

## **Extracted or synthesized soybean isoflavones reduce menopausal hot flash frequency and severity: systematic review and meta-analysis of randomized controlled trials**

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### **Abstract**

**Objective:** This analysis was conducted to determine the efficacy of extracted or synthesized soybean isoflavones in the alleviation of hot flashes in perimenopausal and postmenopausal women.

**Methods:** PubMed and The Cochrane Controlled Clinical Trials Register Database were searched for relevant articles reporting double-blinded randomized controlled trials through December 14, 2010. References within identified articles, as well as peer-reviewed articles that had come to the attention of the authors through other means, were also examined for suitability. This systematic review and meta-analysis, which evaluated the effects of isoflavones on the frequency, severity, or composite score (frequency  $\times$  severity) of hot flashes compared with placebo was conducted according to Cochrane Handbook guidelines.

**Results:** From 277 potentially relevant publications, 19 trials (reported in 20 articles) were included in the systematic review (13 included hot flash frequency; 10, severity; and 3, composite scores), and 17 trials were selected for meta-analyses to clarify the effect of soybean isoflavones on hot flash frequency (13 trials) and severity (9 trials). Meta-analysis revealed that ingestion of soy isoflavones (median, 54 mg; aglycone equivalents) for 6 weeks to 12 months significantly reduced the frequency (combined fixed-effect and random effects model) of hot flashes by 20.6% (95% CI, -28.38 to -12.86;  $P < 0.00001$ ) compared with placebo (heterogeneity  $P = 0.0003$ ,  $I^2 = 67\%$ ; random effects model). Meta-analysis also revealed that isoflavones significantly reduced hot flash severity by 26.2% (95% CI: -42.23 to -10.15,  $P = 0.001$ ) compared with placebo (heterogeneity,  $P < 0.00001$ ,  $I^2 = 86\%$ ; random effects model). Isoflavone supplements providing more than 18.8 mg of genistein (the median for all studies) were more than twice as potent at reducing hot flash frequency than lower genistein supplements.

**Conclusions:** Soy isoflavone supplements, derived by extraction or chemical synthesis, are significantly more effective than placebo in reducing the frequency and severity of hot flashes. Additional studies are needed to further address the complex array of factors that may affect efficacy, such as dose, isoflavone form, baseline hot flash frequency, and treatment duration.

**Key Words:** Soy – Isoflavones – Hot flashes – Genistein – Systematic review – Meta-analysis.

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**H**ot flashes are the most common menopause-related symptom experienced by women.<sup>1,2</sup> A hot flash is a transient vasomotor event consisting of a sensation of warmth, typically accompanied by sweating, flushing, palpitations, and sometimes anxiety.<sup>3</sup> They can persist for several years after menopause and for some women can interfere with daily activities or sleep to such a degree that treatment is sought.<sup>1</sup> Hormone therapy (HT) containing estrogens alone or with progestins in a cyclic or continuous regimen was recommended for many years for the alleviation of hot flashes, but concerns raised about the safety of HT by the results of the Heart and Estrogen/Progestin Replacement Study,<sup>4</sup> the Women's Health Initiative trial,<sup>5</sup> and the Million Women Study<sup>6</sup> have led to recommendations that such preparations should be taken at the lowest dose for the shortest period of time.<sup>2,7</sup> Not surprisingly, the use of HT has decreased

dramatically in recent years,<sup>8</sup> and increasing numbers of women are seeking “natural” alternatives for the relief of hot flashes.<sup>9</sup>

Observational studies showing that hot flashes are considerably less frequent among native Japanese compared with North American women<sup>10,11</sup> and basic science research on isoflavones and the high isoflavone consumption in Japan led to the hypothesis that the estrogen-like effects of soy isoflavones play a role in preventing or minimizing hot flashes.<sup>12</sup> Subsequently published epidemiological studies reported that menopausal symptoms were inversely correlated with soy intake and circulating isoflavone concentrations among Japanese women.<sup>13-16</sup> Since the first clinical trial was conducted in 1995,<sup>17</sup> more than 50 trials have evaluated the effects of soy foods and isoflavone-containing products on the alleviation of hot flashes.

Two reviews and meta-analyses have concluded that soy isoflavones alleviate hot flashes<sup>18,19</sup>; however, most of the others have stated that the data are not sufficiently conclusive to allow definitive conclusions to be made.<sup>20-28</sup> Among these latter analyses, only three evaluated the effects of intervention statistically by combining the data in meta-analyses.<sup>18,20,26</sup> The first to do so included six studies of soy isoflavone extracts and found the combined weighted mean difference in the number of daily hot flashes for soy isoflavone extracts compared with placebo to be  $-1.15$  (95% CI,  $-2.33$  to  $0.03$ ) after 4 to 6 weeks (five trials) and  $-1.22$  (95% CI,  $-2.02$  to  $-0.42$ ) after 6 months (two trials).<sup>20</sup> The second meta-analysis found a significant standardized mean difference (SMD) in percentage change from baseline between isoflavone and control groups of  $-0.34$  (95% CI,  $-0.47$  to  $-0.21$ ,  $P < 0.0001$ ), by combining 12 (six involving soy isoflavone extracts and six involving soy foods/soy protein) parallel-group trials.<sup>18</sup> In the third meta-analysis, which included 19 interventions using “soy dietary supplements” ( $n = 11$ ), “soy extracts” ( $n = 5$ ), or “isolated isoflavones” (genistein or daidzein,  $n = 3$ ), the combined SMD was  $-0.39$  (95% CI,  $-0.53$  to  $-0.25$ ;  $P < 0.0001$ ; for the number of hot flashes, average score of vasomotor symptoms, or average percentage reduction in hot flashes). The authors concluded that the results showed “a significant tendency” in favor of intervention but that conclusions were constrained by the high heterogeneity among the studies.<sup>26</sup>

The SMD was used as a summary statistic for the effect of soy isoflavones on hot flashes in the latter two meta-analyses; however, this method assumes that the differences in SDs among studies reflect differences in measurement scales and not actual differences in variability among study populations.<sup>29</sup> This assumption may be problematic when actual study-related differences in variability occur among participants. In addition, the overall intervention effect can be difficult to interpret when it is reported in units of SD rather than in the units of the measurement scales used in the analyses. Therefore, the published analyses have important limitations. Furthermore, several randomized controlled trials (RCTs) that addressed the effects of soy isoflavone extracts on hot flashes were only recently published and were therefore not included in the above meta-analyses.

The increasing use of soy isoflavone—containing products for the alleviation of menopausal hot flashes underscores the importance of accurate assessments of their efficacy. Therefore, the present systematic review and meta-analysis of RCTs was performed specifically to clarify the effects of ingesting soy isoflavone extracts (not soy foods or soy protein) and synthesized isoflavones on the frequency (number), severity (intensity), and composite score (frequency  $\times$  severity) of hot flashes compared with placebo as expressed as percentage change from baseline in perimenopausal and postmenopausal women. We also sought to evaluate a previous observation that isoflavone-containing products with higher genistein contents are more efficacious than those with lower contents.<sup>21</sup>

## METHODS

### Literature search

The protocol for this systematic review and meta-analysis was based on the Cochrane Handbook for Systematic Reviews of Interventions.<sup>29</sup> PubMed and The Cochrane Controlled Clinical Trials Register Database were searched for published RCTs through December 14, 2010, using complex search strategies containing text and indexing terms ([Fig. 1](#)). As shown in [Figure 1](#), for the PubMed search, free keywords were mapped to the appropriate MeSH terms, and the search strategy that resulted in the most relevant articles was adopted. Reference lists of relevant systematic reviews and meta-analyses<sup>18,20,26</sup> and included RCTs were manually searched. Investigators were also contacted to identify additional studies (including unpublished trials).

### Inclusion and exclusion criteria

Evaluation of the inclusion and exclusion of relevant trials for the systematic review and meta-analysis was independently performed by at least two reviewers, and consensus was reached by discussion when there were disagreements. Studies were included for systematic review if they met all of the following criteria: (1) participants were perimenopausal and/or postmenopausal women with complaints of hot flashes; (2) evaluated soy isoflavone extracts (studies of soy foods, soy protein, or products containing isoflavones from nonsoy sources were excluded) or chemically synthesized isoflavones identical to those found in soy and clearly described isoflavone dose used; (3) contained at least one relevant pairwise comparison of intervention arms (ie, soy isoflavone extracts versus placebo) and the placebo used was identical or similar in appearance and taste to the isoflavone product; (4) reported outcomes for effects on frequency (continuous numerical data), severity (categorical scale data), or composite score (frequency  $\times$  severity) of hot flashes as an individual symptom; and (5) used a parallel-group or crossover design and was reported in English, Chinese, or Japanese.

## **PubMed**

#1: Soy\* OR isoflavone OR phytoestrogen OR genistein OR genistin OR daidzein OR daidzin OR glycitein OR glycitin

#2: Hot flashes OR hot flushes OR menopausal symptoms

#3: Randomized controlled trial [PT]

#4: Random [TIAB] OR randomized [TIAB] OR randomly [TIAB]

#5: Control [TIAB] OR controlled [TIAB] OR placebo [TIAB]

#6: #3 OR (#4 AND #5)

#7: #1 AND #2 AND #6

## **CENTRAL (excluding PubMed)**

#1: Soy\* OR isoflavone OR phytoestrogen OR genistein OR genistin OR daidzein OR daidzin OR glycitein OR glycitin

#2: Hot flashes OR hot flushes OR menopausal symptoms

#3: "accession number" near PubMed

#4: #1 AND #2 NOT #3

**FIG. 1.** Search strategy for PubMed and CENTRAL (excluding PubMed). PT and TIAB are PubMed search field tags of Publication Type and Title/Abstract, respectively.

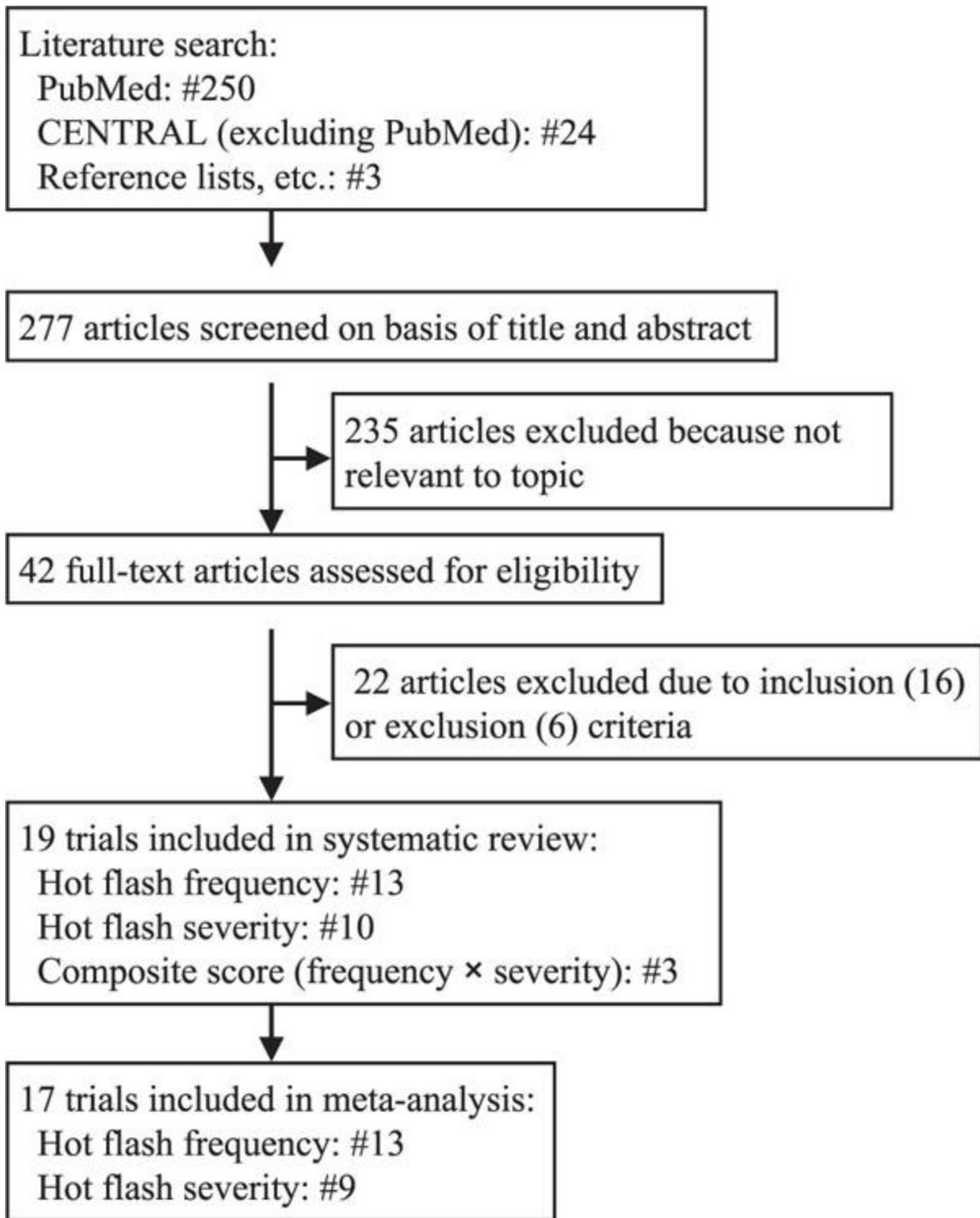
CENTRAL, Cochrane Central Register of Controlled Trials

([http://onlinelibrary.wiley.com/o/cochrane/cochrane\\_clcentral\\_articles\\_fs.html](http://onlinelibrary.wiley.com/o/cochrane/cochrane_clcentral_articles_fs.html)).

Intervention studies that combined soy isoflavones with other treatments that might have effects on hot flashes, such as other phytoestrogens (eg, lignans or isoflavones from red clover), prescription medications,<sup>30</sup> or estrogen, in either or both of the comparison intervention arms, were excluded to eliminate possible interference with the effects of soy isoflavones.<sup>31</sup> Trials that reported only a total score for the Kupperman Menopausal Index (KMI)<sup>32</sup> or for the Greene Climacteric Scale (GCS<sup>33</sup>; score/scale included hot flashes and various other menopausal symptoms) or trials that reported vasomotor sub-scales (including hot flashes and night sweats) of the GCS but did not report individual data on hot flashes were excluded.<sup>34-37</sup>

Meta-analyses based on means require that data are at least approximately normally distributed or are derived from very large trials (n > 100/group). The appropriate paired analysis of continuous data from crossover trials requires that neither carryover nor period effects are identified as a problem.<sup>29</sup> In the current analysis, when at least five trials included in the systematic review provided analyzable percentage mean change from baseline and SD/SE of approximately normally distributed data regarding frequency, severity, or composite score of hot flashes, relevant trials were separately selected for meta-analysis to clarify the effects of soy isoflavones on these specific parameters.

Of the 19 studies included in the systematic review ([Fig. 2](#)), 13 evaluated frequency<sup>38-51</sup>; 10, severity<sup>40,41,44,46,51-56</sup>; and 3, a composite score.<sup>46,50,57</sup> Two articles were published from the same trial,<sup>40,41</sup> and one article reported two studies (study A and B); study B used the same soy preparation as study A but with the concurrent use of a polyunsaturated fatty acid supplement for the entire 24 weeks.<sup>38</sup> Because the polyunsaturated fatty acid supplement was hypothesized to affect the impact of isoflavones on hot flashes, only study A was included in the meta-analysis. In one study that included two phases, a 12-week placebo-controlled phase (phase I) followed by a 12-week open observation phase (phase II) in which all study participants received the active treatment, only phase I was included.<sup>44</sup> In another study that contained a 6-week placebo-controlled phase followed by concurrent use of conjugated equine estrogens for 4 weeks and then a 2-week conjugated equine estrogens—only phase, only the first phase was included.<sup>49</sup>



**FIG. 2.** Schematic diagram of selection of randomized controlled trials. #, number of records.

In 2009, D’Anna et al<sup>41</sup> reported the effects of isolated genistein on hot flash frequency and severity after 12 and 24 months; the 12-month data were reported by these authors in 2007.<sup>40</sup> Because the duration of the other 18 trials included in systematic review (Table 1) were all 12 months or less, only the 12-month data of D’Anna et al<sup>40</sup> were included in the meta-analysis.

Substantial reduction in hot flashes was generally observed with time in the placebo interventions, raising the possibility of carryover and period effects.<sup>2,18</sup> Therefore, as noted previously, crossover trials that did not clearly address carryover and period effects were excluded from meta-analysis<sup>56</sup>; otherwise, only data from the first period were used.<sup>38</sup> Finally, 17 trials were included in the meta-analysis (13 for frequency<sup>38-40,42-51</sup> and 9 for severity<sup>40,44,46,47,51-55</sup>) (Fig. 2).

### Data extraction

Data on study design, number of participants, intervention, and outcomes for hot flashes were independently extracted by at least two reviewers and were compared and confirmed (Table 1). Data from graphs presented in the articles were estimated<sup>40,41,46,47</sup> or authors were contacted to provide the necessary information.<sup>40,41,44,45,51,52</sup> Mean percentage change from baseline and the SD/SEs were calculated from the raw data on hot flash frequency obtained from the author, excluding participants without hot flashes at baseline.<sup>45</sup>

**TABLE 1** *Experimental design of studies included in the systematic review*

First author/year	Location	Design	Participant number			Duration, wk	Mean age (range)	Isoflavone intake, mg/d (aglycone equivalents)		Dose regimen, times per d
			Active initial/final	Placebo initial/final	Total			Genistein		
Albertazzi, <sup>57</sup> 2005	England	X	50	49	6	54 (44–65)	90	90	1	
Campagnoli, <sup>38</sup> 2005	Italy	X	36/29	36/29	12	51 (45–58)	37.1	18.8	1	

First author/year	Location	Design	Participant number		Duration, wk	Mean age (range)	Isoflavone intake, mg/d (aglycone equivalents)		Dose regimen, times per d
			Active initial/final	Placebo initial/final			Total	Genistein	
Cheng, <sup>52</sup> 2007	Sweden	P	26/UC	25/UC	12	58 (49–69)	37.2	19.4	1
Crisafulli, <sup>39</sup> 2004	Italy	P	30/30	30/30	52	52 (47–57)	54	54	1
D’Anna, <sup>40</sup> 2007	Italy	P	198/125	191/122	52	53 (50–70)	54	54	2
Evans, <sup>51</sup> 2011	Canada	P	40/32	42/36	12	53 (40–65)	30	30	1
Faure, <sup>42</sup> 2002	France	P	39/33	36/22	16	53 (NI)	42	8	1
Ferrari, <sup>43</sup> 2009	Italy	P	85/52	95/65	12	54 (40–65)	80	60	1
Gocan, <sup>44</sup> 2008	Austria	P	54/54	82/80	12	54 (45–70)	60	6.8	1

First author/year	Location	Design	Participant number		Duration, wk	Mean age (range)	Isoflavone intake, mg/d (aglycone equivalents)		Dose regimen, times per d
			Active initial/final	Placebo initial/final			Total	Genistein	
Hachul, <sup>45</sup> 2011	Brazil	P	60/37	UC	16	NI (50–65)	80	60.8	1
Han, <sup>53</sup> 2002	Brazil	P	UC/40	UC/40	16	49 (45–55)	100	69.9	2
Jou, <sup>54</sup> 2008	Taiwan	P	66/63	30/26	24	54 (NI)	135	17.1	2
Khaodhiar, <sup>46</sup> 2008	United States	P	L/48	45	12	53 (38–60)	40	4	1
			H/49				60	6	
Nahas, <sup>47</sup> 2007	Brazil	P	40/38	40/38	36	56 (<45)	60	30	2
Petri Nahas, <sup>55</sup> 2004	Brazil	P	25/unknown	25/UC	24	53 (NI)	36	4.8	2
Nikander, <sup>56</sup> 2003	Finland	X	32/28	30/28	12	54 (35–	114	6.8	2

First author/year	Location	Design	Participant number			Duration, wk	Mean age (range)	Isoflavone intake, mg/d (aglycone equivalents)		Dose regimen, times per d
			Active initial/final	Placebo initial/final	Total			Genistein		
										69)
Penotti, <sup>48</sup> 2003	Italy	P	28/22	34/27	24	53 (45–60)	61.1	9.4	2	
Scambia, <sup>49</sup> 2000	Italy	P	20/20	19/19	6	54 (29–63)	39.9	15.6	Unknown	
Upmalis, <sup>50</sup> 2000	United States	P	90/59	87/63	12	55 (≥50)	30.9	15.6	1	

X, crossover; P, parallel; UC, unclear; NI, not indicated; L, low dose; H, high dose.

A variety of outcomes measures for hot flashes were evaluated in the trials included in the systematic review, that is, frequency (daily or weekly numbers), severity (intensity), and/ or composite score (frequency × severity). Of the 13 trials that reported frequency data based on self-report using symptom diaries, one trial (placebo-controlled phase I) reported nonnormally distributed baseline and posttreatment frequency data,<sup>44</sup> one crossover trial (study A) reported only the mean weekly number of hot flashes for both periods but did not clearly address carryover and period effects,<sup>38</sup> one trial (first 6-wk duration) reported a weekly percentage change in the number of hot flashes,<sup>49</sup> and the remaining trials reported the daily number of hot flashes.

A variety of rating scales were used among the 10 trials included in the systematic review that provided self-reported severity data. One study<sup>52</sup> used a 5-point (1, not at all; 2, a little; 3,

medium; 4, much; 5, very much) scale developed by Collins and Landgren,<sup>58</sup> one study used a self-defined 3-point (1, mild; 2, moderate; 3, severe) scale,<sup>40</sup> one study used a 4-point scale (0, not at all; 1, mild; 2, moderate; 3, severe) for hot flashes/ night sweats as a component symptom of the GCS,<sup>44</sup> one study also used a 4-point scale (0-3; 0, none; 3, severe) for severity of hot flash,<sup>51</sup> one study used a 4-point scale (0, no; 1, slight, less than 5; 2, moderate, 5–10; 3, severe, 910) based on the number of hot flashes per day<sup>53</sup> as a component symptom of the KMI, one study used a 10-point scale (1, mild; 10, very severe) that was converted into a 4-point scale (1–3 = 1; 4–6 = 2; 7–9 = 3; 10 = 4),<sup>46</sup> and four studies used a 4-point scale (0, absent; 1, mild or weak; 2, moderate; 3, severe) as a component of the KMI.<sup>47,54–56</sup> Four of the five trials that reported hot flash severity as a component of KMI multiplied a weighting factor of 4,<sup>47,53–55</sup> whereas the remaining trial used the KMI and an overall “menopausal syndrome” severity.<sup>56</sup> Two of these three trials included in the systematic review collected data independently on frequency and intensity/severity but only provided severity data via a composite score that multiplied the number of hot flashes by their severity graded from 1 to 3,<sup>50,57</sup> whereas the third of these trials multiplied the number of hot flashes by their severity graded from 1 to 4.<sup>46</sup>

Some evidence suggests that the efficacy of isoflavones may be influenced by baseline hot flash frequency, that is, the higher the frequency, the greater the efficacy.<sup>18,26,57,59</sup> To minimize the influence of baseline hot flash frequency and differences in severity scales, the outcome of each intervention arm was determined as a percentage change from baseline in hot flash number, severity, or score. The percentage change from baseline outcomes was also preferred because they might have a less skewed distribution than final measurement outcomes.<sup>29</sup> The treatment effect of isoflavones for each trial was estimated as the mean difference between percentage change from baseline in hot flashes for each comparison intervention arm (ie, the percentage change from baseline for participants ingesting soy isoflavones minus that for placebo). Mean percentage change from baseline [(posttreatment mean – baseline mean)/baseline mean × 100%] and its SD (SD of mean change/baseline mean × 100%) was calculated for each intervention arm when the data were not directly reported.

When the SD/SEs of the mean (or percentage mean) changes from baseline were not reported, they were calculated using the reported statistics comparing the changes (eg, CIs, SE, *t* values, *P* values, *F* values). When levels of significance were reported rather than the exact *P* values, the *P* value at the upper limit was used: for example, *P* = 0.05 was used when the article indicated *P* < 0.05. Thirteen of the trials included in the systematic review for hot flash frequency contained sufficient information to calculate treatment effect (mean difference in percentage change from baseline between the two comparison intervention arms) and SE and were included in the meta-analysis. As for trials that did not contain sufficient information to calculate the SD for the percentage change in hot flash severity,<sup>44,46,54</sup> the largest available SD (20.99%–51.32%; averaged, 39.14%) for the same outcome reported in another study<sup>40</sup> in the systematic review

was used as a reasonable imputation.<sup>29</sup> Data for the two isoflavone dose arms in the study by Khaodhiar et al<sup>46</sup> were combined to create one isoflavone arm and compared with placebo.

## Quality assessment

A three-category grading system (A, B, and C) was used to denote the methodological quality of each study as described elsewhere.<sup>28</sup> Category A studies have the least bias, and the results are considered valid; category B studies are susceptible to some bias but not sufficient to invalidate the results; and category C studies (defined as low quality) have significant bias that may invalidate the results (eg, dropout rate 920%, missing baseline data, or irreconcilable apparent differences between data in figures, tables, and text). Concealment of treatment allocation in RCTs was assessed as adequate, inadequate, or unclear.<sup>60</sup> At least two reviewers independently assessed the studies, and consensus was reached by discussion when there were disagreements.

## Meta-analysis and statistical analysis

Two meta-analyses were separately conducted to determine the overall treatment effect of isoflavones on the frequency and the severity of hot flashes using Review Manager 5.1 (Nordic Cochrane Center, Oxford, UK). Percentage change from baseline of categorical severity data reported in the various scales was also analyzed as continuous data similar to the frequency data. When trials contained analyzable repeated measurement data of hot flashes,<sup>39,40,42,43,46-48,50,51,54</sup> the final data were used for the meta-analysis, whereas the interim measurements were used for sensitivity analysis. The data sets for other time points were used for sensitivity analyses. Two studies reported both intention-to-treat (ITT) and per-protocol (PP) analysis data<sup>42,44,51</sup>; we conservatively used ITT data for the primary analysis, but PP data were used for sensitivity analysis. Ferrari et al<sup>43</sup> reported change in the mean daily number of moderate-to-severe hot flashes as the main efficacy variable and change in frequency of hot flashes of any intensity (mild-to-severe). For this study, we used the data for the moderate-to-severe hot flashes for the primary analysis and data for hot flashes of any intensity for sensitivity analysis.

We used both the fixed-effect and the random effects models to calculate mean differences, 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 among results, possible causes were explored by investigating the influence of each trial on the overall meta-analysis estimate and by conducting subgroup analyses and metaregressions. When the cause of the heterogeneity was not determined, the results based on the random effects model were adopted. The *I*<sup>2</sup> statistic (0% to 40%, possibly important; 30% to 60%, may represent moderate heterogeneity; 50% to 90%, may represent substantial heterogeneity; 75% to 100%, may represent considerable heterogeneity) was used for quantifying inconsistency across studies. This process describes the percentage of the variability in effect estimates due to heterogeneity rather than sampling error (chance). Sensitivity analyses were conducted to evaluate the influence of the method of reporting data (using PP data),<sup>42,44</sup> intensity of hot flashes recorded

(using frequency data for hot flashes of any intensity<sup>43</sup>), intervention duration (using interim data from trials with repeated measurements), study quality (eliminating low-quality trials), study design (excluding crossover trials), and imputation approach for missing SD of mean percentage change from baseline in hot flashes (using the averaged SD available from other studies included in the review).

If at least 10 trials were available, subgroup analyses and metaregressions were performed to investigate factors that might relate to the varying effects of isoflavones on hot flashes based on four prespecified factors: dose of isoflavones, dose of genistein, study duration, and baseline hot flash frequency or severity. Statistical difference between the two subgroups was considered when the CIs of the summary estimates in the two subgroups did not overlap or overlapped to a small degree. A significance test was also conducted to investigate the difference between subgroups using the method implemented in RevMan for fixed-effect analyses.

Potential publication bias was examined by using funnel plots and by performing the Egger test to assess the asymmetry of funnel plots. Funnel plots and subgroup analyses were conducted using Review Manager. Metaregressions, tests for asymmetry of funnel plots, and investigation of the influence of each trial on the overall meta-analysis estimate were performed using Stata 10.1 for Windows (StataCorp LP, College Station, TX).

## RESULTS

### Characteristics of included studies

Among the 19 RCTs that met the inclusion criteria for systematic review ([Table 1](#)), 16 trials used a parallel-group design,<sup>39-55</sup> and 3 used a crossover design.<sup>38,56,57</sup> The intervention duration ranged from 6 weeks<sup>49,57</sup> to 24 months.<sup>41</sup> Sixteen trials specified that the participants were postmenopausal women, but the time since the last menstrual period and hormone levels used to define postmenopause status differed across trials.<sup>38-42,45-53</sup> Two trials specified participants as menopausal women,<sup>43,54</sup> and one trial specified participants as premenopausal, perimenopausal, or postmenopausal women.<sup>44</sup> The trials included in this systematic review were conducted in 10 different countries: six trials in Italy, four in Brazil, two in the United States, and one each in Austria, Canada, England, Finland, France, Taiwan, and Sweden. A variety of isoflavone supplements and doses were used as intervention products; the doses ranged from a low of 30 mg<sup>51</sup> to a high of 135 mg.<sup>54</sup> For one trial, which did precisely describe the isoflavone content of the intervention product,<sup>47</sup> and for all others that expressed isoflavone content in glycoside weight, the isoflavone aglycone equivalent weight was estimated by multiplying the total isoflavone dose by 0.6 to correct for molecular weight differences.<sup>61</sup> In this text, isoflavone weights refer to the aglycone equivalents. In another study, because the genistein content of the intervention product (standardized soy extract) was not indicated,<sup>42</sup> it was estimated based on the data presented in another article that used this same product.<sup>47</sup>

Regarding study quality, eight studies were assessed as having “adequate” concealment of treatment allocation,<sup>38,44,46,47,51,53,55,57</sup> five of which were rated “A”<sup>47,51,53,55,57</sup> and three “C” (at high risk of bias).<sup>38,44,46</sup> Because of insufficient information, the allocation concealment of the remaining 11 studies was assessed as “unclear,” of which four trials were rated “A”<sup>39,40,52,56</sup> and seven “C.”<sup>42,43,45,48–50,54</sup>

## Effect of soy isoflavone extracts on frequency of hot flashes

Thirteen trials were included in the systematic review that assessed the effect of isoflavones on hot flash frequency. Both soy isoflavone extracts (–27%<sup>38</sup> to –80%<sup>45</sup>) and placebo (–0.72%<sup>41</sup> to –42%<sup>45,47</sup>) reduced hot flash frequency after the full intervention duration in the 12 parallel-group trials. After subtracting the placebo effect, the reduction in response to isoflavones in these trials ranged from 3%<sup>38</sup> to 57%.<sup>40</sup> Ten of the 13 trials reported statistically significant effects<sup>39–46,49,51</sup>; in one, significance was almost achieved ( $P = 0.0275$  for the first 6 wk and  $P = 0.078$  for the full 12 wk),<sup>50</sup> another was not significant,<sup>48</sup> and the significance of remaining trial was not reported.<sup>47</sup> In the study by Crisafulli et al,<sup>39</sup> the treatment effect was more pronounced in a subgroup of women with more than five hot flashes per day at baseline, but the comparison between the two subgroups was not reported. In four studies, the results of ITT analysis were similar to those of PP analysis,<sup>42,44,51</sup> and in the study by Ferrari et al,<sup>43</sup> the effect on hot flashes of any intensity was similar to the effect on moderate-to-severe hot flashes. Finally, in the crossover trial by Campagnoli et al,<sup>38</sup> which included postmenopausal women with 5 or more moderate-to-severe hot flashes per day, both isoflavones and placebo reduced hot flash frequency, but the difference was not statistically significant.

In the meta-analysis that included 13 trials and 1,196 participants, the fixed-effect model revealed that the daily ingestion of 30 to 80 mg/day of isoflavones (median, 54 mg) for 6 weeks to 12 months significantly reduced hot flash frequency by a net (after subtracting the placebo effect) of 17.42% (95% CI, –21.34 to –13.50,  $P < 0.00001$ ; heterogeneity,  $P = 0.0003$ ,  $I^2 = 67\%$ ; [Table 2](#)). No trial seemed to clearly influence the overall effect ([Fig. 3](#)). The total response (including placebo) in the fixed-effect model was –47.01 (95% CI, –49.73 to –44.28). Meta-analysis using the random effects model resulted in a net reduction of 20.6% (95% CI, –28.38 to –12.86,  $P < 0.00001$ ; [Fig. 4](#), [Table 2](#)). The total response (including placebo) in the random effects model was –50.24 (95% CI, –57.75 to –42.73). The results of the sensitivity analyses based on the four prespecified factors (method of analyzing data, intensity of hot flashes, intervention duration, and study quality) indicate that the effects of isoflavones were robust ([Table 2](#)). Sensitivity analyses using the first interim data of nine trials that contained repeated measurements resulted in nonsignificant heterogeneity among the 13 trials. The results of subgroup analyses and metaregressions of the effects of isoflavones on hot flash frequency are shown in [Table 3](#).

**TABLE 2** Results of meta-analysis and sensitivity analyses evaluating effect soy isoflavone extracts on hot flash frequency

Analyses	Trials	n	Heterogeneity	% Change in frequency of hot flashes (95% CI)	
				Fixed-effect model	Random effects model
Soy isoflavones vs placebo					
Final ITT data <sup>a</sup>	13 trials <sup>38-40,42-51</sup>	1,196	$P = 0.0003$	-17.42 (-21.34 to -13.50)	-20.62 (-28.38 to -12.86)
			$I^2 = 67%$	$P < 0.00001$	$P < 0.00001$
Final PP data (3 of PP <sup>42,44,51</sup> )	13 trials <sup>38-40,42-51</sup>	1,173	$P = 0.0004$	-16.67 (-20.53 to -12.82)	-19.87 (-27.60 to -12.15)
			$I^2 = 66%$	$P < 0.00001$	$P < 0.00001$
Final ITT data (1 of mild-to-severe <sup>43</sup> )	13 trials <sup>38-40,42-51</sup>	1,196	$P = 0.0003$	-17.31 (-21.15 to -13.46)	-20.60 (-28.24 to -12.97)
			$I^2 = 67%$	$P < 0.00001$	$P < 0.00001$
Final ITT data of A-, B-category trials	4 trials <sup>39,40,47,51</sup>	449	$P = 0.01$	-32.95 (-41.12 to -24.78)	-32.96 (-48.93 to -16.99)
			$I^2 = 73%$	$P < 0.00001$	$P < 0.0001$
First ITT data <sup>b</sup>	13 trials <sup>38-40,42-51</sup>	1,223	$P = 0.46$	-16.29	Same as using

Analyses	Trials	<i>n</i>	Heterogeneity	% Change in frequency of hot flashes (95% CI)	
				Fixed-effect model	Random effects model
				(-20.16 to -12.41)	the fixed-effect model
			$I^2 = 0\%$	$P < 0.00001$	
First PP data (3 of PP <sup>42,44,51</sup> )	13 trials <sup>38-40,42-51</sup>	1,209	$P = 0.43$	-16.07 (-19.89 to -12.25)	-16.11 (-19.98 to -12.23)
			$I^2 = 1\%$	$P < 0.00001$	$P < 0.00001$
First ITT data (1 of mild-to-severe <sup>43</sup> )	13 trials <sup>38-40,42-51</sup>	1,223	$P = 0.50$	-16.60 (-20.44 to -12.75)	Same as using the fixed-effect model
			$I^2 = 0\%$	$P < 0.00001$	
First ITT data of A-, B-category trials	6 trials <sup>39,40,42,47,48,51</sup>	596	$P = 0.36$	-21.51 (-27.91 to -15.11)	-21.12 (-27.97 to -14.28)
			$I^2 = 9\%$	$P < 0.00001$	$P < 0.00001$
Second ITT data <sup>c</sup>	13 trials <sup>38-40,42-51</sup>	1,212	$P < 0.0001$	-16.31 (-19.82 to -12.81)	-18.46 (-25.97 to -10.94)
			$I^2 = 73\%$	$P < 0.00001$	$P < 0.00001$
Second PP data	13 trials <sup>38-40,42-51</sup>	1,201	$P < 0.0001$	-15.76	-17.93 (-25.42)

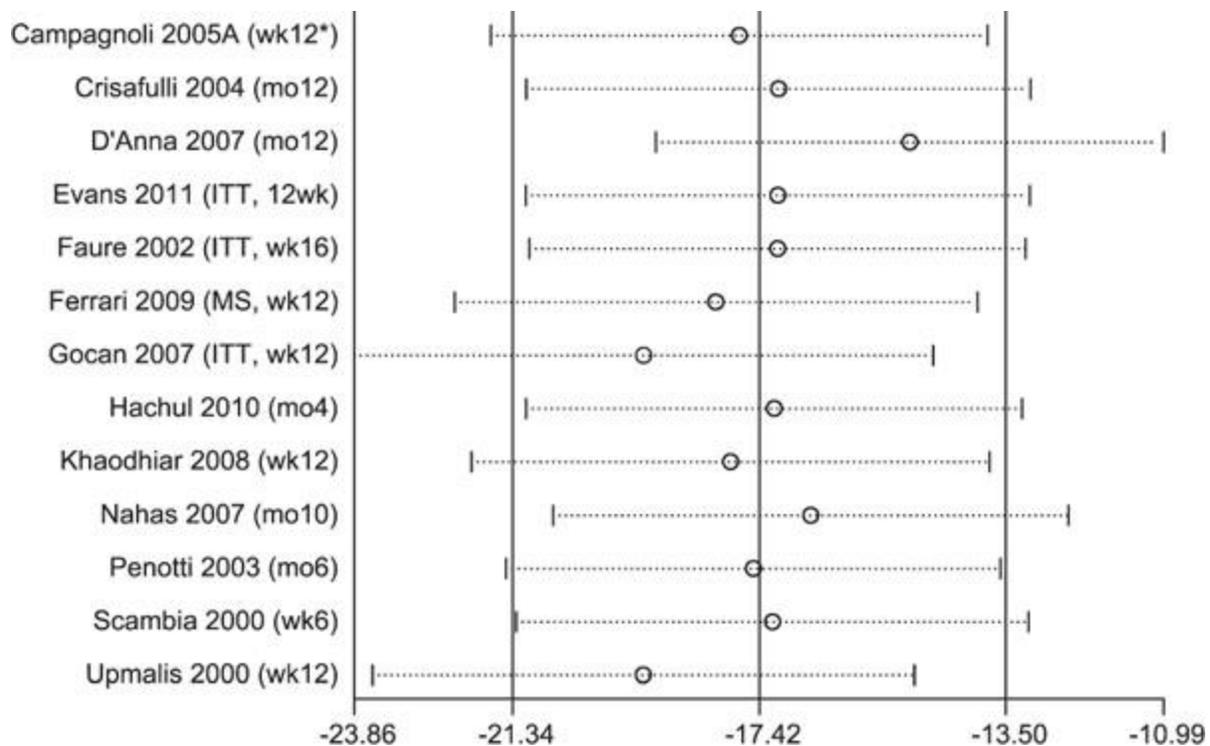
Analyses	Trials	<i>n</i>	Heterogeneity	% Change in frequency of hot flashes (95% CI)	
				Fixed-effect model	Random effects model
(3 of PP <sup>42,44,51</sup> )				(-19.21 to -12.32)	to -10.44)
			$I^2 = 73%$	$P < 0.00001$	$P < 0.00001$
Second ITT data (1 of mild-to-severe <sup>43</sup> )	13 trials <sup>38-40,42-51</sup>	1,212	$P < 0.0001$	-16.26 (-19.71 to -12.81)	-18.48 (-25.88 to -11.08)
			$I^2 = 73%$	$P < 0.00001$	$P < 0.00001$
Second ITT data of A- and B-category trials	6 trials <sup>39,40,42,47,48,51</sup>	586	$P < 0.00001$	-22.22 (-27.78 to -16.67)	-24.41 (-40.74 to -8.07)
			$I^2 = 84%$	$P < 0.00001$	$P = 0.003$

ITT, intention to treat; PP, per protocol.

<sup>a</sup>Using primary ITT % change from baseline data of three trials<sup>42,44,51</sup> that also contained PP data and using primary data of moderate-to-severe hot flashes in one trial<sup>43</sup> that also contained data of mild-to-severe hot flashes.

<sup>b</sup>Using first interim ITT data of 10 trials<sup>39,40,42,43,45-48,50,51</sup> that contained repeated measurements and using final data of remaining three trials.

<sup>c</sup>Using second interim ITT data of six trials<sup>39,40,42,47,48,51</sup> that contained three or more repeated measurements and using final data of remaining seven trials.



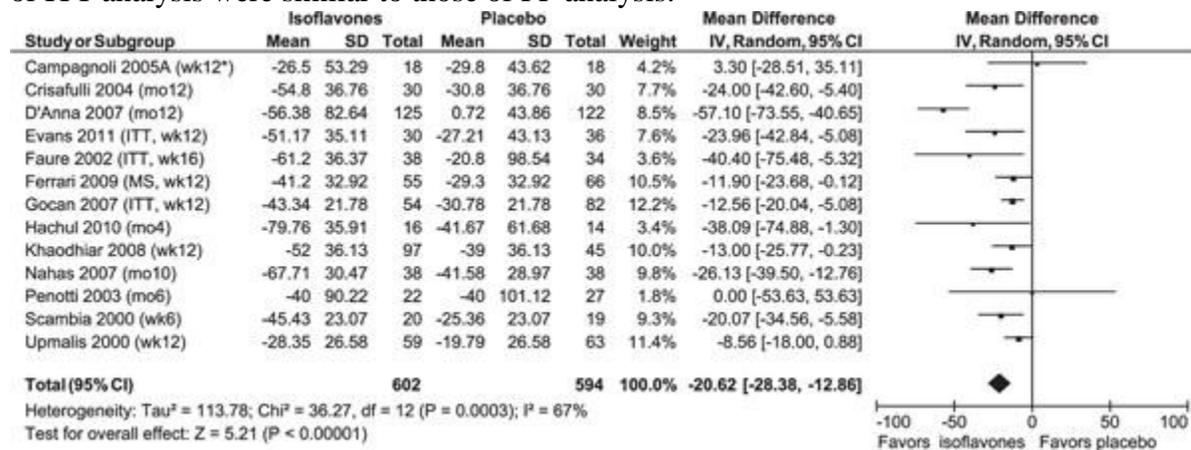
**FIG. 3.** Influence of each study on the overall effect of soy isoflavones on frequency of hot flash (% change from baseline). ITT analysis data were used for this figure. The vertical center line and the two lines on each side of it denote the combined overall effect and 95% CI for all included studies (fixed-effect model). The open circles (^) and horizontal dotted lines denote the combined overall effect and 95% CI when each study is omitted, respectively, thereby demonstrating the influence of this study on the overall effect. ITT, intention to treat; MS, moderate-to-severe.

When final primary data were used, the subgroup of trials that used isoflavone doses that provided 18.8 mg/day genistein or more (median genistein intake among studies) and were at least 12 weeks in duration (median) resulted in significantly larger reductions in hot flash frequency than did those trials that were shorter in duration and that provided lesser amounts of genistein. More specifically, trials that used a higher genistein-content isoflavone product reduced hot flash frequency more than twice as much as trials that used low-genistein supplements (fixed-effect model: high genistein,  $-26.50$ ; low genistein,  $-12.47$ ; test for subgroup difference,  $P = 0.0008$ ; random effects model: high genistein,  $-29.13$ ; low genistein,  $-12.47$ , test for subgroup difference,  $P = 0.03$ ). Metaregression based on the two-category data (1, emedian; 2, 9median;  $P = 0.065$ ) and on continuous data ( $P = 0.116$ ) indicated that the difference between the two pairwise subgroups did not quite achieve statistical significance. Using a lower genistein cutoff (e15 mg vs 915 mg) also showed supplements with higher genistein contents to be more effective ([Table 3](#)).

On the effect of duration, in longer-term studies (>12 wk), isoflavones reduced frequency about 3 times more (fixed-effect model:  $-34.63$  vs  $-12.66$ ,  $P < 0.00001$ ; random effects model:  $-34.29$  vs  $-12.66$ ,  $P < 0.006$ ) than shorter-term studies. Finally, metaregression fitting the effects on frequency and the continuous length of intervention duration revealed a significant relationship between the two variables (Fig. 5). Importantly, the funnel plot (Fig. 6) and Egger test ( $P = 0.232$ ) did not indicate obvious publication bias.

### Effect of soy isoflavone extracts on severity of hot flashes

Ten trials were included in the systematic review of the effect of isoflavones on hot flash severity/intensity. The reduction in the placebo groups ranged from 0%<sup>52</sup> to  $-78\%$ <sup>54</sup> and, in the isoflavone groups, from  $-6.98\%$ <sup>51</sup> to  $-69.89\%$ <sup>47</sup>. The net percentage change (minus placebo) resulting from isoflavone exposure ranged from 9.5%<sup>54</sup> to  $-57\%$ <sup>52</sup>. The results from two of the ten trials were statistically significant,<sup>41,52</sup> one almost achieved significance,<sup>50</sup> two were not significant,<sup>48,51</sup> and the significance of the remaining studies was unclear.<sup>44,46,47,53-55</sup> The results of ITT analysis were similar to those of PP analysis.<sup>44</sup>



**FIG. 4.** Effects of soy isoflavone extracts on frequency of hot flashes (% change from baseline). Mean difference, mean percentage changes (%) in frequency of hot flashes from baseline for soy isoflavones minus that for placebo. Random indicates random effects model. ■ denotes the effect estimate of each study (size of the square corresponds to its weight), horizontal line denotes the 95% CI, and ◆ denotes the combined overall effect. ITT, intention to treat; MS, moderate-to-severe.

**TABLE 3** Subgroup analyses and metaregressions of the effects of soy isoflavone extracts on frequency of hot flashes

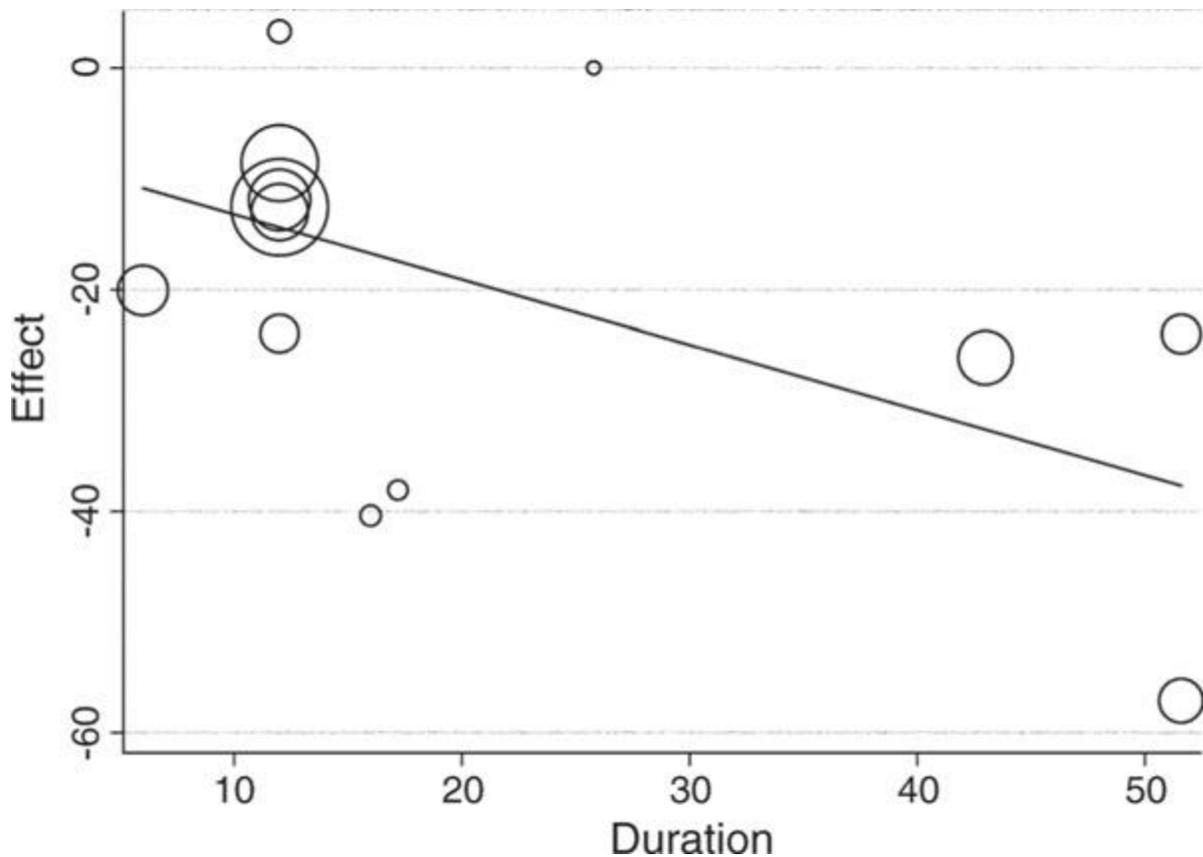
Variables	Trials	n	Heterogeneity	Mean difference in hot flash frequency, % (95% CI)
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				Fixed-effect model	Random effects model
<b>Isoflavone dose</b>					
≤54 mg/d (median)	8 trials <sup><a href="#">38-40,42,46,49-51</a></sup>	784	$P = <0.0001, I^2 = 77\%$	-19.47 (-24.95 to -13.99), $P < 0.00001$	-22.74 (-35.13 to -10.36), $P = 0.0003$
>54 mg/d	5 trials <sup><a href="#">43-45,47,48</a></sup>	412	$P = 0.27, I^2 = 22\%$	-15.26 (-20.87 to -9.65), $P < 0.00001$	-16.12 (-23.40 to -8.85), $P < 0.0001$
Test for subgroup difference				$P = 0.29$	$P = 0.37$
Metaregression				2-category data, $P = 0.61$ ; continuous data, $P = 0.727$	
<b>Genistein dose</b>					
≤18.8 mg/d (median)	7 trials <sup><a href="#">38,42,44,46,48-50</a></sup>	596	$P = 0.50, I^2 = 0\%$	-12.47 (-17.34 to -7.60), $P < 0.00001$	Same as using the fixed-effect model
>18.8 mg/d	6 trials <sup><a href="#">39,40,43,45,47,51</a></sup>	600	$P = 0.001, I^2 = 75\%$	-26.50 (-33.11 to -19.90), $P < 0.00001$	-29.13 (-43.09 to -15.17), $P < 0.0001$
Test for subgroup difference				$P = 0.0008$	$P = 0.03$

Variables	Trials	n	Heterogeneity	Mean difference in hot flash frequency, % (95% CI)	
				Fixed-effect model	Random effects model
Metaregression				2-category data, $P = 0.065$ ; continuous data, $P = 0.116$	
$\leq 15$ mg/d	4 trials <a href="#">42,44,46,48</a>	399	$P = 0.46, I^2 = 0\%$	-13.39 (-19.70 to -7.09), $P < 0.0001$	Same as using the fixed-effect model
$> 15$ mg/d	9 trials <a href="#">38-40,43,45,47,49-51</a>	797	$P = 0.0001, I^2 = 74\%$	-19.95 (-24.96 to -14.95), $P < 0.00001$	-22.74 (-33.44 to -12.05), $P < 0.0001$
Test for subgroup difference				$P = 0.11$	$P = 0.14$
Intervention duration					
$\leq 12$ wk (median)	7 trials <a href="#">38,43,44,46,49-51</a>	662	$P = 0.66, I^2 = 0\%$	-12.66 (-17.09 to -8.23), $P < 0.00001$	Same as using the fixed-effect model
$> 12$ wk	6 trials <a href="#">39,40,42,45,47,48</a>	534	$P = 0.04, I^2 = 57\%$	-34.63 (-43.06 to -26.20), $P < 0.00001$	-34.29 (-49.07 to -19.50), $P < 0.00001$
Test for subgroup difference				$P < 0.00001$	$P = 0.006$

Variables	Trials	<i>n</i>	Heterogeneity	Mean difference in hot flash frequency, % (95% CI)	
				Fixed-effect model	Random effects model
Metaregression				2-category data, <i>P</i> = 0.004; continuous data, <i>P</i> = 0.009	
Baseline frequency					
≤8.34/d (median)	7 trials <sup>38-40,43,44,46,49</sup>	781	<i>P</i> = 0.0001, <i>I</i> <sup>2</sup> = 78%	-17.52 (-22.31 to -12.72), <i>P</i> < 0.00001	-20.12 (-31.47 to -8.78), <i>P</i> = 0.0005
>8.34/d	6 trials <sup>42,45,47,48,50,51</sup>	415	<i>P</i> = 0.12, <i>I</i> <sup>2</sup> = 43%	-17.21 (-24.03 to -10.04), <i>P</i> < 0.00001	-20.80 (-31.85 to -9.75), <i>P</i> = 0.0002
Test for subgroup difference				<i>P</i> = 0.94	<i>P</i> = 0.93
Metaregression				2-category data, <i>P</i> = 0.829; continuous data, <i>P</i> = 0.387	

Using final primary intention-to-treat % change from baseline data of three trials<sup>42,44,51</sup> that also contained per-protocol data, final primary data of moderate-to-severe hot flashes in one trial<sup>43</sup> that also contained data of mild-to-severe hot flashes, and final data of remaining nine trials. One month is equivalent to 4.3 weeks to unify intervention duration.



**FIG. 5.** Bubble plot of metaregression between effects of soy isoflavone extracts on the frequency of hot flashes and intervention duration. The line denotes the fitted regression line between effect (% change from baseline) of soy isoflavones and continuous intervention duration (weeks; 1 mo = 4.3 wk); the circles represent the estimates from each study, sized (fixed-effect model) according to the precision of each estimate (the inverse of its within-study variance).

Nine trials were selected for a meta-analysis of the effect of soy isoflavones on hot flash severity.<sup>40,44,46,47,51-55</sup> Three of the 9 trials did not provide sufficient data to calculate the SD/SE of mean percentage change from baseline after isoflavone or placebo interventions.<sup>44,46,54</sup> The largest available SD (21.99% to 51.32%; average, 39.14%) from one included trial was used for these three.<sup>40</sup> Meta-analysis of the nine trials, which included 988 women, resulted in a significant 30.52% (95% CI, -35.91% to -25.12%,  $P < 0.00001$ ; heterogeneity,  $P < 0.00001$ ,  $I^2 = 86\%$ ) reduction in severity in response to isoflavones when compared with placebo using the fixed-effect model. The total response (including placebo) in the fixed-effect model was -43.54 (95% CI, -47.17 to -39.91). Meta-analysis using the random effects model revealed that daily ingestion of an average of 62.8 mg/day of isoflavones (range, 30-135 mg; median, 54 mg) for 12 weeks to 12 months significantly reduced hot flash severity by 26.2% (95% CI, -42.23% to -10.15%,  $P = 0.001$ ) compared with placebo (Fig. 7). The total response (including placebo) in

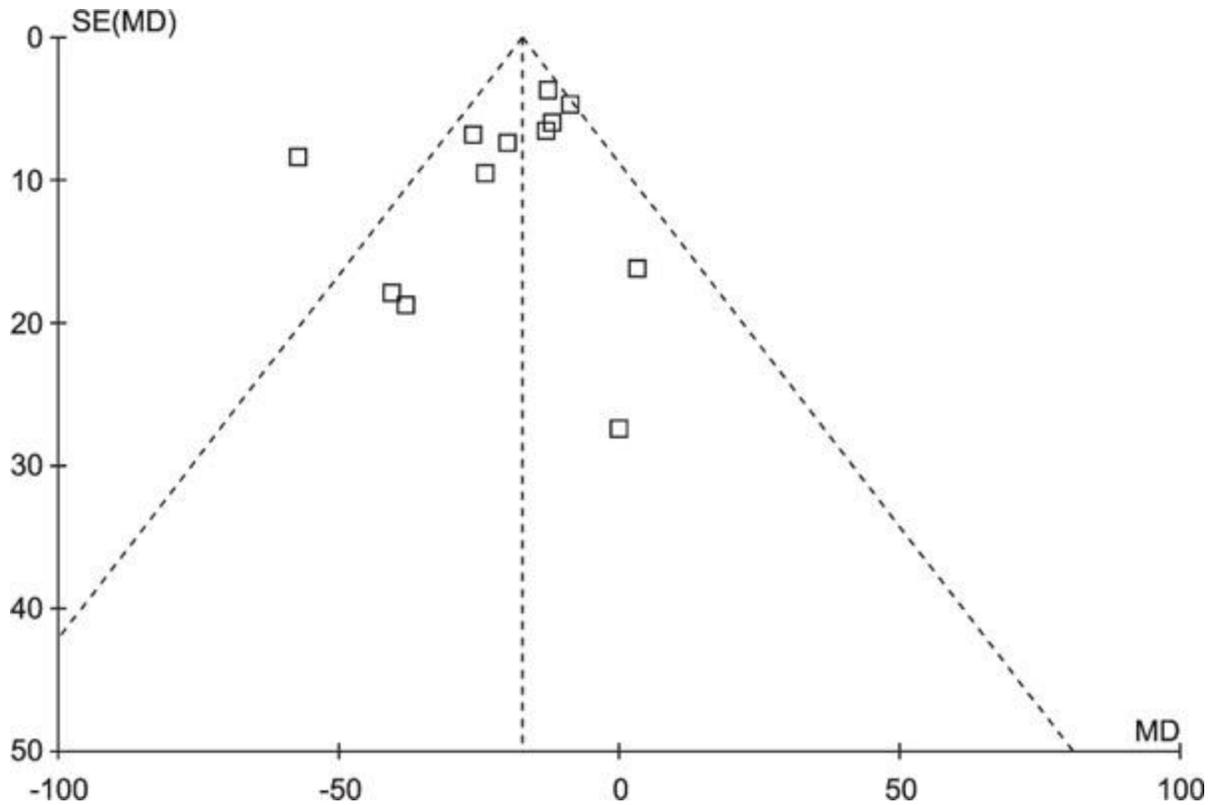
the random effects model was  $-47.61$  (95% CI,  $-63.78$  to  $-31.44$ ). Eight of nine trials resulted in negative mean difference in percentage change from baseline between isoflavone and placebo interventions, indicating a beneficial effect of isoflavones.

The following prespecified sensitivity analyses were conducted: (1) using PP analysis data from two trials<sup>44,51</sup> ( $-26.78\%$ ; 95% CI,  $-43.29\%$  to  $-10.27\%$ ,  $P = 0.001$ ; heterogeneity,  $P < 0.00001$ ,  $I^2 = 87\%$ , random effects model), (2) using the first interim data from seven trials with repeated measurements<sup>40,44,46,47,51,54,55</sup> ( $-18.81\%$ ; 95% CI,  $-33.15\%$  to  $-4.47\%$ ,  $P = 0.01$ ; heterogeneity,  $P < 0.00001$ ,  $I^2 = 88\%$ , random effects model), (3) using the second interim data from six trials with two or more interim measurements<sup>40,44,46,47,51,55</sup> ( $-23.39\%$ ; 95% CI,  $-38.15\%$  to  $-8.63\%$ ,  $P = 0.002$ ; heterogeneity,  $P < 0.00001$ ,  $I^2 = 85\%$ , random effects model), (4) eliminating three low-quality trials<sup>44,46,54</sup> ( $-32.16\%$ ; 95% CI,  $-51.97\%$  to  $-12.35\%$ ,  $P = 0.001$ ; heterogeneity,  $P < 0.00001$ ,  $I^2 = 86\%$ , random effects model), and (5) using the averaged SD available from other studies included in the review ( $-25.74\%$ ; 95% CI,  $-42.02\%$  to  $-9.45\%$ ,  $P = 0.002$ ; heterogeneity  $P < 0.00001$ ,  $I^2 = 89\%$ , random effects model). Because of the limited number of available trials, subgroup analyses and metaregressions were not conducted for the possible factors relating to the varying effects across trials. The funnel plots (Fig. 8) and the Egger test ( $P = 0.494$ ) did not indicate obvious publication bias.

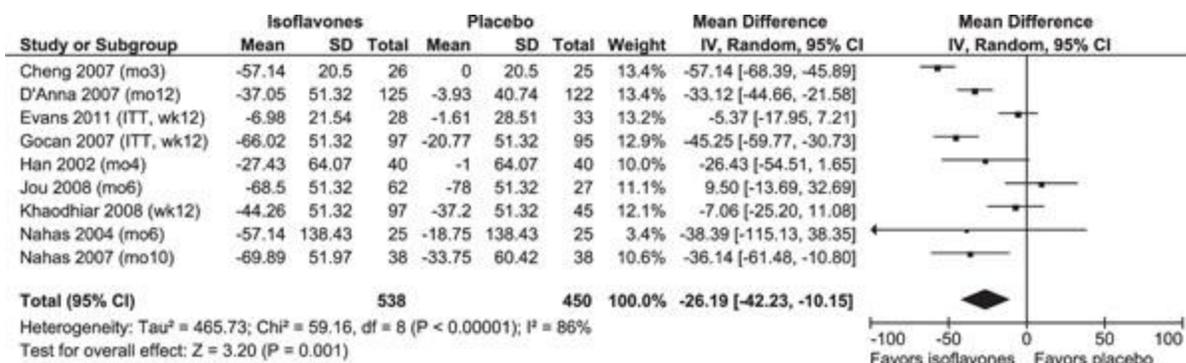
To investigate the within-arm effect of soy isoflavones and placebo on the severity of hot flashes, meta-analyses using the inverse generic variance method were conducted. The combined changes from baseline resulting from the ingestion of isoflavones and placebo for 12 weeks to 12 months were significant at  $-47.61\%$  (95% CI,  $-63.78\%$  to  $-31.44\%$ ,  $P < 0.00001$ ; heterogeneity,  $P < 0.00001$ ,  $I^2 = 94\%$ , random effects model) and  $-20.66\%$  (95% CI,  $-34.28\%$  to  $-7.04\%$ ,  $P = 0.003$ ; heterogeneity,  $P < 0.00001$ ,  $I^2 = 90\%$ , random effects model), respectively. The funnel plots and Egger tests ( $P = 0.703$  and  $P = 0.105$  for isoflavones and placebo, respectively) for both intervention arms did not indicate obvious biases.

### **Effects of soy isoflavone extracts on hot flash composite scores**

Three studies were included for a systematic review of the effects of isoflavones on hot flash “composite score.”<sup>46,50,57</sup> Because Albertazzi et al<sup>57</sup> had many participants with few or no hot flashes, they conducted an analysis with the subset of their study population who had a score (hot flash intensity  $\times$  number) of more than 9 ( $n = 41$ ). In this group, genistein reduced the hot flash score by 11% more than the placebo ( $-31\%$  vs  $-20\%$ ,  $P = 0.02$ ).<sup>57</sup> Although the trend of the median (SD/SE not shown) variations of composite score for both periods of this crossover trial were presented, the carryover and period effects were not clearly addressed, and the mean data were unavailable for both periods.



**FIG. 6.** Funnel plots of the effects of soy isoflavone extracts on the frequency of hot flashes (% change from baseline). The vertical center broken line and two broken lines on both sides of it denote the combined overall effect and 95% CI (fixed-effect model), respectively. MD, mean percentage change (%) in frequency of hot flashes from baseline for soy isoflavones minus that for placebo.



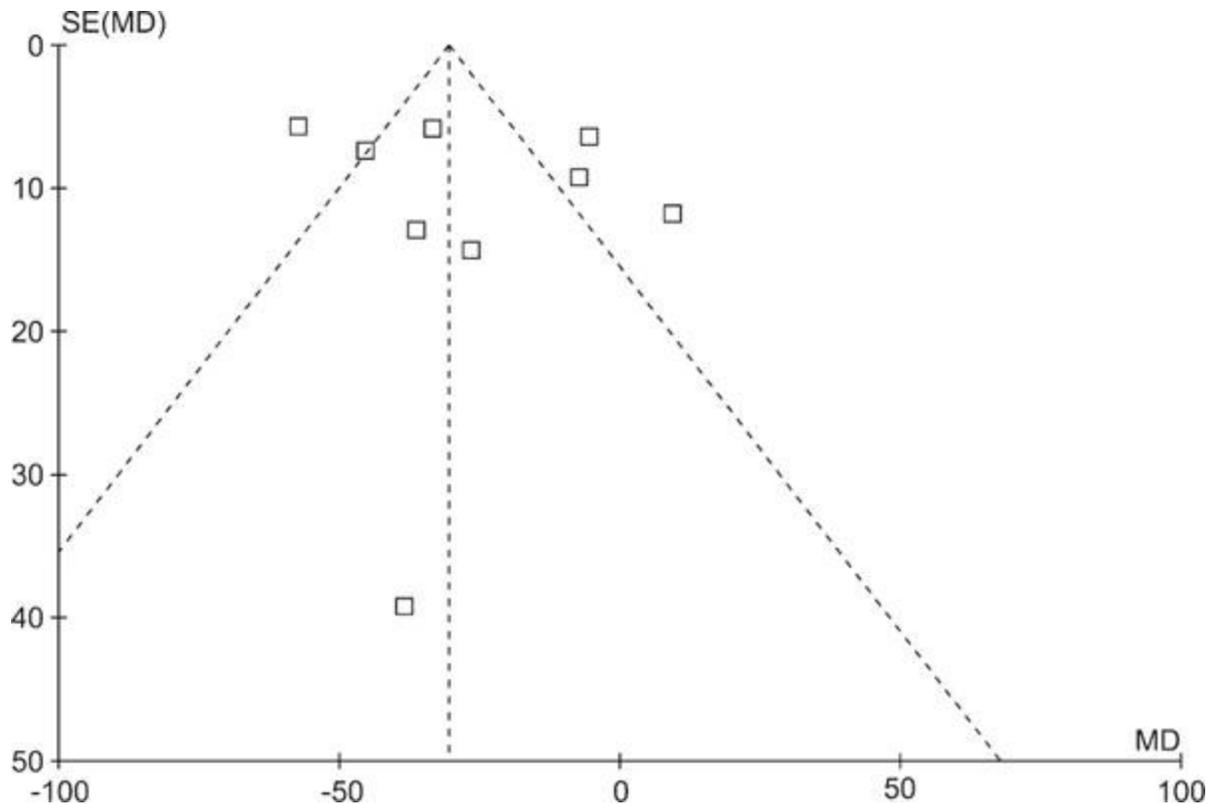
**FIG. 7.** Effects of soy isoflavone extracts on the severity of hot flashes (% change from baseline). “Mean difference” denotes the mean percentage changes (%) in severity of hot flashes from baseline for soy isoflavones minus that for placebo, whereas “Random” denotes random effects model. ■, effect estimate of each study (size of the square)

corresponds to its weight); horizontal line, 95% CI; ◆, combined overall effect. ITT, intention to treat.

In the study by Khaodhriar et al,<sup>46</sup> the mean percentage reductions from baseline in composite score after 12 weeks of intervention for placebo and 40 and 60 mg/day isoflavones were 53%, 63%, and 61%, respectively (data obtained from a graph in which SD/SEs were not shown); the reductions after 4 weeks were 32%, 34%, and 34%, respectively, and the reductions after 8 weeks were 44%, 54%, and 54%, respectively.<sup>46</sup> However, the comparison among the three intervention arms and the comparison between placebo and combined soy isoflavone groups were not reported. In the study by Upmalis et al,<sup>50</sup> there was a significant percentage change from baseline in the composite score for hot flashes in both the placebo (−20%) and isoflavone (−28%) groups, the difference being statistically significant ( $P = 0.01$ ).<sup>50</sup> Because of the limited number of available trials, meta-analysis was not conducted to clarify the effect on composite score.

## DISCUSSION

The present systematic review and meta-analysis found that intake of isoflavones extracted from soy or synthesized to match those in soy caused a greater alleviation of hot flashes than placebo. Although a common perception in the literature is that the results from trials examining the effect of isoflavone-containing products on the alleviation of hot flashes have been mixed, the forest plot (Fig. 4) clearly shows that at least for soy-derived or synthesized isoflavones, there is a clear and consistent pattern in favor of isoflavones over placebo.



**FIG. 8.** Funnel plots of effects of soy isoflavone extracts on severity of hot flashes (% change from baseline). The vertical center broken line and the two broken lines on each side of it denote the combined overall effect and 95% CI (fixed-effect model), respectively. MD, mean percentage changes (%) in the severity of hot flashes from baseline for soy isoflavones minus that for placebo.

The net reduction in response to isoflavones for frequency and severity (random effects model) were  $-20.6\%$  (95% CI,  $-28.38\%$  to  $-12.86\%$ ,  $P < 0.00001$ ) and  $-26.2\%$  (95% CI,  $-42.23\%$  to  $-10.15\%$ ,  $P = 0.001$ ), respectively. Sensitivity analyses indicated that the effects of isoflavones on both the frequency and severity of hot flashes were robust and that there was no obvious publication bias. With regard to frequency, subgroup analyses and metaregressions indicated a dose-response effect of genistein and a time-responsive effect. Depending on the cutoff (18.8 or 15 mg/d) and the analysis model used (fixed or random effects), higher-genistein—containing isoflavone products were approximately 50% to 200% more potent than lower-genistein—containing isoflavone products (Table 3). The higher genistein cutoff (18.8 mg/d) was used because it was the median for all studies, and the lower (15 mg) cutoff was used because of a previous observation.<sup>19</sup> Metaregression also revealed a significant relationship between effect magnitude and intervention duration. The decrease in hot flash frequency in longer duration studies ( $>12$  wk) was approximately threefold greater than that in shorter-duration trials.

The current evaluation is the first systematic review and meta-analysis to separately clarify the effects of soy isoflavones on frequency, severity, and composite score of hot flashes in terms of percentage change from baseline. In contrast with some previous evaluations, the current analysis included studies that involved women with breast cancer and were as short as 6 weeks in duration because data indicate that patients not receiving active cancer treatment respond the same as healthy women to interventions that alleviate hot flashes and because research shows that for interventions that alleviate hot flashes, efficacy is likely to be apparent within 4 weeks of treatment initiation.<sup>62</sup> This having been said, some previously published data indicate that the benefit of isoflavones increases with time beyond 4 weeks, and one recent review suggested that hot flash trials should be a minimum of 8 weeks in duration.<sup>63</sup> As already noted, the observation that longer-duration trials show greater efficacy of isoflavones has been confirmed in the current analysis.

A previous analysis of 11 studies found that only one of six lower-genistein supplement trials ( $\leq 15$  mg/d) significantly reduced hot flashes, whereas all five higher-genistein trials did.<sup>19</sup> The current results are consistent with that analysis and support the observation that genistein is more potent than daidzein and glycitein, the other two isoflavones in soybeans, at least for the alleviation of hot flashes. This observation is consistent with the higher estrogen receptor binding affinity and transcriptional activity of genistein.<sup>64</sup>

The mechanism by which soy isoflavones alleviate hot flashes has not been established, and in fact, a complete understanding of the etiology of hot flashes has not yet been achieved, although the decline in estrogen levels as women enter menopause is almost certainly a contributing factor. Consequently, it has been speculated that the effect of isoflavones on hot flashes is related to the chemical and biological similarity of isoflavones with mammalian estrogens, which have been shown to alleviate hot flashes in perimenopausal and postmenopausal women.<sup>2</sup> However, although isoflavones are classified as phytoestrogens, because of their preferential binding to and transactivation of estrogen receptor  $\beta$  in comparison with estrogen receptor  $\alpha$ , they are also classified as selective estrogen receptor modulators.<sup>65</sup> In support of this classification is evidence indicating that isoflavones exert estrogen-like effects in some but not all estrogen-sensitive tissues.<sup>66,67</sup> For example, Carmignani et al<sup>68</sup> showed that isoflavone-rich soy protein providing 53 mg isoflavones alleviated hot flashes to a similar extent as HT (1 mg estradiol and 0.5 mg norethisterone acetate) but, unlike HT, did not increase the vaginal maturation index.

A limitation of the current meta-analysis was the significant heterogeneity among the findings with regard to both hot flash frequency (Fig. 4) and severity (Fig. 7). In most systematic reviews and meta-analyses, studies differ in terms of population, age, health status, and other covariates, not all of which were assessed. Variations in such covariates, as well as variation in study design and treatment form (eg, study duration, isoflavone concentration and composition), may lead to variations in the magnitude of observed effects. A random effects model assumes not one true effect but a distribution of true effect sizes, incorporating heterogeneity, and produces an effect

size that represents the mean of the population of true effects. Sensitivity analysis using interim measurement data indicated that differences in study duration may have contributed to heterogeneity. Subgroup analyses and metaregressions indicated that the dose of genistein and the length of intervention influenced the effects of soy isoflavones on the frequency of hot flashes. The ability to identify other factors contributing to inconsistency in results was limited by the small number of studies that could be included in subanalyses.

The US Food and Drug Administration recommends that hot flash studies enroll women who have at least seven hot flashes per day. Despite the fact that the current analysis included some studies involving women with less than this number, baseline hot flash frequency did not affect efficacy. This finding is consistent with research from the Mayo Clinic involving a variety of intervention products,<sup>62</sup> although it is inconsistent with previously published evaluations of the efficacy of isoflavones that found greater efficacy in studies involving women with more, rather than fewer, hot flashes.<sup>18,26,59</sup> Our ability to detect an effect of baseline hot flash frequency may have been limited because in the current analysis, the median hot flash number, which was used as the cutoff for analysis, was quite high (8.34/d). However, with use of a cutoff of only 5 hot flashes or fewer per day based on a previous observation to examine the effect of baseline hot flash frequency on efficacy,<sup>59</sup> the results unexpectedly showed that isoflavones reduced frequency to a greater extent in those studies below rather than above this cutoff (fixed-effect model:  $-33.13$  vs  $-14.09$ ,  $P = 0.0003$ ; random effects model:  $-33.69$  vs  $-14.58$ ,  $P = 0.12$ ). This finding is probably of little relevance because only three trials<sup>39,40,49</sup> were included in the low baseline hot flash group and the results were dominated by one particular trial.<sup>40</sup> Therefore, at this point, the effect of baseline hot flash frequency on efficacy is unclear.

Recently, two bodies have issued opinions on the efficacy of isoflavones for alleviating menopausal symptoms. One comes from a round table held on October 9 to 10, 2010, composed of 22 clinicians and researchers acknowledged to be experts in the field, convened under the auspices of The North American Menopause Society/Utian Translational Science Symposium.<sup>69</sup> The expert panel concluded that “soy-based isoflavones are modestly effective in controlling hot flashes, as demonstrated to date in predominantly Caucasian women in early post-menopause who have at least four hot flashes a day.” The primary basis for this conclusion appears to be work by Bolanos et al.<sup>26</sup> The current research supports the conclusion about efficacy but, as discussed previously, not as related to baseline hot flash frequency, a concept originally promoted by Messina and Hughes.<sup>59</sup>

The second opinion comes from the European Food Safety Authority (EFSA), which did not conduct a formal meta-analysis of the data, but in response to an Article 13 health claim petition, concluded that “the evidence provided is insufficient to establish a cause and effect relationship between the consumption of soy isoflavones and reduction of vasomotor symptoms associated with menopause.”<sup>70</sup> The reason this conclusion differs from the conclusion of the current

research is because, as described below, the datasets upon which each conclusion was based differed quite markedly.

The EFSA based their opinion on the results of 12 human intervention studies included in the petition. Of those, current research excluded three because the intervention involved soy protein or soy foods, not isoflavone supplements.<sup>71-73</sup> The current research included six trials that were rejected by the EFSA.<sup>44,48,49,54-56</sup> Two of these were rejected because they involved women with breast cancer.<sup>55,56</sup> These were included in the current research because, as already noted, research conducted at the Mayo Clinic indicates that women with breast cancer respond to hot flash treatments similarly to women without breast cancer.<sup>62</sup> The trial by Gocan et al<sup>44</sup> was rejected by the EFSA because the abstract to which it had access did not include sufficient data for evaluation. The authors of the current research were provided all necessary data. The study of Penotti et al<sup>48</sup> was rejected because the use of HT or other medication was not reported to be an exclusion criterion; however, because an inclusion criterion was that participants had seven or more hot flashes per day, we assumed that none of the women were using HT, and this has been confirmed by the author (M. Penotti, personal communication, August 7, 2011). Scambia et al<sup>49</sup> was rejected partly for the reason noted for Penotti et al<sup>48</sup> but also because it was said that no information was provided on whether participants were comparable at baseline with regard to menopausal symptoms. However, none of the women were using HT (G. Scambia, personal communication, August 1, 2011), and the authors do provide baseline hot flash frequency data for the placebo and isoflavone groups. Finally, Jou et al<sup>54</sup> was rejected because randomization did not take into account the pre-planned subgroup analysis of equol- and non—equol-producing participants, and no overall results were reported. We did not believe this to be a valid reason for exclusion. However, elimination of this study from the current research did not appreciably affect the results because the net change in hot flash severity without Jou et al<sup>54</sup> was -30.64 (-46.50 to -14.78); with Jou et al,<sup>54</sup> it was -26.19 (-42.23 to -10.15).

The current analysis included four trials not included in the EFSA evaluation. Two of these were published subsequent to the submission of the health claim petition.<sup>45,51</sup> One of the four was rejected because the intervention included a combination of treatments; however, the current analysis included data from the treatment arm in that trial involving isoflavones only.<sup>38</sup> For unknown reasons, the fourth study by Ferrari et al<sup>43</sup> was not part of health claim petition.

Finally, it is important to recognize that the EFSA did not subanalyze the data according to the genistein content of the intervention product. As discussed, higher-genistein—containing supplements reduced menopausal symptoms more than twice as much as lower-genistein-containing supplements.

Studies evaluating the efficacy of various forms of estrogen show the reduction in hot flashes to be greater than that found in response to isoflavones; typically, there is an 80% to 90% total improvement with the former.<sup>74</sup> However, these studies also generally reported a much higher placebo response than in the isoflavone studies, such that the net improvement due to estrogen is about 30% to 40%.<sup>75</sup> For example, in studies evaluating the dose-response effects of oral equine

estrogens and oral and transdermal 17 $\beta$ -estradiol, the reduction in the frequency of hot flashes in the placebo groups was 44%,<sup>76</sup> 55%,<sup>77</sup> and 45%,<sup>78</sup> respectively. In one study in the current analysis, genistein (54 mg/d) reduced hot flash frequency by 29% compared with placebo, whereas HT (1 mg 17 $\beta$ -estradiol plus 0.5 mg norethisterone acetate) reduced frequency by 27% compared with genistein.<sup>39</sup> The differences between genistein and placebo ( $P < 0.001$ ) and HT and genistein ( $P < 0.05$ ) were statistically significant. However, as noted previously, another study found that isoflavone-rich soy protein was as efficacious as HT.<sup>68</sup> The current analysis clearly indicates that for women seeking alleviation of hot flashes who are unable or unwilling to take HT, isoflavones are a reasonable alternative.

Previous analyses have suggested that gastrointestinal disturbances may be more common in response to isoflavone exposure than placebo.<sup>79,80</sup> Beyond that, there is little evidence that in healthy women, exposure to isoflavones poses any significant risk when consumed for the time periods that have been studied to date, but relatively few long-term studies ( $\geq 3$ y) have been conducted.<sup>81-83</sup> The most controversial aspect to isoflavones is their effect on breast cancer risk and the prognosis of women with breast cancer.<sup>84</sup> However, clinical studies indicate that isoflavone exposure (see Reference 85 for review), whether from supplements or soy foods, does not adversely affect breast tissue, and recently published prospective epidemiological studies suggest that isoflavone exposure from soy foods may actually improve prognosis.<sup>86-89</sup> Although research shows that more processed genistein-containing products stimulate tumors in ovariectomized athymic mice to a greater extent than less processed ones,<sup>90</sup> the mechanism responsible for this effect, which is that processing leads to higher circulating levels of unconjugated (biologically active) levels of genistein,<sup>91</sup> is now known not to apply to humans.<sup>92</sup> Therefore, there seems to be little scientific basis for differentiating between the effects of extracts and soy foods given equal isoflavone exposure, at least in health outcomes affected by isoflavones.

In one long-term trial, after 5 years of exposure to isoflavone supplements (90 mg/d), there was a slightly increased risk of simple endometrial hyperplasia<sup>83</sup>; however, several limitations of this study have been identified,<sup>93,94</sup> and the incidence of hyperplasia in the isoflavone group was similar to that observed in the placebo group in other long-term trials not involving isoflavones.<sup>95</sup> Therefore, the implication of this finding remains to be determined. Recent data also raise questions about isoflavone exposure by women with subclinical hypothyroidism.<sup>96</sup> Preliminary data indicate that in a small subset of women with subclinical hypothyroidism, isoflavones caused progression to overt hypothyroidism although in the group of participants overall, in response to isoflavone-rich soy protein, there were marked reductions in blood pressure, inflammation, and insulin resistance.

## CONCLUSIONS

Soy isoflavones extracted from soybeans or chemically synthesized reduced hot flash frequency and severity significantly more than did placebo. The effect size varied across trials and was related to the dose of genistein used and trial duration. Further studies are needed to

identify additional factors relating to the observed heterogeneity among study results, such as dose, isoflavone form, baseline hot flash frequency, and duration of treatment. Adding to the complexity of interpreting study results was the large number (14) of intervention products used and the often inadequate description of the dose provided, both in terms of total aglycone content and isoflavone profile, and the lack of clarity about whether the assessment of hot flash symptom referred to frequency or severity. In many cases, it was necessary to contact the authors to obtain the information needed for a given study to be included in the current research. Journal reviewers and editors as well as researchers themselves need to pay more attention to these types of details so that the literature can be more easily and accurately evaluated.

Finally, many women seek natural alternatives to HT for the relief of menopausal hot flashes. A recently conducted small survey of such women found that about 70% would be satisfied with a nonhormonal intervention that provided at least a 50% reduction in hot flashes.<sup>97</sup> Therefore, the results of this systematic review and meta-analysis clearly justify health professionals recommending that women who do not want to use HT try isoflavones for the relief of menopause-related hot flashes. For women with moderate-to-severe hot flashes who are seeking relief from their symptoms, the reduction in the number and severity of hot flashes observed in this analysis in response to isoflavones could result in a significant improvement in their quality of life.

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