Effect of oral isoflavone supplementation on vascular endothelial function in postmenopausal women: a meta-analysis of randomized placebo-controlled trials[1–3]

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ABSTRACT

Background: The effect of isoflavone on endothelial function in postmenopausal women is controversial.

Objective: The objective of this study was to evaluate the effect of oral isoflavone supplementation on endothelial function, as measured by flow-mediated dilation (FMD), in postmenopausal women.

Design: A meta-analysis of randomized placebo-controlled trials was conducted to evaluate the effect of oral isoflavone supplementation on endothelial function in postmenopausal women. Trials were searched in PubMed, Embase, the Cochrane Library database, and reviews and reference lists of relevant articles. Summary estimates of weighted mean differences (WMDs) and 95% CIs were obtained by using random-effects models. Meta-regression and subgroup analyses were performed to identify the source of heterogeneity.

Results: A total of 9 trials were reviewed in the present meta-analysis. Overall, the results of the 9 trials showed that isoflavone significantly increased FMD (WMD: 1.75%; 95% CI: 0.83%, 2.67%; $P = 0.0002$). Meta-regression analysis indicated that the age-adjusted baseline FMD was inversely related to effect size. Subgroup analysis showed that oral supplementation of isoflavone had no influence on FMD if the age-adjusted baseline FMD was $\geq$5.2% (4 trials; WMD: 0.24%; 95% CI: −0.94%, 1.42%; $P = 0.69$). This improvement seemed to be significant when the age-adjusted baseline FMD levels were <5.2% (5 trials; WMD: 2.22%; 95% CI: 1.15%, 3.30%; $P < 0.0001$), although significant heterogeneity was still detected in this low-baseline-FMD subgroup.

Conclusions: Oral isoflavone supplementation does not improve endothelial function in postmenopausal women with high baseline FMD levels but leads to significant improvement in women with low baseline FMD levels. *Am J Clin Nutr* 2010;91:480–6.

INTRODUCTION

The risks of cardiovascular diseases increase with the decline in estrogen production after menopause in women (1). Dietary intake of compounds with estrogenic properties reduces the incidence of cardiovascular events, according to recent epidemiologic studies (2–4). Isoflavone, mainly produced by soybeans, has been suggested to have estrogenic and potentially cardioprotective effects and improved endothelial dysfunction in many experimental studies (5–7).

Endothelial dysfunction is an early pathophysiologic feature, and an independent predictor of poor prognosis, in most forms of cardiovascular diseases (8, 9). Experimental studies indicate that isoflavone can stimulate the production of nitric oxide (NO) via estrogen receptor–mediated activation of endothelial NO synthase (eNOS) (10). Therefore, the effect of oral isoflavone supplementation on endothelial function in postmenopausal women has been investigated by many studies (11–33). However, the results of these studies were not consistent, and the sample sizes were relatively small. As a result, the precise effect of isoflavone supplementation has not been established.

In most of these studies, endothelial function was measured by flow-mediated dilation (FMD), which has been widely investigated and proved to be sensitive and accurate in reflecting endothelial function (34, 35). In the present study, we identified all published, double-blind, randomized, placebo-controlled trials of isoflavone and performed a meta-analysis to determine the effect of isoflavone supplementation on FMD in postmenopausal women.

METHODS

Search strategy and selection criteria

In our present study, we conducted a systematic review of the available studies according to the QUORUM (Quality of Reporting of Meta-analyses) guidelines for the conduction of meta-analyses (36). We searched PubMed (http://www.ncbi.nlm.nih.gov/pubmed) (from 1950 up to March 2009), Embase (http://www.embase.com) (from 1966 up to March 2009), the Cochrane Library database (http://www.cochrane.org), and reviews and reference lists of relevant articles using the relevant text words "isoflavone" paired with "endothelial" or "endothelium." Our search was limited to completed, published, double-blind, randomized, and placebo-controlled trials of oral isoflavone supplementation studies. Meanwhile, because the FMD mea-

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surement has been proven to be sensitive and accurate to reflect the endothelial function (34, 35), the included studies in the meta-analysis should perform the FMD measurement. Participants must have been treated with isoflavone for ≥3 d, because we did not want to estimate the acute effect of isoflavone on endothelial function.

Data extraction and quality assessment

The search, data extraction, and quality assessment were completed independently by 2 reviewers (S-HL and X-XL) according to the inclusion criteria. The 2 reviewers extracted data, including the number of participating subjects, population characteristics (age, sex, and baseline comorbidities), duration of treatment, source and dose of isoflavones, baseline cholesterol concentration, and percentage change in FMD.

The quality of the studies was judged by concealment of treatment allocation, quality of randomization, blinding, reporting of withdrawals, and generation of random numbers. Trials scored one point for each area addressed, with a possible score of between 0 and 5 (highest level of quality) (37).

Statistical analysis

Our meta-analysis and statistical analyses were performed with Stata software (version 10.0; Stata Corporation, College Station, TX) and REVMAN software (version 5.0; Cochrane Collaboration, Oxford, United Kingdom). The primary outcome was the percentage change in FMD between baseline and final levels due to isoflavone supplementation. If the percentage change in FMD was not reported in the study, we calculated it according to the Cochrane Handbook for Systemic Review and Follman D's theory for overview of clinical trials with continuous variables (38). We assumed equal variance among trials and between intervention and controls.

Summary estimates of weighted mean differences (WMDs) and 95% CIs were obtained by using random-effect models (39). Statistical heterogeneity of treatment effects between studies was formally tested with Cochran's test ($P < 0.1$). The I^2 statistic was also examined, and we considered an I^2 value >50% to indicate significant heterogeneity between the trials (40). Potential heterogeneity in estimates of treatment effect attributable to each potential source of heterogeneity was explored by univariate meta-regression. Meanwhile, subgroup analyses were performed to further identify the possible sources of heterogeneity by comparing summary results obtained from subsets of studies grouped by age, duration of supplementation, source and dose of oral isoflavone, baseline cholesterol concentration, and age-adjusted baseline FMD level. Because FMD was correlated significantly with age (41), the baseline FMD value was adjusted by age in the present meta-analysis. Potential publication bias was assessed with the Egger test (42) and represented graphically by use of Begg's funnel plots of the effect size compared with its SE.

RESULTS

Search results

The method used to select the studies is shown in **Figure 1**. In total, 561 articles were identified in a combined search of the PubMed, Embase, and Cochrane Library databases and by using a manual approach (articles cited in previous reviews or references in the identified articles). Of the 561 articles, 536 were excluded because they were animal experiments (not controlled trials in humans) or because the objectives of the articles were not related to our present meta-analysis. Therefore, 25 potentially relevant articles (5, 6, 11–33) were selected for full text evaluation. After the evaluation, 9 eligible randomized controlled studies (15, 17, 23, 24, 26, 28–30, 33) were enrolled in our present meta-analysis. The remaining 16 articles (5, 6, 11–14, 16, 18–22, 25, 27, 31, 32) were excluded for several reasons: because FMD measurements were not performed in 5 trials (6, 21, 22, 25, 27), FMD values were not reported in 3 trials (11, 18, 20), male participants were also enrolled in 4 trials (14, 16, 19, 32), the studies were not randomized placebo-controlled studies (5, 12, 13), and the results reflected only the acute effect of isoflavone on endothelial function because participants were only given one test meal enriched with isoflavone or placebo and then FMD was measured 7 h (not > 3 d) after the test meal (31).

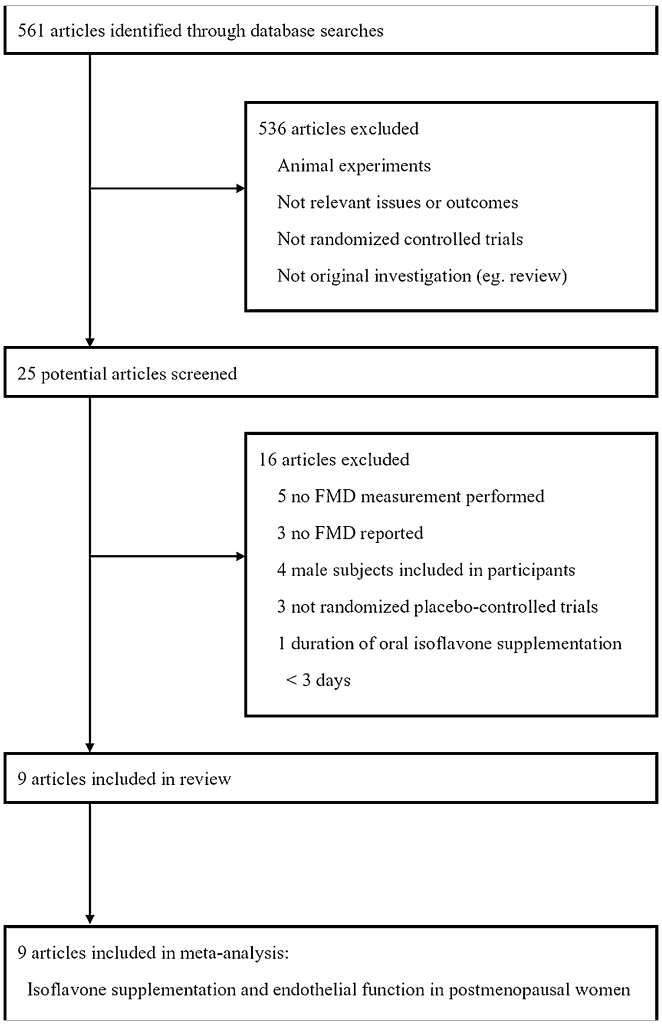

FIGURE 1. Identification process for eligible studies. FMD, flow-mediated dilation.

Study characteristics

Nine studies were included in our present meta-analysis. The characteristics of these studies are shown in **Table 1**. All of the

TABLE 1
Characteristics of the population, intervention, and outcomes in the included trials[1]

Reference	Year	Study design	Participants	No. of subjects	Mean age	Isoflavone dose	Source of isoflavone	Study duration	Mean basal cholesterol	Baseline FMD[2]	Outcomes: FMD reported	Outcomes: Side effects reported
					y	*mg/d*			*mg/dL*	%		
Simons et al (15)	2000	R, DB, PC, CO	Healthy PoW	20	59	80	Tablets, from Blackmores Ltd (Warriewood, Australia)	8 wk	227	2.1 ± 0.5	Yes	No
Hale et al (17)	2002	R, DB, PC	Healthy PoW	29	57	80	Soy protein, from Archer Daniel Midland Inc (Decatur, IL)	2 wk	195	8.9 ± 5.7	Yes	No
Cuevas et al (33)	2003	R, DB, PC, CO	Hypercholesterolemic PoW	18	59	80	Soy protein, from Protein Technologies International (St Louis, MO)	4 wk	286	5.3 ± 1.2	Yes	No
Lissin et al (23)	2004	R, DB, PC	Hypercholesterolemic PoW	40	61.5	90	Tablets, from Archer Daniel Midland Inc	6 wk	240	2.6 ± 1.2	Yes	No
Colacurci et al (26)	2005	R, DB, PC	Healthy PoW	57	55.4	60	Tablets, from Estromineral (Neptune, Italy)	6 mo	Not reported	3.4 ± 0.5	Yes	No
Kreijkamp-Kaspes et al (24)	2005	R, DB, PC	Healthy PoW	202	66.7	99	Soy protein, from Solae Company (St Louis, MO)	12 mo	236	4.6 ± 4.2	Yes	Yes
Hallund et al (28)	2006	R, DB, PC, CO	Healthy PoW	30	57	50	Soy protein, from Solbar Plant Extracts Ltd (Ashdod, Israel)	8 wk	232	2.2 ± 0.8	Yes	No
Evans et al (29)	2007	R, DB, PC, CO	Healthy PoW	22	61.5	Soy protein 25 g/d		4 wk	180	8.6 ± 7.2	Yes	No
Katz et al (30)	2007	R, DB, PC, CO	Healthy PoW	22	58.5	55	Soy protein, from Eli Lilly and Company (Indianapolis, IN)	6 wk	224	9.6 ± 6.4	Yes	Yes

[1] R, randomized; DB, double-blind; PC, placebo-controlled; CO, crossover; PoW, postmenopausal women; FMD, flow-mediated dilation.
[2] All values are the means ± SDs reported in the trials, not age-adjusted baseline FMD levels.

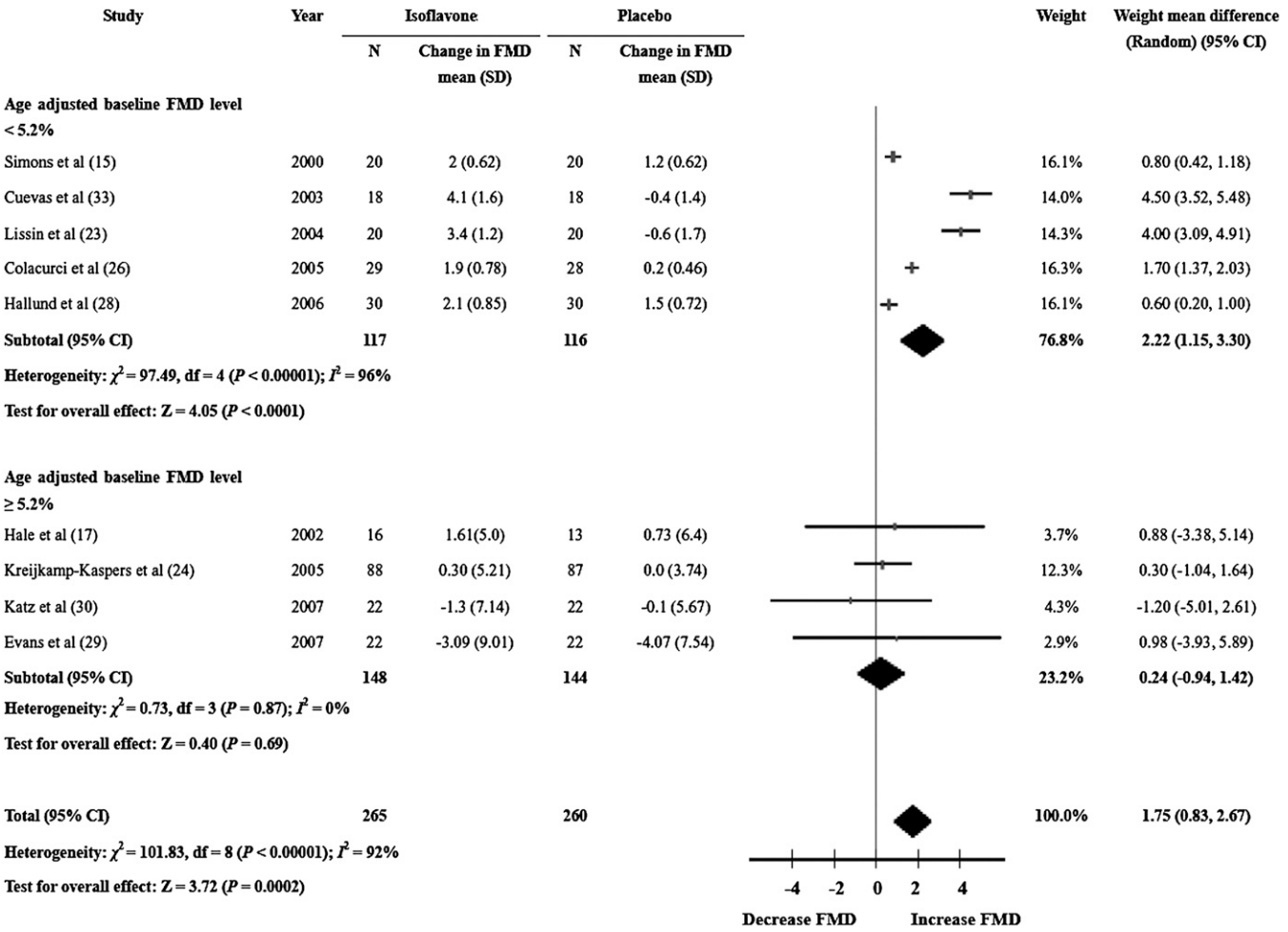

FIGURE 2. Meta-analysis of the effect of oral isoflavone supplementation on flow-mediated dilation (FMD) as compared with placebo. The sizes of the data markers indicate the weight of each study in the analysis. The subgroups were differentiated by FMD at baseline (<5.2% and ≥5.2%).

studies were randomized, double-blind, and placebo-controlled; 5 had a crossover design (15, 28–30, 33). The sample size of the 9 trials ranged from 18 to 202. The subjects in 7 trials (15, 17, 24, 26, 28–30) were healthy postmenopausal women, and in the other 2 trials were hypercholesterolemic postmenopausal women (23, 33). The average age of these subjects ranged from 55.4 to 66.7 y. The source of isoflavone was soy protein in 6 trials (17, 24, 28–30, 33). The other 3 trials (15, 23, 26) used tablets of isoflavone purchased from different companies. Doses of isoflavone in these trials ranged from 50 to 99 mg/d. The duration of treatment ranged from 2 wk to 12 mo. The average baseline cholesterol concentration ranged from 180 to 286 mg/dL. The baseline FMD levels varied from 2.1 ± 0.5% to 9.6 ± 6.4%.

Data quality

The quality of these 9 studies ranged from 3 to 5 (maximum score), and they were all randomized, double-blind, and placebo-controlled. Exact details of withdrawals were reported in 6 studies (15, 17, 24, 28, 30, 33) but not in the 3 other studies.

Effects of oral supplementation of isoflavone on FMD

The primary outcome was the percentage change in FMD between baseline and final levels due to isoflavone supplementation. The effect of oral isoflavone supplementation on FMD levels was well investigated by the 9 trials. Two trials (17, 23) directly reported the percentage change in FMD, but the remaining 7 trials (15, 24, 26, 28–30, 33) only provided the baseline and final FMD levels due to isoflavone or placebo supplementation. Therefore, the percentage changes in FMD in the 7 trials were calculated according to the Cochrane Handbook for Systemic Review and Follman D's theory (38).

The data were extracted and pooled from the 9 studies, and the meta-analysis showed that the percentage change in FMD levels was significantly higher in the isoflavone-supplemented subjects than in the placebo-treated subjects (9 trials, 525 subjects; WMD: 1.75%; 95% CI: 0.83, 2.67; $P = 0.0002$) (**Figure 2**). Significant heterogeneity for this outcome was found (heterogeneity $\chi^2 = 101.83$, $I^2 = 92\%$, $P < 0.00001$). To detect the sources of heterogeneity, meta-regression and subgroup analyses were performed in our present study. Meta-regression analysis of the data showed that age-adjusted baseline FMD was negatively related to effect size (regression coefficient = −0.22; 95% CI: −0.43, −0.01; $P = 0.036$), which largely explained the heterogeneity of the effect. The source, dose (range: 50–99 mg), and duration (range: 2 wk to 12 mo) of isoflavone supplementation and the baseline cholesterol concentration (range: 180–286 mg/dL) were not effect modifiers.

TABLE 2
Subgroup analyses for the effect of oral isoflavone supplementation on endothelial function[1]

	Intervention group	Effect (95% CI)	*P*
	n		
Mean age			
<59 y, low median	4	1.00 (0.01, 2.00)	0.10
≥59 y, high median	5	2.27 (0.27, 4.27)	
Source of isoflavone			
Soy protein	6	1.24 (−0.74, 3.23)	0.24
Tablets purchased from companies	3	2.08 (0.83, 3.33)	
Isoflavone dose			
<80 mg/d, low median	4	1.01 (0.01, 2.01)	0.11
≥80 mg/d, high median	5	2.24 (0.26, 4.22)	
Study duration			
<8 wk, low median	5	1.66 (−1.55, 4.87)	0.21
≥8 wk, high median	4	0.94 (0.31, 1.58)	
Age-adjusted baseline FMD			
<5.2%, low median	5	2.22 (1.15, 3.30)	0.001
≥5.2%, high median	4	0.24 (−0.94, 1.42)	
Mean baseline cholesterol[2]			
<228 mg/dL, low median	4	0.78 (0.40, 1.16)	0.07
≥228 mg/dL, high median	4	2.35 (0.12, 4.59)	

[1] FMD, flow-mediated dilation.
[2] Colacuri et al's trial (26) was excluded because it did not report the baseline cholesterol value.

To clarify the heterogeneity, subgroup analyses were performed to investigate the source of heterogeneity. The results are shown in **Table 2**. We identified no evidence of heterogeneity of effect in the subgroup analyses including mean age, the source and dose of isoflavone, the baseline cholesterol concentration, or the duration of the studies, except for age-adjusted baseline FMD level. In our present meta-analysis, we calculated the median values of age-adjusted baseline FMD levels and defined the age-adjusted baseline FMD level <5.2% (lower median) for all included trials to be the low baseline FMD subgroup; the age-adjusted baseline FMD ≥5.2% (upper median) was assigned as the high subgroup (Table 2). Therefore, a subgroup meta-analysis was performed according to the baseline FMD level. The oral supplementation of isoflavone significantly increased FMD levels when the age-adjusted baseline FMD levels were <5.2% (5 trials, 233 subjects; WMD: 2.22%; 95% CI: 1.15, 3.30; $P < 0.0001$); isoflavone supplementation had no influence on FMD if the age-adjusted baseline FMD was ≥5.2% (4 trials, 292 subjects; WMD: 0.24%; 95% CI: −0.94, 1.42; $P = 0.69$). Meanwhile, the heterogeneity of effect size in 9 trials was largely explained by the different baseline FMD levels. The subgroup meta-analysis showed that there was no heterogeneity (heterogeneity $\chi^2 = 0.73$, $I^2 = 0\%$, $P = 0.87$) in the 4 trials with the high age-adjusted baseline FMD (≥5.2%) (Figure 2).

Publication bias

A statistical analysis of the Egger test and funnel plots was performed in all 9 studies. The results detected no publication bias (Egger test, $P = 0.674$; **Figure 3**). Meanwhile, Egger tests were also done in the 2 subgroups divided by age-adjusted median baseline FMD level, which also indicated there was no publication bias in these subgroups (Egger test, low-FMD subgroup: $P = 0.129$; high-FMD subgroup: $P = 0.929$).

DISCUSSION

The present meta-analysis of 9 trials showed that oral supplementation with isoflavone in postmenopausal women significantly increased FMD levels compared with the placebo controls. However, the significant heterogeneity detected among the 9 trials might influence the confidence of this final result. To find the source of heterogeneity, meta-regression and subgroup analyses were performed. Meta-regression indicated that age-adjusted baseline FMD was negatively related to effect size. In other words, the effect of isoflavone supplementation was gradually attenuated as the age-adjusted baseline FMD increased. The subgroup analyses indicated that oral supplementation of isoflavone significantly increased FMD levels when the age-adjusted baseline FMD levels were <5.2%, whereas it had no effect on FMD if the baseline FMD level was high (FMD ≥ 5.2%). The heterogeneity could be largely explained by the differences in baseline FMD levels. These data suggest that the intervention with oral isoflavone supplementation may not improve the endothelial function if the baseline FMD is already high (FMD ≥5.2%). Potentially, oral isoflavone supplementation should be applied to those targeted subjects, but not to all postmenopausal women.

Menopause increases the risks of cardiovascular disease as a result of the loss of estrogen protection (1, 43). Dietary intake of compounds with estrogenic and cardioprotective properties have been introduced to help postmenopausal women prevent the development of heart diseases (2–4). Isoflavone, mainly from soybeans, has shown a positive effect on arterial compliance and endothelial function in animal studies (10, 44). Because endothelial dysfunction is an early pathologic feature and an independent predictor of most forms of cardiovascular diseases, the effect of isoflavone on endothelial function in postmenopausal women has been investigated by many researchers (15, 17, 23, 24, 26, 28–30, 33). However, the results were controversial and the

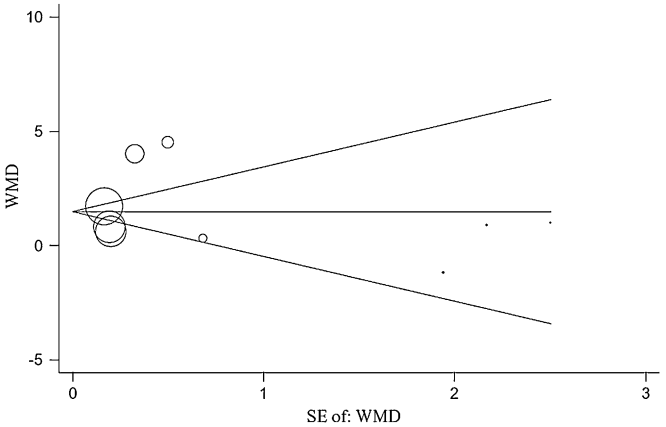

FIGURE 3. Begg's funnel plot (with pseudo 95% CIs) of all individual studies in the meta-analysis. Studies that evaluated the effect of oral isoflavone supplementation on flow-mediated dilation were plotted with weighted mean differences (WMDs) on the vertical axis and the SEs of the WMDs along the horizontal axis. Graph symbols were sized by weights.

conclusions were inconsistent. Our meta-analysis indicated that the effect of oral isoflavone supplementation was mainly dependent on the baseline FMD level. High baseline FMD levels might result in less or no influence of isoflavone on the improvement of endothelial function. Teede et al (19), who investigated the effect of isoflavone on endothelial function in healthy men and postmenopausal women with a high baseline FMD level (8.7 ± 0.7%), also showed no significant change in FMD after isoflavone supplementation for 6 wk, which was consistent with our present conclusion.

Previous animal experiments showed that isoflavone produced an effect on endothelial function via the stimulation of NO production (10). Another study (45) suggested that it might be the antiinflammatory action of isoflavone that resulted in the improvement of endothelial dysfunction. On the basis of these findings, it is not surprising that isoflavone has less of an effect in subjects with increased FMD levels and a normal endothelial profile, because subjects with high FMD levels may already have sufficient NO activity and do not have severe inflammatory reactions in the endothelial regions. Conversely, subjects with low FMD levels already have endothelial dysfunction with insufficient NO production and severe regional inflammatory reactions; thus, isoflavone supplementation might significantly increase FMD levels and restore the endothelial function. Chan et al's (32) recent study was the first randomized, double-blinded, placebo-controlled trial to investigate the effect of oral isoflavone supplementation on FMD levels in patients with ischemic stroke, although this trial was not involved in our present meta-analysis because male and female subjects were combined in this study. The baseline FMD level was 2.0 ± 1.8%, and a significant change in FMD level was observed after the supplementation with oral isoflavone (80 mg/d) for 12 wk. This study provides new evidence to support the conclusion of our meta-analysis, which indicates that the effect of isoflavone supplementation may, indeed, be influenced by the baseline endothelial profile.

Despite the intriguing results of the present meta-analysis, some potential limitations should be addressed. First, because isoflavone belongs to a group of estrogen-like plant compounds, it is mainly used by women. Therefore, the studies included in our present meta-analysis were all investigating the effect of isoflavone on endothelial function in postmenopausal women. Male subjects with oral isoflavone supplementation might experience a different effect on endothelial function. Teede et al (16) have already shown that a significant change in FMD can be detected in men after oral isoflavone supplementation for 3 mo. Future studies should include more male subjects to determine the effect of isoflavone on endothelial function in men.

Second, the baseline cholesterol concentration ranged from 180 to 286 mg/dL in the present meta-analysis, and only 2 included trials enrolled hypercholesterolemic women. High cholesterol might impair the endothelial cells and result in endothelial dysfunction and atherosclerosis (46). Previous studies showed that isoflavone significantly reduces cholesterol concentrations (47). Therefore, oral isoflavone supplementation in subjects with high cholesterol concentrations might significantly improve endothelial function. Our subgroup analysis showed a significant trend ($P = 0.07$) between the low and high baseline cholesterol subgroups, which indicated that baseline cholesterol concentrations may influence the effect of oral isoflavone on endothelial function. More studies focusing on hypercholesterolemic subjects should be performed in the future to clarify this issue.

Third, the subgroup analysis showed that the dose of isoflavone was not an effect modifier. This result might be ascribed to a normal but narrow range of doses (50–100 mg/d) in the 9 trials. Therefore, future studies focusing on the effect of different doses of oral isoflavone on endothelial function are needed to modify our present results.

Fourth, the sources of isoflavone used in the trials were not consistent. Of the 9 trials included in our present meta-analysis, the source of isoflavone in 6 trials was soy protein, but the tablets of isoflavone used in the other 3 trials were purchased from different companies. These companies might obtain isoflavone not only from soy protein, but also from other plants or by other means. Therefore, different sources of isoflavone may potentially influence the effect of oral isoflavone on FMD, although no significant difference was found in the subgroup analysis of different sources of isoflavone. Future studies should evaluate the influence of different sources of isoflavone on endothelial function.

In conclusion, the present meta-analysis indicates that oral isoflavone supplementation cannot improve endothelial function in postmenopausal women with high baseline FMD levels (≥5.2%). This improvement was significant when the baseline FMD levels were low (<5.2%), although significant heterogeneity was still detected. The baseline endothelial profile may be an important and potential factor influencing the effect of oral isoflavone supplementation on endothelial function. Additional high-quality rigorous studies, especially in women with cardiovascular diseases and in men, should be performed to confirm our results and explore the exact mechanisms of isoflavone in the improvement of endothelial function.

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