Original Article

Effect of soy isoflavone extract supplements on bone mineral density in menopausal women: meta-analysis of randomized controlled trials

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This study was conducted to clarify the effect of ingesting soy isoflavone extracts (not soy protein or foods containing isoflavones) on bone mineral density (BMD) in menopausal women. PubMed, CENTRAL, ICHUSHI, CNKI, Wanfang Data, CQVIP, and NSTL were searched for randomized controlled trials published in English, Japanese, or Chinese reporting the effects of soy isoflavone extracts on lumbar spine or hip BMD in menopausal women. Trials were identified and reviewed for inclusion and exclusion eligibility. Data on study design, participants, interventions, and outcomes were extracted. Eleven, seven, five, and five trials were finally selected for estimation of the effects on spine, femoral neck, hip total, and trochanter BMD, respectively. Meta-analysis including data from1240 menopausal women revealed that daily ingestion of an average of 82 (47–150) mg soy isoflavones (aglycone equivalent) for 6–12 months significantly increased spine BMD by 22.25 mg/cm^2 (95% CI: 7.62, 32.89; p=0.002), or by 2.38% (95% CI: 0.93, 3.83; p=0.001) compared with controls (random-effects model). Subgroup analyses indicated that the varying effects of isoflavones on spine BMD across trials might be associated with study characteristics of intervention duration (6 vs. 12 months), region of participant (Asian vs. Western), and basal BMD (normal bone mass vs. osteopenia or osteoporosis). No significant effects on femoral neck, hip total, and trochanter BMD were found. Soy isoflavone extract supplements increased lumbar spine BMD in menopausal women. Further studies are needed to address factors affecting the magnitudes of effect on spine and to verify the effect on hip.

Key Words: meta-analysis, isoflavones, dietary supplements, menopause, bone density

INTRODUCTION

Osteopenia and osteoporosis are major health problems in postmenopausal women, who experience a sharp decrease in estrogen concentration that leads to an increased rate of bone remodeling.[1,2] The yearly decline in bone mineral density (BMD) of the lumbar spine and hip in postmenopausal women is reported to be at least 1% and up to 2.4%.[1,3] Although hormone replacement therapy (HRT) has positive effects in increasing BMD in postmenopausal women with low bone mass,[1,4] it is associated with a higher risk of hormone-related cancer[5-7] and other unfavorable adverse events.[8,9]

Epidemiological studies indicate that women who have high soy intake have a lower risk of osteoporosis than women who consume a typical Western diet.[10-12] Consequently, many menopausal women use phytoestrogens to maintain their BMD because they are unlikely to cause the undesirable effects associated with steroid hormones.[8,13] The primary dietary phytoestrogens ingested are soy isoflavones, which have structures similar to that of estrogen.[14]

A meta-analysis of randomized controlled trials (RCTs) has estimated the effect of ingesting soy isoflavones on lumbar spine BMD.[15] This included 10 RCTs of both soy isoflavone tablets and isolated soy protein containing isoflavones, and revealed a significant increase of BMD by 20.6 mg/cm^2 (magnitude in term of percentage and effect on hip not presented) resulting from soy isoflavones. Given the result in units of mg/cm^2, whether the magnitude of increase can prevent the naturally occurring postmenopausal bone loss remains unclear. Subgroup analysis of three trials testing isoflavone tablet revealed no significant effect, however one trial testing soy isoflavone extract was mistakenly included in the isolated soy

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protein subgroup.[16] In addition, two[17] and three[18] comparisons from the same trial respectively with two and three soy isoflavone groups compared to the same control group were included simultaneously as separate studies in the meta-analysis. This is not recommended because it is considered to induce a serious unit-of-analysis problem.[19] Another recently published meta-analysis included 10 RCTs of soy isoflavones supplementation of at least one year duration (four RCTs testing isoflavones extracts), and did not find significantly beneficial effects of soy isoflavones on spine and hip BMD.[20]

Supplements of soy isoflavone extracts were easily ingested by the people who want to benefit from soy isoflavones, but are unable to usually consume and/or do not like to intake products of soy protein or soy foods containing isoflavones. In addition, the beneficial effects of soy protein might require synergistic reactions between isoflavones and other soy components.[15] Thus, clarifying the effects of extracted soy isoflavones (not as a constituent part in soy protein) is of more clinically important. However, both the two meta-analyses failed to reveal significant effects of soy isoflavone extracts in subgroup analysis, which might be due to the fact that only data from four RCTs were included.[15,20] We have identified 12 RCTs of soy isoflavone extracts (not of soy protein or foods containing isoflavones) that reported effects on spine BMD in menopausal women,[8,16-18,21-29] and performed the present meta-analysis to clarify the effects of soy isoflavone extract both in terms of change (mg/cm^2) and percentage change (%) from baseline for lumbar spine and hip BMD, without influence on the same parameters by soy protein per se or other components in soy protein.

MATERIALS AND METHODS

PubMed (1966–2008), CENTRAL (1966–2008), ICHUSHI (1983–2008), and CNKI (1979–2008) were searched for relevant studies that had been published by September 2008. We also searched Wanfang Data, CQVIP and NSTL, which are other major search engines in China. Reference lists of relevant studies were manually searched. Studies were eligible for inclusion if they met all of the following criteria: (1) randomized parallel-group controlled trials published in English, Japanese, or Chinese; (2) trials with a crossover design that contained data for the first period;[19,30] (3) tested the effects of ingesting supplements of soy isoflavone extracts (not of soy protein or foods containing isoflavones) on lumbar spine or hip (femoral neck, total hip, or trochanter) BMD in menopausal women; and (4) BMD data were measured by dual X-ray absorptiometry. When duplicate data were reported for the same study subjects, only the article with the largest sample was included.[19] Two reviewers independently reviewed and evaluated the studies, and consensus was reached by discussion when there were disagreements.

Data on study design, number of participants, interventions, and outcomes for BMD were also independently extracted by two reviewers and confirmed by each other. When necessary, data on outcomes for BMD were obtained from graphs. If possible, we obtained necessary data not reported in the articles by contacting to the authors. We calculated mean change (follow-up − baseline) and percentage change [(follow-up − baseline) ÷ baseline × 100%] from baseline in BMD, when the data were not directly available. We primarily determined missing SD of the changes if statistical analyses comparing the changes themselves were presented (e.g., confidence intervals, standard errors, *t* values, *p* values, F values). Alternatively, we imputed them by computing mean correlations between the baseline and final values from included trials in which SD for change, as well as for baseline and final measurements were available.[19] Standard deviation for percentage change was calculated by dividing SD for change with mean baseline value.

We used the Jadad scale to assess the quality of included RCTs, a score of < 3 indicating low quality.[31] We also used a 3-category grading system (A, B, C) to denote the methodological quality of each study.[32] Category A studies have the least bias and results are considered valid; B studies are susceptible to some bias, but not sufficient to invalidate the results; and C studies have significant bias that may invalidate the results. We arbitrarily defined category C as of low quality. Concealment of treatment allocation in RCTs was assessed as adequate, inadequate or unclear.[33] Two reviewers independently assessed the studies, and consensus was reached by discussion when there were disagreements.

We performed meta-analysis to determine the overall treatment effect of soy isoflavones on BMD, using the weighted mean difference method in Review Manager (version 5.0.20; Nordic Cochrane Center, Oxford, England). Treatment effect of each trial was estimated as the mean difference between changes (or percentage changes) from baseline in BMD for each comparison group (i.e., the change from the baseline for participants ingesting soy isoflavones minus that for controls). When data of more than one time points for the same trial were reported in one article or reported separately in two articles, we primarily used the data set for the short duration in order not to induce unit-of-analysis error. The data set for other time points were used for sensitivity analysis to prevent reporting bias. For trials had more than one isoflavone group compared with one control group, we combined the multiple isoflavones groups into a single group for each of these trials without inducing unit-of-analysis error.[34]

We used both a fixed effect model or a random effects model to calculate weighted mean differences (WMD), 95% CIs for each comparison, a combined overall effect with *p*-value, and the *p*-value for testing heterogeneity ($p<0.1$ was considered significant); when there was significant heterogeneity across included trials, the results based on the random effects model were shown.[19,30,35]

We conducted sensitivity analyses to evaluate the effects of degree of correlation between baseline and final values, time point of measurement (using data for long duration instead of data for short duration in trials with multiple time points of evaluation), study design (selecting only placebo-controlled trials), and study quality (eliminating low-quality trials). If at least 10 trials were available, subgroup analyses and meta-regressions were performed to investigate possible factors that might related to varying effects of soy isoflavones on BMD across trials, on the basis of pre-specified factors of intervention

duration, isoflavone dosage, region of participants, and basal BMD.[15,20] We used a cut-off point of 75 mg/day in subgroup analysis for isoflavone dosage, because daily isoflavone intake of up to 75 mg (aglycone form) is considered safe by the Japan Food Safety Commission. Significant tests based on test for heterogeneity, chi-squared statistics, were performed to investigate differences between two subgroups.[19,34] We examined potential publication bias by using funnel plots and by performing Egger's test to assess the asymmetry of funnel plots. Meta-regressions and Egger's test were respectively performed with the use of user-written "metareg" and "metabias" commands for Stata 10.1 for Windows (StataCorp LP, College Station, Tex).

RESULTS

The search strategy (Figure 1) yielded 16 potentially appropriate reports of RCTs to be included in the meta-analysis. After excluding one article[36] reporting only duplicate femoral data that had appeared in another article,[25] and two articles[37,38] describing a smaller sample than that analyzed in another article,[23,17] 13 articles on 12 trials were included for meta-analysis.[8,16-18,21-29] Two articles reported outcomes for durations of six months[27] and one year[28] for the same trial participants.

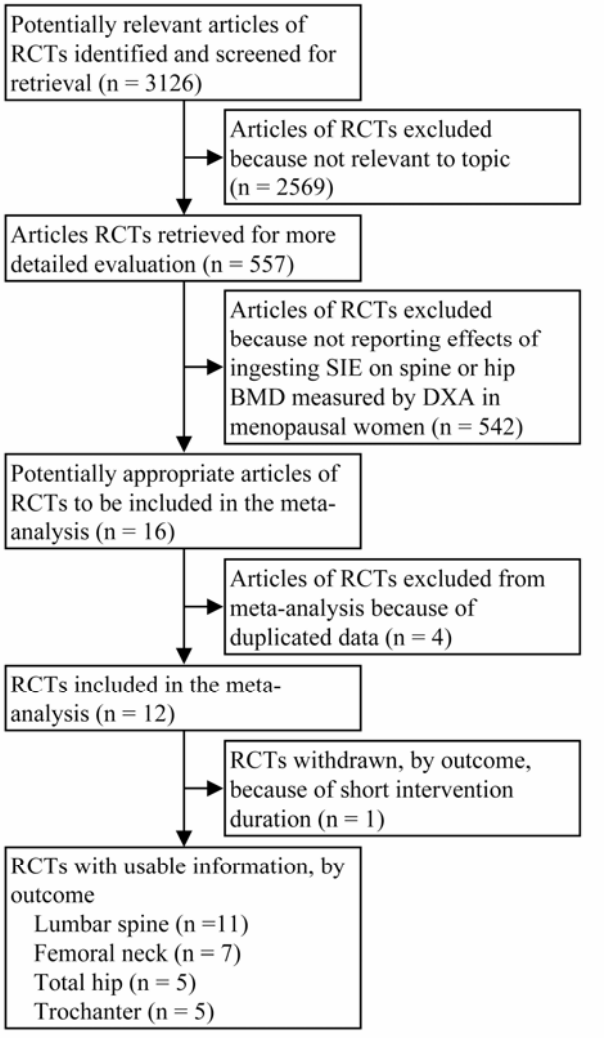

Figure 1. Search and selection of trials. Abbreviations: RCTs, randomized controlled trials; BMD, bone mineral density; SIE, soy isoflavone extracts, DXA, dual X-ray absorptiometry.

The characteristics of 12 trials are summarized in Table 1. Two articles for each trial contained data for two time points.[21,25] Three trials tested two isoflavone groups[17,22,24] and one tested three isoflavone groups[18] compared with one identical control group. One trial did not address the form and composition of soy isoflavones tested,[18] we assumed the dose as aglycone equivalent to calculate the mean dosage. Four, six, and two trials included participants of normal bone mass (T-score > –1 SD, corresponds to BMD > 0.937g/cm^2), low bone mass or osteopenia (–1 SD ≥ –2.5, corresponds to 0.937 g/cm^2 ≥ BMD ≥ 0.772 g/cm^2), and osteoporosis (T-score < –2.5 SD, corresponds to BMD < 0.772 g/cm^2) on the basis of averaged basal spine BMD, respectively.[39] Only one trial was assessed as "adequate" for concealment of treatment allocation,[22] and the remaining trials were assessed as "unclear" due to insufficient information. Participants in the comparison groups had similar dietary intakes of soy isoflavones, calcium, and vitamin D and physical activities. Most of the studies were designed to maintain the participants' usual diets, lifestyle and body weight. Adverse events were generally similar for both the isoflavone and control groups and no serious adverse events were noted in the included trials, although they were not well addressed in several trials.

Because bone is a slowly responding organ, a complete bone remodeling cycle takes up to 6 months, and therefore a study duration of less than 6 months is not sufficient to evaluate the effect of any intervention on bone BMD.[28] Thus, one 3-month trial of low-quality that reported negative effect of soy isoflavones on spine BMD was then withdrawn.[26] From 3126 relevant articles identified, 11,[8,16-18,21-25,27-29] 7,[8,17,22-25,27,28] 5,[16,17,22,27-29] and 5[17,22-24,27,28] trials were finally selected for estimating the effects on lumbar spine, femoral neck, total hip, and trochanter BMD, respectively (Figure 1, Table 1). Fourteen correlation coefficients between baseline and follow-up values were calculated from 5 reports of 4 trials,[17,23,24,27,28] which were consistent and resulted in a mean value of 0.98 (0.96–1).

Meta-analysis of the 11 trials with 1240 participants using the fixed effect model resulted in significant heterogeneity (p<0.001), and revealed that daily ingestion of an average of 82 (47–150) mg (aglycone equivalent) soy isoflavones for 6 months to one year significantly increased lumbar spine BMD by 12.08 mg/cm^2 (95% CI: 9.83, 14.33 mg/cm^2; p<0.001), or by 1.47% (95% CI: 1.21, 1.74%; p<0.001) compared with controls. Meta-analysis using the random effects model, revealed an significant overall effect of soy isoflavones in increasing spine BMD by 20.25 mg/cm^2 (95% CI: 7.62, 32.89 mg/cm^2, p=0.002), or by 2.38% (95% CI: 0.93, 3.83%, p=0.001; Figure 2). Of the11 selected trials, 7 trials revealed significant positive mean difference between changes or percentage change from baseline in spine BMD for isoflavone and control groups (favors isoflavone). The mean difference was negative at 27-week time point and was positive at 53-week time point in one trial,[21] the mean difference at 2-year duration was about two times of that at 1-year time point;[25] whereas, the

Table 1. Characteristics of included randomized controlled trials

Study	Design[†]	Follow-up	Participants[‡]	Intervention[§]	Baseline mean BMD outcomes (g/cm^2)[¶]	Jadad scale	Quality category
Brink 2008[21]	P; R, DB+, WD	27, 53 wk	N: 300/237 (21%) PoW; mean age: 53 y; TSM = 33 (12–60) mo; non-osteoporotic (spine Z-score ≥ 2); Netherlands, Italy, France	110 mg IAE [25–35% De, 60–75% Ge, 1–5% Gle] vs. placebo	L1–4: 0.990, mean (SD) T-score = −0.0 ± 1.1	4	C (dropout > 20%)
Chen 2003[22]	P; R+, DB+, WD	1 y	N: 203/175 (14%) PoW; mean age: 54.2 y; TSM = 4.1 (1–10) y; Hong Kong	40 and 80 mg IAE [46% De, 15% Ge, 39% Gle] vs. placebo	L1–4: 0.860; FN: 0.682; TH: 0.819; Tr: 0.605	5	A
Dong 2008[23]	P; R, WD	12 mo	N: 60/52 (13%) PoW; mean age: 54.7 y; TSM = 6.2 (≥ 1) y; T-score < −1.5 China	100 mg IC [66 mg IAE: 39% De, 61% Ge, 1% Gle] + calcium vs. calcium only (control)	L2–4: 0.756; FN: 0.719; Tr: 0.552	2	B
Gao 2006[18]	P; R	24 wk	N: 50/50 PoW; age: 48–62 y; TSM ≥ 1y; China	60, 90, and 150 mg IF vs. no-treatment (control)	L1–4: 0.974	1	B
Harkness 2004[16]	CO; R+, DB, WD	6 mo × 2	N: 20/19 (5%) PoW; mean age: 70.6 y; TSM = 19.1 (> 8) y; T-score < 2.5; USA	110 mg IAE [40% De, 52% Ge, 9% Gle] vs. placebo	L1–4: 0.881; TH: 0.800	4	B
Huang 2006[24]	P; R, OL, WD	1 y	N: 43/42 (2%) PoW; mean age: 52.4 y; TSM = 4.4 (1–13) y; Taiwan	100 and 200 mg IAE [29% De, 71% Ge] vs. regular diet only (control)	L1–4: 0.881; FN: 0.812; Tr: 0.715	2	B
Marini 2007[25]	P; R+, DB+, WD	12, 24 mo	N: 389/389 (10, 22%) PoW; mean age: 54.5 y; TSM = 63 mo (≥ 1 y); femoral neck BMD < 0.795 g/cm^2 (−1.0 T-score); Italy	54 mg pure Ge vs. placebo	L: 0.840; FN: 0.670	5	A, C (dropout > 20%)
Morabito 2002[8]	P; R, DB+	1 y	N: 90/90 PoW; mean age: 51.5 y; TSM = 6.5 (≥ 1) y; femoral neck BMD < 0.795 g/cm^2 (−1.0 T-score); Italy	54 mg pure Ge vs. placebo	L: 0.925; FN: 0.688	3	A
Uesugi 2003[26]	P; R, WD	3 mo	N: 22/21 (4%) PoW; mean age: 53.7 y; TSM = 6 (5–10) y; non-osteoporosis; Japan	62 mg IC [38 mg IAE: 52% De, 11% Ge, 37% Gle] vs. placebo	L2–4: 1.040	2	C (unclear analyzed N)
Wu 2006a[27], b[28]	P; R, DB+, WD	6, 12 mo	N: 136/128, 108 (6, 21%); mean age: 54.4 y; TSM = 3.2 (1–5) y; Japan	75 mg IC [47 mg IAE: 54% De, 13% Ge, 34% Gle] vs. placebo	L2–4: 0.899; FN: 0.672; TH: 0.782; Tr: 0.595	4	A, C (dropout > 20%)
Xin 2006[29]	P; R, DB	6 mo	N: 76 MW; age: 45–55 y; TSM ≤ 5 y; China	50 mg pure De + calcium vs. calcium only (control)	L2–4: 0.715; TH: 0.643	2	C (unclear analyzed N)
Ye 2006[17]	P; R+, SB, WD	6 mo	N: 90/84 (7%) PoW; mean age: 52.3 (1–5) y; TSM = 2.6 (1–5) y; China	84 and 126 mg IAE [52% D(e), 15% G(e), 33% Gl(e)] vs. placebo	L1–4: 0.864; FN: 0.702; TH: 0.800; Tr: 0.588	3	B

[†]CO, crossover; DB, double-blinded (gives 1 point to Jadad scale); DB+, double-blinded by appropriate method (gives 2 point); OL, open-labeled; P, Parallel; R, randomized (give 1 point); R+, randomized by appropriate method (gives 2 point); SB, single-blinded; WD, withdrawals and dropouts described (gives 1 point).
[‡]BMD, bone mineral density; N, randomize/analyzed number (dropout rate) of participants; MW, menopausal women; PoW, postmenopausal women; TSM, averaged time since menopause.
[§]IAE, isoflavone aglycone equivalents; IC, isoflavone conjugate containing glycoside and aglycone forms; IF, isoflavones (form and composition unknown); D(e), daidz(e)in; De, daidzein; Ge, genistein; G(e), genist(e)in; Gl(e), glycit(e)in; Gle, glycitein.
[¶]FN, femoral neck; L, lumbar spine; TH, total hip; Tr, trochanter.

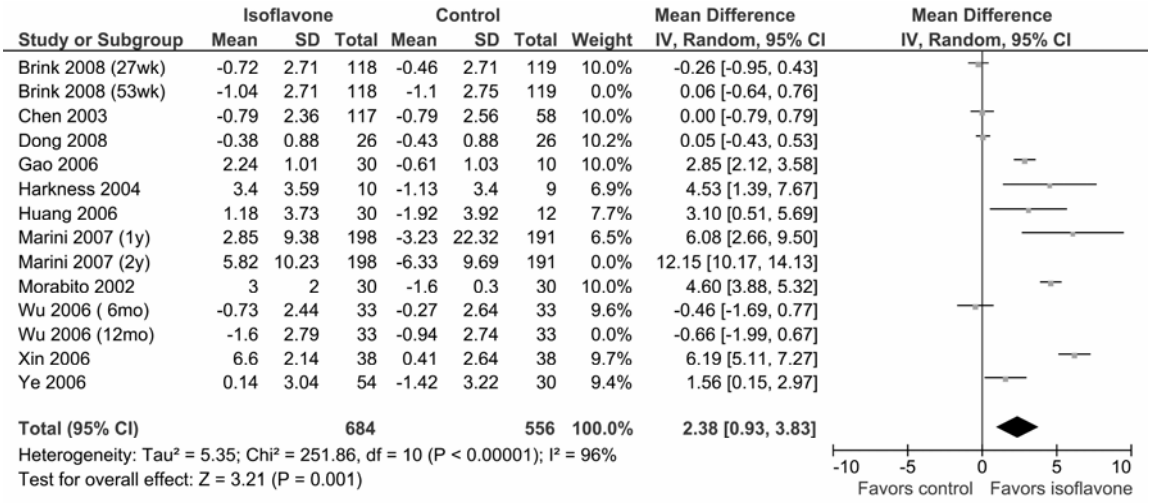

Figure 2. Effects of soy isoflavones on spine BMD (%). Mean Difference, weighted mean difference between percentage changes (%) of spine bone mineral density (BMD) from baseline for isoflavone and control groups; random, random effects model. Horizontal lines denote the 95% CI. Data sets for long duration not included in the meta-analysis were signed 0.0% Weight. ■ Point estimate (size of the square corresponds to its weight); ♦ Combined overall effect.

mean difference for 6 months duration[27] was similarly negative to that for 1 year duration.[28]

Sensitivity analyses assuming the level of correlation coefficient between baseline and follow-up values to be 0.75 and 0.5, using data sets of longer duration instead of short duration for trials with two time points of measurements, selecting only placebo-controlled trials, and eliminating low-quality trials (Jadad scale < 3 or Category C) did not result in significantly different overall effects of soy isoflavones on spine BMD.

Results of subgroup analyses of the effects of soy isoflavones on spine BMD were shown in Table 2. Each subgroup analysis resulted in significant heterogeneity and revealed significant effect of soy isoflavones in increasing spine BMD compared with controls using the fixed effect model. Results based on fixed effect model revealed that effects of soy isoflavones on spine BMD in subgroups of 6 months duration and of Asian region were significantly different with the effects in subgroup of 1 year duration and of Western region, respectively. Two subgroups of each subgroup analysis using the random effects model, show similarly significant effects of soy isoflavones in increasing spine BMD, except for a subgroup of participants with normal bone mass at baseline. Meta-regressions analyzing each of or all of the four pre-specified categorical study characteristics (intervention duration, isoflavone dosage, region of participants, and basal spine BMD), did not reveal that these pre-specified factors were significantly associated with the varying effects of soy isoflavones on spine BMD across trials. The funnel plots (Figure 3) and Egger's test of effects of soy isoflavones on spine BMD among the 11 trials (p=0.251 and p=0.267 for effects in terms of change and percentage change, respectively) did not indicate any obvious publication bias.

Meta-analysis of the 7 trials with 868 participants using the fixed effect model resulted in significant heterogeneity (p<0.001). Meta-analysis using the random effects model, revealed that daily ingestion of an average of 76 (47–150) mg (aglycone equivalent) soy isoflavones for 6 months to one year non-significantly increased femoral neck BMD by 10.24 mg/cm^2 (95% CI: −3.73, 24.20 mg/cm^2, p=0.15), or by 1.48% (95% CI: −0.54, 3.50%, p=0.15) compared with controls. Sensitivity analysis assuming the level of correlation coefficient between baseline and follow-up values to be 0.75 and 0.5, did not result in significantly different overall effects of soy isoflavones on femoral neck BMD. Whereas, sensitivity analysis using data sets of longer duration for trials with two time points of measurements, found that ingestion of soy isoflavones for 6 months to 2 years tended to increase femoral neck BMD by 16.89 mg/cm^2 (95% CI: −2.34, 36.11 mg/cm^2, p=0.09), or by 2.45% (95% CI: −0.31, 5.21, p=0.08; Figure 4) compared with controls (random effects model). Sensitivity analyses selecting only placebo-controlled trials and eliminating low-quality trials were not performed because of the small number of available trials.

Meta-analysis of the 5 trials with 420 participants using the fixed effect model resulted in non-significant heterogeneity (p≥0.1), revealed that daily ingestion of an average of 74 (47–110) mg (aglycone equivalent) soy isoflavones for 6 months to one year non-significantly change total hip BMD by 2.45 mg/cm^2 (95% CI: −1.41, 6.30 mg/cm^2, p=0.21), or by 0.05% (95% CI: −0.53, 0.63%, p=0.86) compared with controls. Sensitivity analyses assuming the level of correlation coefficient between baseline and follow-up values to be 0.75 and 0.5 and using data sets of longer duration for trials with two time points of measurements, did not result in significantly different overall effects of soy isoflavones on total hip BMD.

Meta-analysis of the 5 trials with 419 participants revealed that daily ingestion of an average of 85 (47–150) mg (aglycone equivalent) soy isoflavones for 6 months to one year non-significantly change trochanter BMD by −0.40 mg/cm^2 (95% CI: −6.58, 5.78 mg/cm^2, p=0.90), or by −0.07% (95% CI: −1.15, 1.02%, p=0.91) compared with controls (random effects model). Sensitivity analyses assuming the level of correlation coefficient between

Table 2. Subgroup analyses of the effects of soy isoflavones on spine BMD[†]

Variables	No. of trials	Sample size	*p* for heterogeneity	Fixed effect model			Random effects model	
				WMD (95% CI)	*p*-value	*p*-value (diff)	WMD (95% CI)	*p*-value
Intervention duration								
6 months	6[16-18, 21, 27, 29]	522	< 0.00001	17.72 (14.03, 21.41) mg/cm^2	< 0.00001	= 0.0002	18.74 (1.25, 36.23) mg/cm^2	0.04
			< 0.00001	1.81 (1.40, 2.21) %	< 0.00001	= 0.03	2.31 (0.16, 4.47) %	0.04
1 year	5[8, 22-25]	718	< 0.00001	8.74 (5.90, 11.58) mg/cm^2	< 0.00001		22.64 (1.54, 43.74) mg/cm^2	0.04
			< 0.00001	1.23 (0.88, 1.58) %	< 0.00001		2.52 (0.17, 4.87) %	0.04
Isoflavone dose								
≤ 75 mg/d	6[8, 22, 23, 25, 27, 29]	818	< 0.00001	11.70 (9.10, 14.30) mg/cm^2	< 0.00001	= 0.57	20.79 (1.48, 40.09) mg/cm^2	0.03
			< 0.00001	1.53 (1.20, 1.85) %	< 0.00001	= 0.59	2.59 (0.26, 4.92) %	0.03
> 75 mg/d	5[16-18, 21, 24]	422	< 0.00001	13.21 (8.73, 17.69) mg/cm^2	< 0.00001		19.49 (2.64, 36.34) mg/cm^2	0.02
			< 0.00001	1.37 (0.91, 1.83) %	< 0.00001		2.10 (0.31, 3.90) %	0.02
Region of participants								
Asian	7[17, 18, 22-24, 27, 29]	535	< 0.00001	9.01 (6.44, 11.59) mg/cm^2	< 0.00001	< 0.00001	15.06 (0.89, 29.23) mg/cm^2	0.04
			< 0.00001	1.17 (0.86, 1.49) %	< 0.00001	= 0.0006	1.85 (0.16, 3.54) %	0.03
Western	5[8, 16, 21, 25]	705	< 0.00001	21.97 (17.34, 26.60) mg/cm^2	< 0.00001		31.46 (0.56, 62.37) mg/cm^2	0.05
			< 0.00001	2.20 (1.71, 2.68) %	< 0.00001		3.56 (0.13, 6.99) %	0.04
Basal spine BMD								
Normal bone mass	3[18, 21, 24]	319	< 0.00001	12.31 (7.42, 17.20) mg/cm^2	< 0.00001	= 0.92	17.06 (−7.55, 41.66) mg/cm^2	0.17
			< 0.00001	1.27 (0.78, 1.76) %	< 0.00001	= 0.33	1.78 (−0.74, 4.29) %	0.17
Osteopinia or osteoporosis	8[8, 16, 17, 22, 23, 25, 27, 29]	921	< 0.00001	12.02 (9.48, 14.55) mg/cm^2	< 0.00001		21.70 (5.43, 37.97) mg/cm^2	0.009
			< 0.00001	1.56 (1.24, 1,87) %	< 0.00001		2.64 (0.69, 4.60) %	0.008

[†]BMD, bone mineral density; WMD, weighted mean difference; *p*-value, test for overall effect of each subgroup; *p*-value (diff), test for subgroup differences.

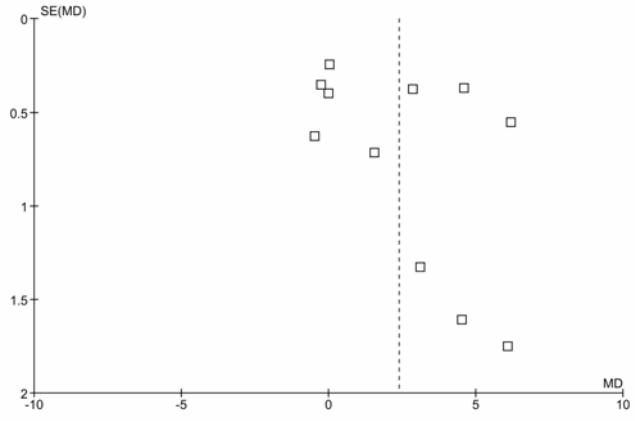

Figure 3. Funnel plots of effects of soy isoflavones on spine BMD (%). MD, weighted mean difference between percentage changes (%) of spine bone mineral density (BMD) from baseline for isoflavone and control groups; SE (MD), standard error of MD; fixed, fixed effect model.

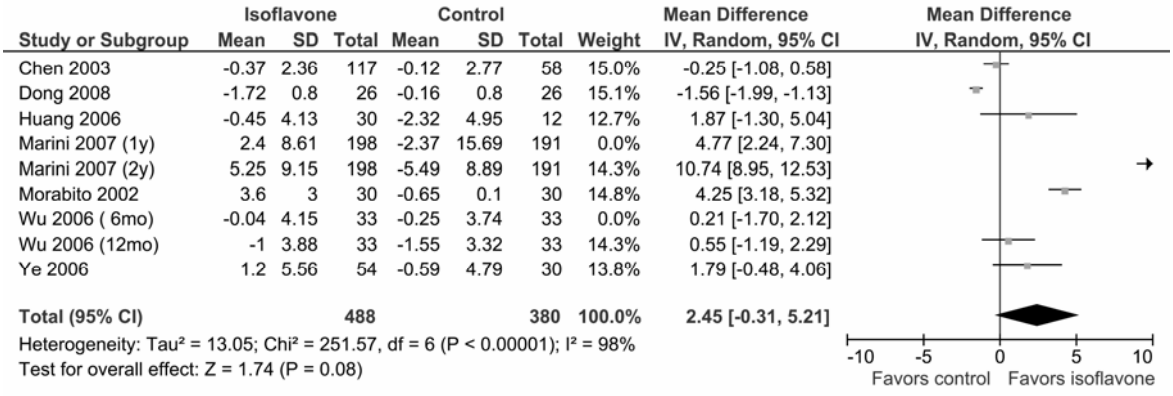

Figure 4. Effects of soy isoflavones on femoral neck BMD (%). Mean Difference, weighted mean difference between percentage changes (%) of femoral neck bone mineral density (BMD) from baseline for isoflavone and control groups; random, random effects model. Horizontal lines denote the 95% CI. Data sets for long duration not included in the meta-analysis were signed 0.0% weight. ■ Point estimate (size of the square corresponds to its weight); ♦ Combined overall effect.

baseline and follow-up values to be 0.75 and 0.5 and using data set of longer duration for trials with two time points of measurements, did not result in significantly different overall effects of soy isoflavones on trochanter BMD.

DISCUSSION

The present meta-analysis found that ingestion of about 82 mg of extracted soy isoflavones (in the aglycone form) per day for 6 months to 1 year significantly increased lumbar spine BMD by 2.38% compared with controls without isoflavones, in menopausal women. Results of sensitivity analyses indicated that the effect of soy isoflavone extracts in increasing lumbar spine BMD was robust. This magnitude of beneficial effect of soy isoflavones appears to almost completely offset naturally occurring postmenopausal bone loss. Effect of soy isoflavones in increasing femoral neck BMD seems to take more time than spine BMD. Our meta-analysis did not reveal significant effects on total hip and trochanter BMD, which might be due to the limited number of five trials.

An intake of 82 mg soy isoflavones/day (in the aglycone form) is approximately equivalent to 1.7 times the amount consumed habitually in Japan (mean: 47.2 mg/day).[40] The mechanism mediating the improvement of BMD at these skeletal sites by soy isoflavones is not well understood, but it may be a result of their chemical and biological similarity to mammalian estrogens, which are known to increase BMD in menopausal women.[1, 4]

Results of subgroup analyses indicated that the varying effects of soy isoflavone extracts on spine BMD across the 11 trials were associated with study characteristics of intervention duration, region of participants, and basal BMD. The heterogeneity of effects of soy isoflavones on spine BMD across the 11 trials might also be induced by differences in habitual dietary intake of soy isoflavones,[28] time since menopause,[3] intervention duration,[25] isoflavone dosage,[17,41] chemical forms and proportions of individual soy isoflavones,[42-44] and participants' ethnicity. Isoflavone glycosides are not absorbed intact across the enterocytes of healthy adults, and their bioavailability requires initial hydrolysis by intestinal β-glucosidases for uptake into the peripheral circulation.[44] Asian and Western populations are reported to have differences in the capacity of intestinal flora to convert daidzein to its metabolite, equol.[45] Equol is easily absorbed and possesses substantial estrogenic activity because of its affinity for both the estrogen α and β receptors.[43] Equol is suggested to be the single most important factor that influences the clinical efficacy of soy isoflavones in preventing bone

loss.[46] Because of the limited number of trials and insufficient data available, our meta-analysis was also unable to evaluate possible influences on the varying effects of soy isoflavones on spine BMD across trials of dietary intake of soy isoflavones, time since menopause, chemical forms and proportions of individual soy isoflavones, blood isoflavone concentration, urinary isoflavone excretion, and equol producer status.

Since there was significant heterogeneity in effects of soy isoflavones on spine BMD, we preferably presented the results by incorporating heterogeneity into the random effects model in this meta-analysis. A random effects meta-analysis model involves an assumption that the effects being estimated in the different studies are not identical, but follow some distribution. The model represents our lack of knowledge about why real, or apparent, treatment effects differ by considering the differences as if they were random.[19]

The magnitude of effect of soy isoflavone extracts in increasing spine BMD by 20.25 mg/cm^2 revealed in our present meta-analysis, were consistent with the results (by 20.6 mg/cm^2) from the previous meta-analysis that included 10 RCTs testing both extracted soy isoflavones and isolated soy protein containing isoflavones.[15] Thus, soy isoflavones ingested either alone in extracted form or as constituent part of isolated soy protein have been demonstrated to exert a mild but significant effect in increasing lumbar spine BMD in menopausal women. Our meta-analysis also revealed that ingestion of soy isoflavones for 6 months appears to be enough to exert beneficial effect on spine BMD in menopausal women. The present meta-analysis did not reveal influences of isoflavone dosage on the effect on spine BMD, possibly due to the fact that trials tested various forms and compositions of soy isoflavones likely possessing different bioavailability and effects on bone mass; other explanations might be the limited number of trials or of some other factors inducing the heterogeneity.

CONCLUSION

The effect of soy isoflavones in increasing spine BMD in menopausal women are not as strong as those of approved pharmacologic therapies involving estrogen or bisphosphonates.[1,4,47,48] However, the present meta-analysis revealed that soy isoflavone extract supplements did result in a significant improvement of lumbar spine BMD with good tolerance and no induction of notable adverse events. Our meta-analysis suggested that soy isoflavone supplements can be used not only to offset the bone loss that occurs naturally in women after menopause, but are also applicable for complementary or alternative use in patients with postmenopausal osteopenia or osteoporosis who are unable to tolerate the side effects of estrogen or/and bisphosphonate therapies. Furthers studies are needed to address factors affecting the magnitudes of the effect of soy isoflavones on spine BMD and to verify the effect on hip BMD.

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AUTHOR DISCLOSURES

Kyoko Taku, Melissa K. Melby, Jun Takebayashi, Shoichi Mizuno, Yoshiko Ishimi, Toyonori Omori and Shaw Watanabe, disclose no conflicts of interest.

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Original Article

Effect of soy isoflavone extract supplements on bone mineral density in menopausal women: meta-analysis of randomized controlled trials

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大豆異黃酮抽取物的補充劑對停經後婦女骨質密度的效果：隨機對照試驗的後設分析

本研究旨在確認攝取大豆異黃酮抽取物(並非大豆蛋白或含有異黃酮的食品)對停經後婦女骨質密度(BMD)的效果。我們從 PubMed，CENTRAL，ICHUSHI，CNKI，Wanfang Data，CQVIP，和 NSTL 檢索，以英語，日語，或中文發表，並報告大豆異黃酮抽取物對停經後婦女腰椎或髖關節 BMD 效果的隨機對照試驗論文。依照納入和排除標準，對試驗論文進行鑑別和評閱來判定是否採用。有關研究設計，對象，介入，和結果的數據被抽取出進行分析。最終分別有 11、7、5、和 5 個試驗被採用來評估對腰椎、大腿骨頸部、髖關節全體、和股骨大轉子 BMD 的效果。包括 1240 名停經後婦女的後設分析(隨機效果模型)顯示，與對照組相比，每日平均攝取 82 (47-150) mg 的大豆異黃酮(苷元當量)持續 6-12 個月，顯著地提高腰椎 BMD 22.25 mg/cm^2 (95%信賴區間: 7.61，32.89；p=0.002)，或提高 2.38% (95%信賴區間: 0.93，3.83；p=0.001)。亞組分析顯示，不同試驗間大豆異黃酮對腰椎 BMD 的效果各異，可能與介入期間(6 或 12 個月)，對象的區域(亞洲或西方)，和基礎 BMD(正常骨質或骨質減少症或骨質疏鬆症)的研究特徵相關。我們的後設分析沒有發現對大腿骨頸部，髖關節全體，和股骨大轉子 BMD 的效果。大豆異黃酮抽取物的補充劑提高了停經後婦女的腰椎 BMD。需要更深入的研究去闡明影響其對腰椎效果程度的因素，以及驗證其對髖關節的效果。

關鍵字：後設分析、異黃酮、膳食補充劑、停經、骨密度