99.8%
Isoflavone dose												
<90 mg/day	16	1.02 (−0.10, 2.14)	0.07	99.6%	7	−0.02 (−0.26, 0.22)	0.85	90.8%	11	4.34 (2.19, 6.48)	<0.001	99.9%
≥90 mg/day	14	0.59 (0.08, 0.89)	0.02	95.6%	4	0.54 (0.23, 0.85)	0.001	82.6%	11	0.00 (−0.43, 0.44)	0.98	94.1%
Intervention duration												
<1 year	13	0.03 (−1.03, 1.09)	0.95	98.7%	4	−0.20 (−1.06, 0.65)	0.64	76.4%	8	0.53 (−0.15, 1.22)	0.13	92.5%
≥1 year	17	1.30 (0.43, 2.16)	0.003	99.7%	7	0.38 (0.23, 0.54)	<0.001	88.3%	14	3.16 (1.57, 4.75)	<0.001	99.8%

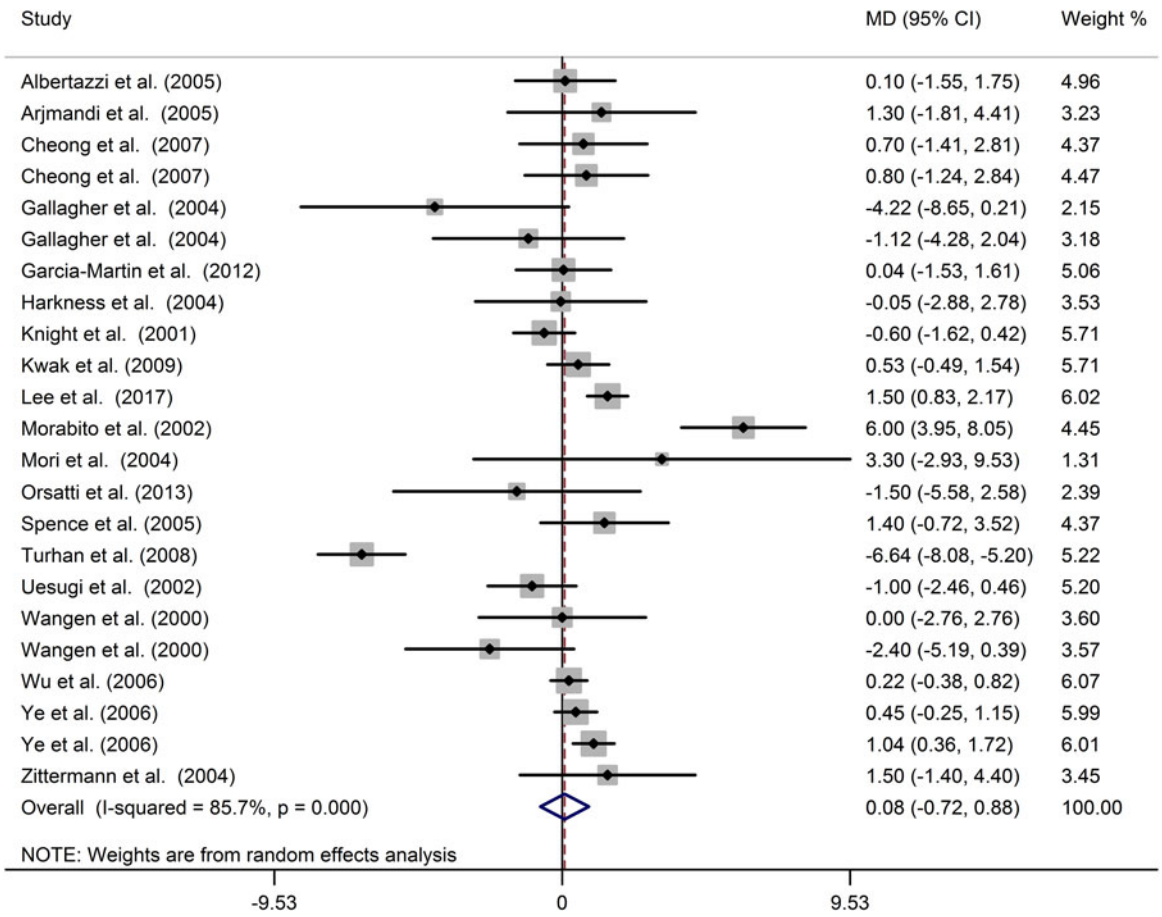

Figure 2. Forest plot of clinical trials examining the effect of soy isoflavones on osteocalcin. Data are mean difference and 95% CI using the random-effects model.

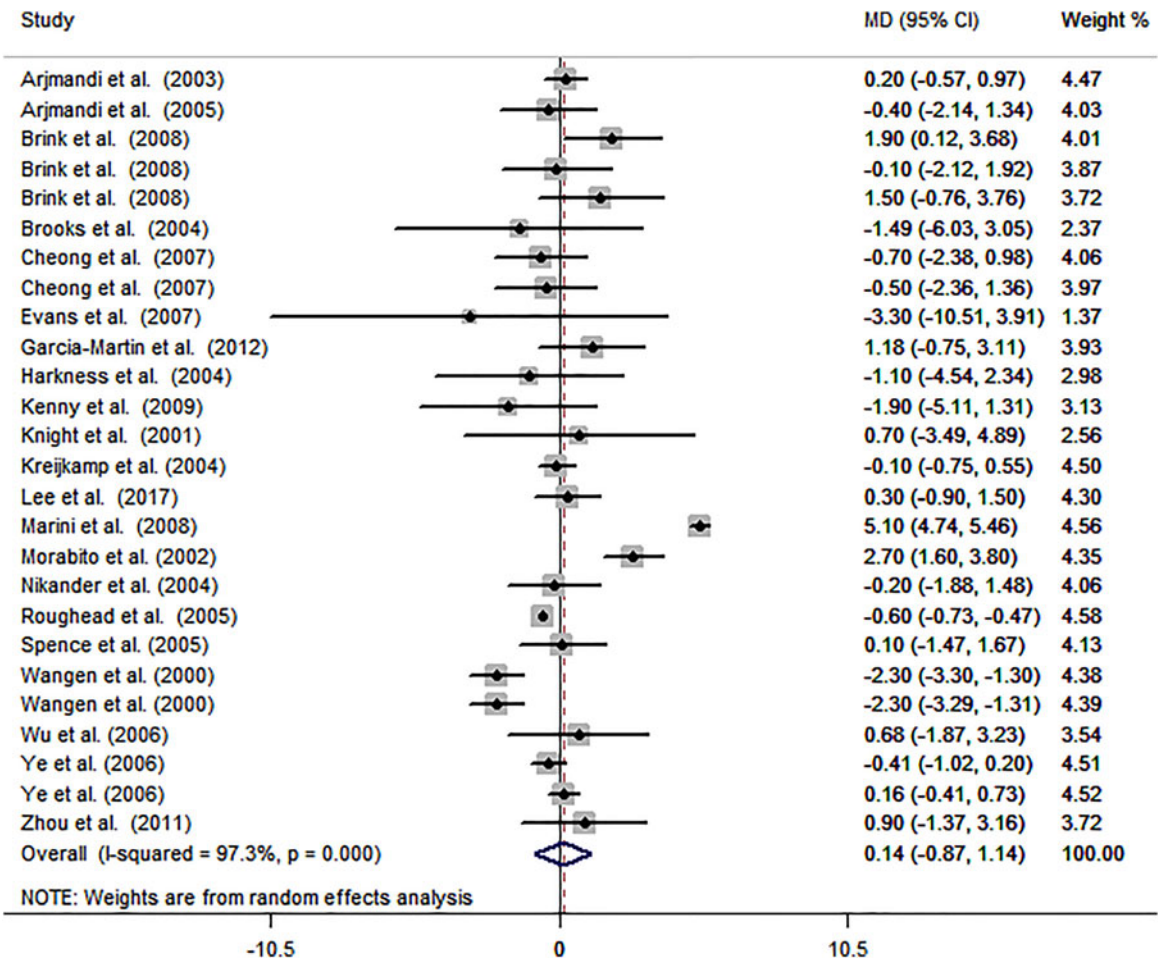

Figure 3. Forest plot of clinical trials examining the effect of soy isoflavones on bone-specific alkaline phosphatase. Data are mean difference and 95% CI using the random-effects model.

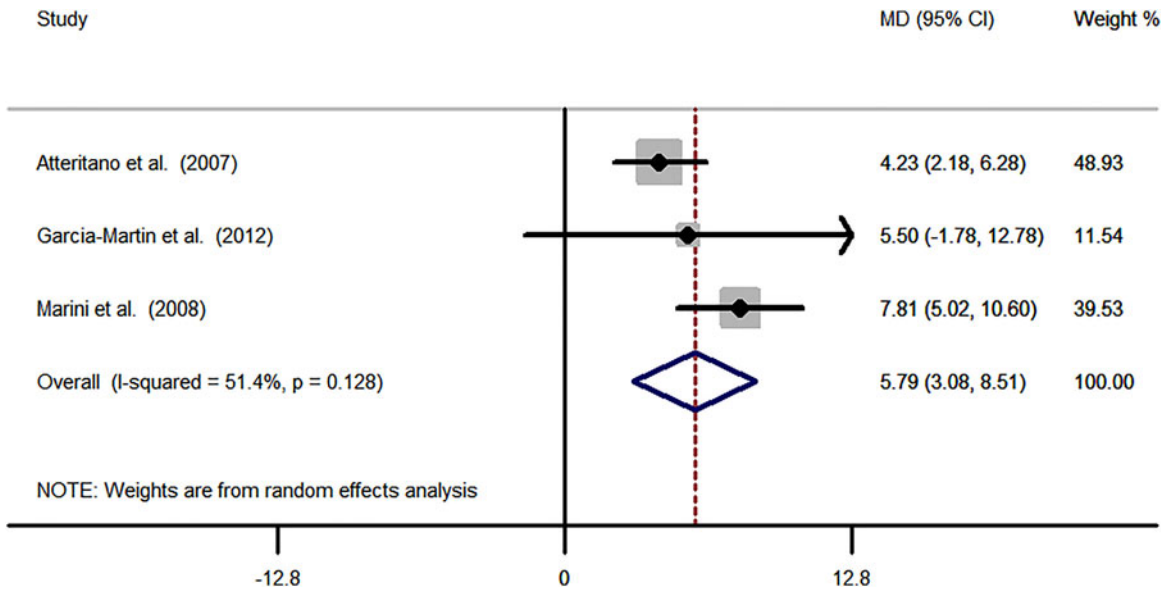

Figure 4. Forest plot of clinical trials examining the effect of soy isoflavones on osteoprotegerin. Data are mean difference and 95% CI using the random-effects model.

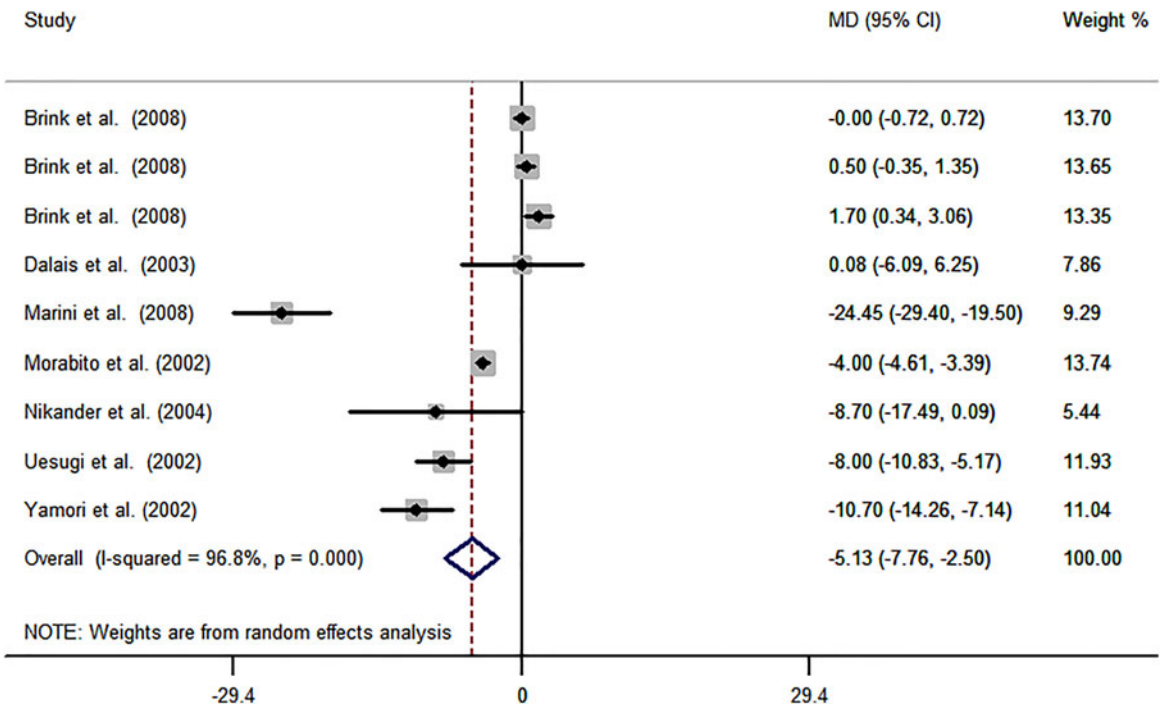

Figure 5. Forest plot of clinical trials examining the effect of soy isoflavones on pyridinoline. Data are mean difference and 95% CI using the random-effects model.

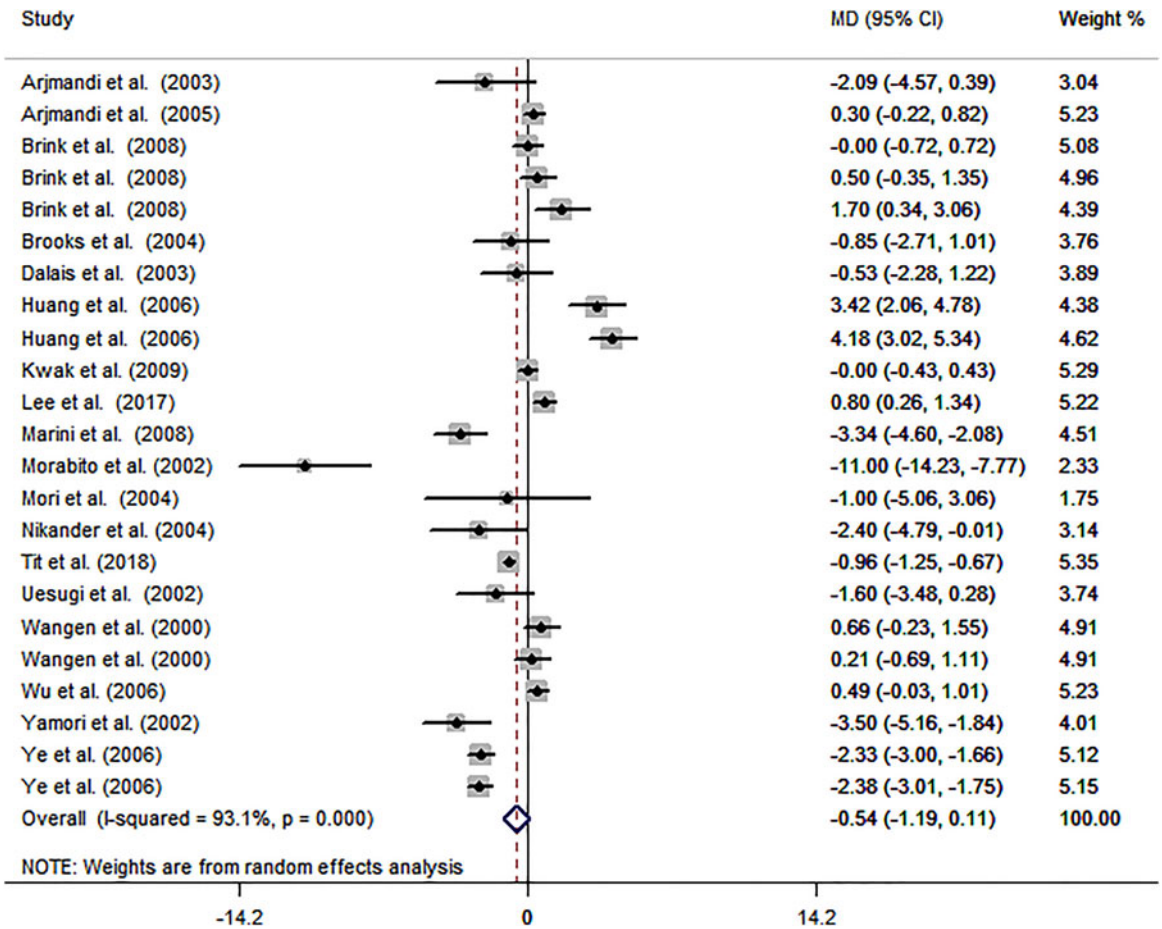

Figure 6. Forest plot of clinical trials examining the effect of soy isoflavones on deoxypyridinoline. Data are mean difference and 95% CI using the random-effects model.

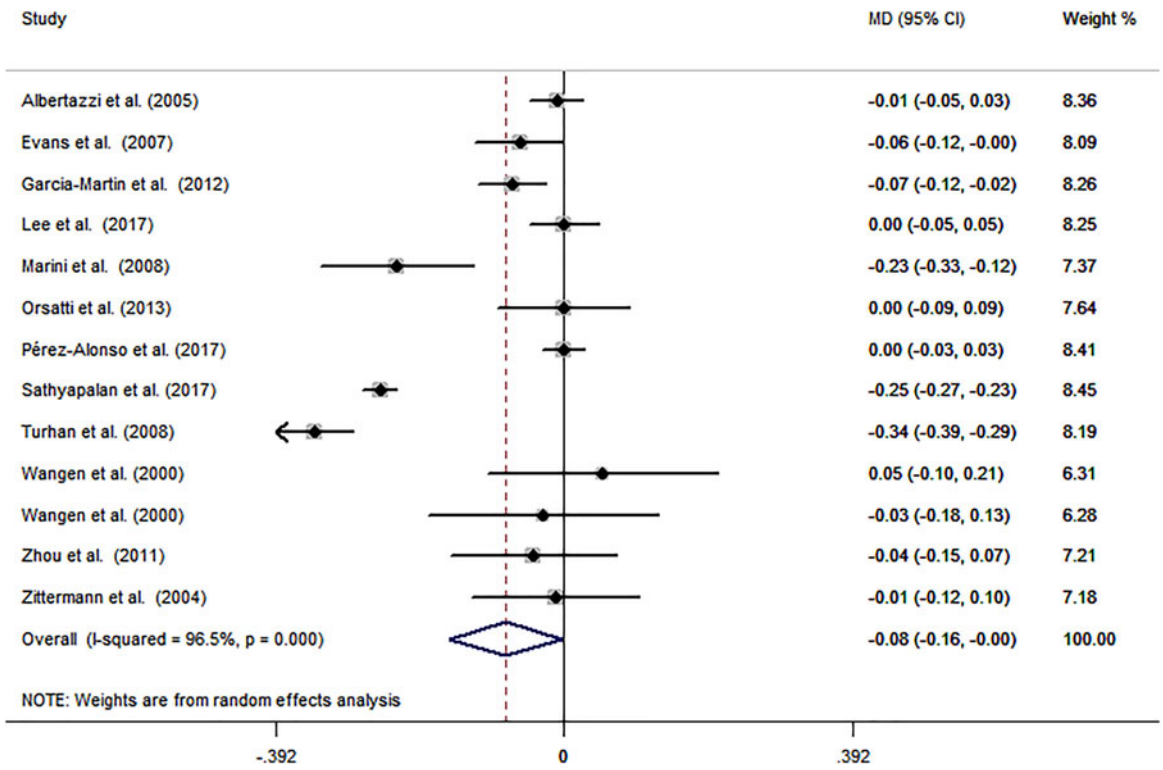

Figure 7. Forest plot of clinical trials examining the effect of soy isoflavones on C-telopeptide. Data are mean difference and 95% CI using the random-effects model.

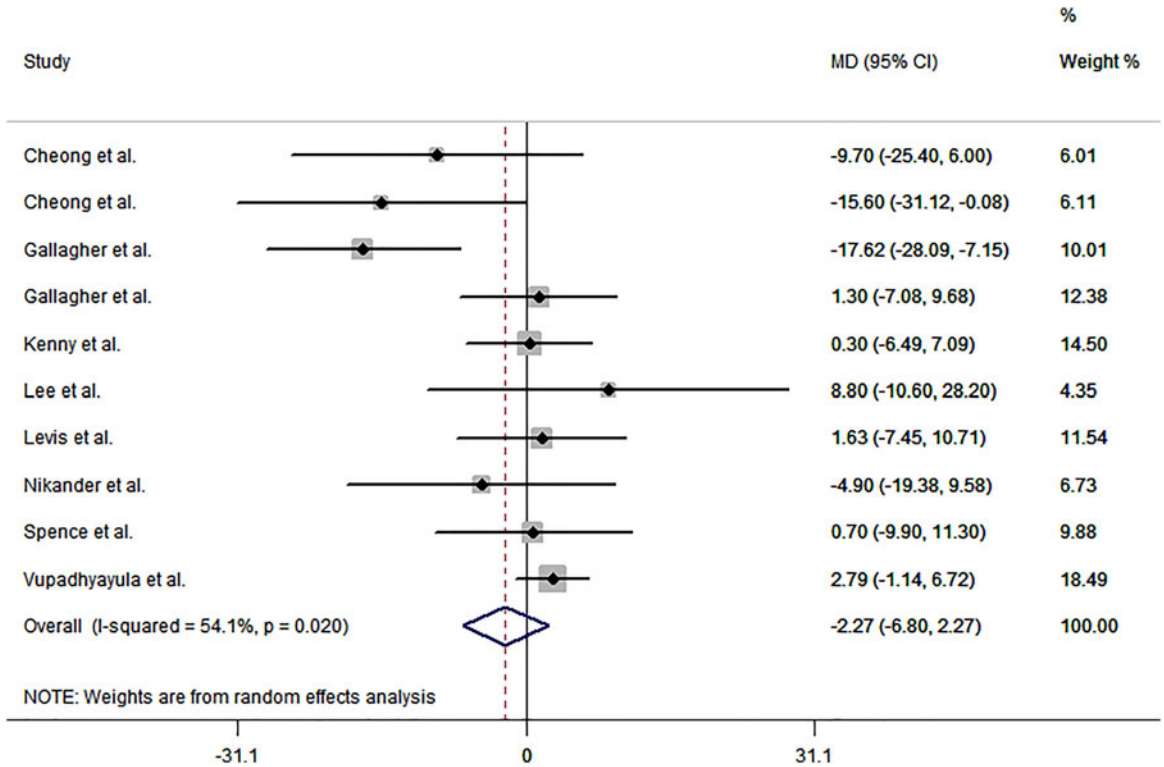

Figure 8. Forest plot of clinical trials examining the effect of soy isoflavones on N-telopeptide. Data are mean difference and 95% CI using the random-effects model.

Table 3. Subgroup analysis for the effect of soy isoflavones on pyridinoline, deoxypyridinoline, and C-telopeptide.

	Pyridinoline				Deoxypyridinoline				C-telopeptide			
	n	Mean difference (95% CI)	*p*	I^2	*n*	Mean difference (95% CI)	*p*	I^2	*n*	Mean difference (95% CI)	*p*	I^2
All studies	9	−5.13 (−7.76, −2.50)	<0.001	96.8%	23	−0.54 (−1.19, 0.11)	0.11	93.1%	13	−0.08 (−0.16, −0.00)	0.043	96.5%
Trial region												
Asia	1	−8.00 (−10.83, −5.17)	<0.001	–	9	0.23 (−1.00, 1.45)	0.72	95.8%	2	−0.17 (−0.50, 0.16)	0.32	98.9%
West	8	−4.71 (−7.46, −1.95)	0.001	97.0%	14	−1.01 (−1.78, −0.23)	0.01	89.3%	11	−0.06 (−0.14, 0.02)	0.13	96.0%
Participants' BMI												
<25 kg/m^2	5	−1.75 (−4.27, 0.78)	0.18	97.2%	14	−0.17 (−1.16, 0.81)	0.73	94.5%	4	−0.01 (−0.08, 0.05)	0.70	0
≥25 kg/m^2	4	−11.11 (−21.02, −1.19)	0.03	92.5%	9	−1.19 (−2.10, −0.29)	0.01	89.6%	9	−0.11 (−0.20, −0.01)	0.02	97.6%
Isoflavone dose												
<90 mg/day	5	−9.37 (−15.27, −3.47)	0.002	95.3%	14	−1.34 (−2.15, −0.52)	0.001	92.1%	8	−0.12 (−0.22, −0.02)	0.02	95.9%
≥90 mg/day	4	0.49 (−0.57, 1.56)	0.36	66.2%	9	0.61 (−0.59, 1.81)	0.32	94.3%	5	−0.01 (−0.03, 0.01)	0.31	0
Intervention duration												
<1 year	4	−7.25 (−11.26, −3.25)	<0.001	66.0%	13	−1.04 (−1.90, −0.19)	0.02	89.7%	11	−0.07 (−0.16, 0.02)	0.14	97.1%
≥1 year	5	−3.84 (−6.95, −0.74)	0.02	98.1%	10	−0.00 (−1.09, 1.08)	0.99	95.4%	2	−0.14 (−0.29, 0.01)	0.07	86.3%

Sukumar 2012; Dimitri et al. 2012; Kim et al. 2017). A part of the protective effect of weight on bone is suggested to be due to mechanical loading of excess body weight (Shapses and Sukumar 2012). Furthermore, adipose tissue releases hormones such as estrogen and leptin which have shown to stimulate osteoblasts and/or inhibit osteoclasts. Nonetheless, a number of investigations have documented that fat mass may not have beneficial effect on bone mass, but instead may have a deleterious impact (Messina et al. 2019; Zhu et al. 2017; Zhao et al. 2007). We also found that in overweight/obese individuals isoflavones caused significant reduction in bone resorption markers, pyridinoline, deoxypyridinoline, and C-telopeptide, indicating that consumption of isoflavones also benefits the bone of people with extra weight. These results suggest that the protective effect of isoflavones on bone is not limited to specific weight.

Intervention duration

Only interventions longer than 1 year showed significant improvement in BMD of all sites, but both intervention durations revealed diminution of bone resorption markers although merely some of these subgroups were statistically significant. This suggests that any intervention duration may be efficacious to inhibit bone resorption but interventions ≥1 year are long enough to affect BMD.

Bone remodeling consists of five phases: osteoclast activation, bone resorption, osteoblast recruitment, bone matrix formation, and mineralization (termination phase) (Katsimbri 2017). The first 3 phases last for about 6 weeks with resorption phase taking one third of this time, formation phase takes approximately 4 months, and mineralization continues for 3–4 months (Katsimbri 2017; Kenkre and Bassett 2018). The shortest intervention time among the trials of this meta-analysis was 1 month, which is longer than bone resorption phase, suggesting that all of the interventions have been long enough to allow affecting bone resorption by isoflavones. In addition, calcium kinetic studies have documented that even interventions as short as 3 days can affect different bone remodeling phases (Shahnazari et al. 2010). Human trials have also shown that interventions as short as 7 days can modify bone turnover markers including osteocalcin (Sirtori et al. 1996). Therefore, the lack of effect on bone formation biomarkers could not be due to short intervention length in some trials although subgroups analysis for bone alkaline phosphatase showed that interventions shorter than 4 months diminished alkaline phosphatase while longer interventions made no change (data not shown). Such result was not observed for osteocalcin.

Dosage

The effect of dose may be explained by the balance in differentiation of bone marrow stromal cells into osteoblasts and adipocytes (Shapses and Sukumar 2012). Cell culture studies have shown that lower doses of daidzein stimulated osteogenesis and decreased adipogenesis but higher doses behaved contrarily (Dang and Löwik 2004). This type of dose-dependent effect was observed in bone turnover markers (pyridinoline, deoxypyridinoline, and C-telopeptide) of the present meta-analysis and also BMD of lumbar spine and femoral neck but not in total hip BMD, suggesting that doses less than 90 mg/day might prevent bone resorption and improve BMD of lumbar spine and femoral neck but higher doses may also be beneficial for hip and lumbar spine BMD.

Trial region

Subgroup analysis based on region suggested that both Asians and non-Asian populations may benefit from isoflavones in BMD of different sites but non-Asians may get more advantages in decreasing bone resorption markers, pyridinoline and deoxypyridinoline. There are reasons that explain the effectiveness of isoflavones in various populations. For instance, compared to Western populations, Asians are reported to have a higher percentage of equol producers who are individuals capable of intestinal conversion of isoflavones to their active and absorbable metabolite, equol (Song et al. 2006; Vergne et al. 2009). On the other hand, the habitual intake of soy by Asians may diminish the effectiveness of collateral soy isoflavone consumption and lower benefits of isoflavones supplements in this population (Maskarinec et al. 2017). Hence, people in each ethnicity can benefit from isoflavones for either BMD or bone

turnover markers and there are genetic or non-genetic reasons that justify this responsiveness.

High heterogeneity

We observed a high heterogeneity between trials which was not resolved by subgroup analysis, suggesting a multifactorial essence for the heterogeneity. Although the majority of the trials recruited peri- or postmenopausal women, the participants were not all in the same menopausal age. It is very likely that small difference in the level of estrogen in these women have affected the results (Rapuri, Gallagher, and Haynatzki 2004). As explained earlier, participants' weight and ethnicity also affect bone metabolism. Diversities in study design, such as form of isoflavones (glycosides or aglycones), isoflavones composition (proportion of different flavonoids in the supplements), dosage, form of treatment (pure compounds vs. soy foods), intervention length, and habitual intake of isoflavones in the study populations added more to the extent of the heterogeneity. It is likely that the influence of these factors on the results is too broad and complicated to be solved by subgroup analysis.

This high heterogeneity and controversy also exists in the results of different meta-analyses published on the issue. For instance, while some meta-analyses found no effect from isoflavones on lumbar spine BMD (Ricci et al. 2010; Liu et al. 2009) others reported benefits for spine (Ma et al. 2008a) and both femoral neck and lumbar spine (Lambert, Hu, and Jeppesen 2017). Results of subgroup analysis have also been conflicting. For instance, Ma et al. (2008a) reported more significant favorable effects in doses >90 mg/day, interventions >6 months, and Western populations while Liu et al. (2009) and Ricci et al. (2010) found no effect from subgroups of dose, ethnicity, and intervention duration on lumbar spine BMD. The controversy in the results of these meta-analyses is likely due to differences in their inclusion/exclusion criteria, publication date, and cutoff points used for subgroup analysis.

Clinical significance

Although results of this and other relevant meta-analyses are positive for the beneficial effects of isoflavones on BMD and bone resorption markers, the clinical significance of the effects is unclear. What is clinically important is whether isoflavones can protect bone from osteoporotic fractures. Although no meta-analysis has yet evaluated such effect, cohort studies have shown the inverse association between soy/isoflavone consumption and bone fracture. For instance, a prospective cohort of 63,257 Chinese men and women living in Singapore indicated that in follow-up durations of more than 5 years, compared to women, but not men, in the lowest quartile of intakes for tofu equivalents (<49.4 g/day), soy protein (<2.7 g/day), and isoflavones (<5.8 mg/1000 kcal/day), those in the second to fourth quartiles exhibited 21% to 31% reduction in the risk of hip fracture (Koh et al. 2009). Considering the minimum dose of 5.8 mg/1000 kcal reported by Koh et al., the doses tested in trials of this meta-analysis are likely to have potential for protecting bones against fracture.

Among isoflavones, genistein has the highest binding affinity for estrogen receptor, with having 13%–87% of the estradiol potential for binding to estrogen receptor (Kuiper et al. 1998; Pilšáková, Riečanský, and Jagla 2010). At the highest accessible levels which occur after consumption of soy-rich meals, genistein may exhibit estrogenic activity comparable to endogenous estradiol. This effect is especially dominant in estrogen-deficient individuals, such as postmenopausal women, for whom isoflavones can be used as an alternative for hormone replacement therapy (Pilšáková, Riečanský, and Jagla 2010). In this regard, a meta-analysis was performed to evaluate and compare the effect of estrogen or isoflavones on the incidence of osteoporotic fractures in postmenopausal women (Bolaños and Francia 2010). The results indicated that there was no statistically significant difference between hormone therapy and isoflavones in reducing osteoporosis-related fractures.

Adverse effects

Structural and functional similarity between isoflavones and estrogen poses the concern about possible adverse effect of isoflavones for increasing the risk of breast cancer. However, available evidence supports the opposite: recent meta-analyses of cohort (Zhao et al. 2019) or observational (Qiu and Jiang 2018) studies have shown an inverse association between intake of soy and isoflavones with the risk of development (Zhao et al. 2019) or overall survival from breast cancer (Qiu and Jiang 2018). However, caution needs to be taken for use of high doses of isoflavones in women with a family history of breast cancer (Touillaud et al. 2019).

Limitations

As stated, broad between-study heterogeneity was the major limitation in this and similar meta-analyses. Furthermore, to study the dose effect, a number of trials with two isoflavone groups were included in the meta-analysis, and thus the control group was used twice, causing an overestimation of the statistical significance. This meta-analysis had advantages over the previous works due to assessing BMD of three important sites with a rather extensive view on bone turnover markers, and subgroup analysis on both BMD and bone turnover markers. It is an update on the previous works and contains almost twice the number of trials of the last meta-analysis.

Conclusion

Results of this meta-analysis showed that consumption of soy isoflavones benefits BMD in normal weight people and diminishes bone resorption in overweight/obese individuals. Although bone resorption can be decelerated over short-term isoflavone consumption, periods longer than a year are probably needed to affect BMD. Isoflavones also appear benefits on bone in any dose and subjects' ethnicity. More

studies, however, are needed to confirm the lack of the effect of isoflavones on bone formation biomarkers and clarify underlying mechanisms in humans.

Disclosure statement

No potential conflict of interest was reported by the authors.

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