CHAPTER 38

Isoflavones for Menopausal Vasomotor Syndrome

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38.1 Pharmacology Aspects

Isoflavones, coumestans, lignans and some resorcinol derivatives are considered to be phytoestrogens, molecules that perform important functions in certain vegetables and share properties similar to human estrogens. Because of this structural and functional similarity, they have been studied for many years to establish their clinical usefulness to manage menopausal syndrome. Isoflavones and coumestans (coumestrol, metoxycoumestrol) are flavonoid compounds, whereas lignans (enterodiol, enterolactone) and resorcinol derivatives are non-flavonoids (Figure 38.1). The latter abound in grapes and in grape products, such as wine. Greater emphasis has been placed on the study of this type of phytoestrogen because soy (*Glycine max*) isoflavones are distributed in many sources of human consumption and there are certain epidemiological associations described in populations with a high consumption of these foods.

There are various types of isoflavones but those most studied in connection with human health are genistein, daidzein and glycitein, which are the names given to the non-glycosidic forms to distinguish them from the corresponding

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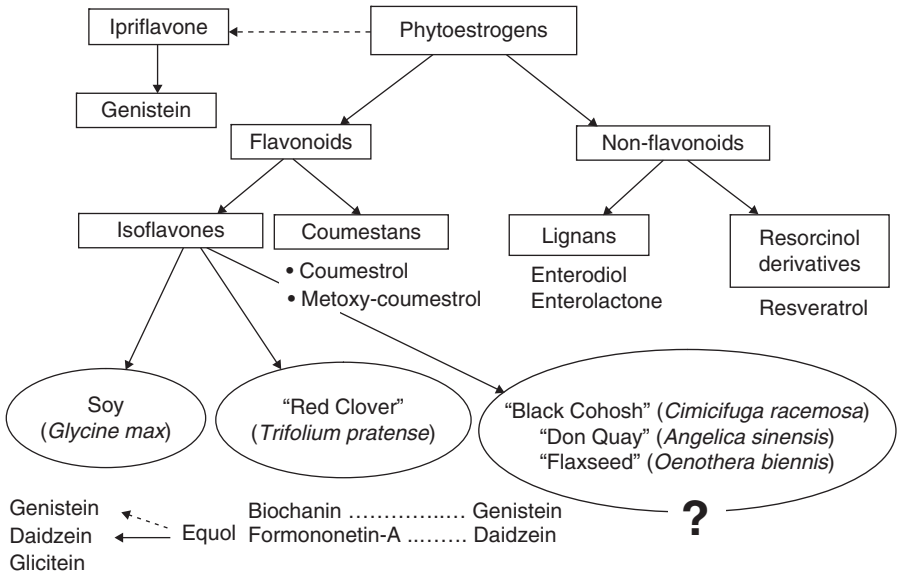


Figure 38.1 Chemical types of phytoestrogens.

glycosidic forms (linked to glucose): genistin, daidzin and glycitin. Other less active forms, such as formononetin and biochanin A, are partially metabolized by intestinal flora to obtain genistein and daidzein in a highly variable proportion that depends on individual intestinal enzymatic capacity. A synthetic form of isoflavone (ipriflavone) has been used in several clinical trials, as it is estimated that its intestinal metabolism produces genistein in approximately 10% of the total load contributed by ipriflavone. These compounds generally have a chemical structure based on two phenolic rings joined by a heterocyclic ring (forming the basic structure of isoflavones) and with various hydroxyl substitutions (-OH) at different points of the heterocyclic chain, generating a specific chemical name for each isoflavone (Figure 38.2). The presence of these hydroxyl radicals provides these compounds with a significant antioxidant capacity (Button and Patel 2004; Palacios 2002; Yildiz 2006).

Trifolium pratense (red clover), a vegetable species used to feed equines but not apt for human consumption, is a rich source of formononetin and biochanin A. Nevertheless, the clinical efficacy of this source on menopausal vasomotor syndrome has been evaluated for a long time, although the results were non-conclusive due to the high heterogeneity of the studies. Other sources, such as *Cimicífuga racemosa* (black cohosh), *Angelica sinensis* (dong quai) or *Oenothera bienis* (evening primrose), have been described, but their benefits on the climacteric syndrome are more based on experience than on formal scientific evidence. However, the phytoestrogen composition in these sources has not been well determined (Button and Patel 2004; Low Dog 2005).

Studies on phytoestrogens have accumulated enough evidence to support their alternative use in the management of climacteric vasomotor symptoms,

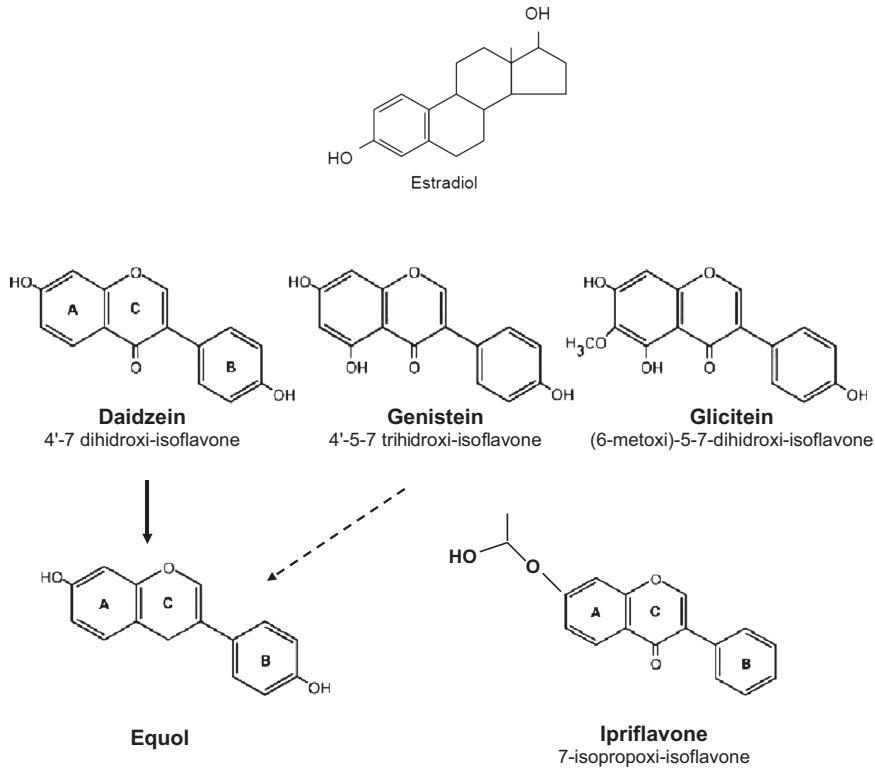


Figure 38.2 Chemical structures of isoflavones.

and such benefit seems to be supported by its isoflavone content. However, the main sources (soy, *T. pratense*, *C. racemosa* and *A. sinensis*) differ in isoflavone composition, and such difference may explain the heterogeneity in the results of the studies when analyzed as a whole (Button and Patel 2004; Low Dog 2005; Mahady 2003). Such heterogeneity can be minimized with subgroup analysis. For this reason, it is important that studies with phytoestrogens determine the exact composition of the intervention under study.

Genistein, daidzein and glycitein are the predominant isoflavones in soy (*G. max*), in contrast with the composition of red clover (*T. pratense*) based on formononetin and biochanin, its main flavonoids. Other sources, such as black cohosh (*C. racemosa*) and dong quai (*A. sinensis*), are popularly used but there is much uncertainty to their exact isoflavone composition (Mahady, 2003). For this reason, the results of studies that evaluate the efficacy of all these sources should not be extrapolated and it would more consistent to conduct separate analyses. Moreover, considering that the proportion of isoflavones in soy may vary in accordance with the preparation administered, we prefer to classify this source into three types, ''dietary isoflavone intake'', ''soy extract'' and ''soy isoflavone concentrate'' (genistein or daidzein), to try to reduce the heterogeneity observed in all the studies.

The study of equol has been a focus of attention. This active intestinal metabolite of daidzein and genistein has shown a significant biological activity. The clinical response has been related to the intestinal enzymatic capacity for generating equol. It is presumed that an important proportion of menopausal women could not produce sufficient equol, which represents a disadvantage to show a significant clinical result. This hypothesis is still under research and ongoing studies would enable us to draw more definitive conclusions in this regard. Other metabolites, such as *O*-desmethyl-angolensin (*O*-DMA) (Atkinson *et al*. 2005; Jou 2008; Yuan *et al*. 2007) have been described, but they do not perform an important biological function. Although it has been proposed that intestinal enzymatic capacity to form equol in the metabolism of phytoestrogens would be a plausible cause to predict the clinical response, some clinical studies have not been able to prove this hypothesis. New trials on the use of an equol concentrate are awaited to define the actual impact which the metabolism of isoflavones would have on the predicted clinical efficacy.

Some pharmacokinetic aspects in the metabolism of isoflavones must be highlighted. Although the intestinal absorption rate is still uncertain, preliminary studies indicate that it would be approximately $0.5\,mg\,kg^{-1}\,dose^{-1}$, which would be consistent with most of the doses used in clinical trials. That is, loads above $0.5\,mg\,kg^{-1}\,dose^{-1}$ would progressively saturate intestinal absorption receptors up to generating the fecal loss of the excess dose. However, there is some population variability about the calculation of this absorption rate, and today it is believed that the absorption range would be broader than believed up to now (Setchell 2001).

Isoflavones have affinity for the estrogen receptors (ER) α and β, but, for ERα, they behave like competitive antagonists, showing a blocking effect with regard to ovarian estrogens. Nevertheless, they have an intense activity in ERβ and their biological effects are shown through them. The distribution of ERs varies; thus, for example, ERα predominate in mammary and endometrial tissue, while ERβ predominate in bone, nervous and endothelial tissues. However, this distribution of receptors varies among individuals. This would (at least partially) explain the variability of their efficacy when evaluating the clinical effect of these compounds. Vaginal tissue does not have a clear predominance of any type of these receptors, making uncertain the use of local phytoestrogens to manage the vaginal dryness, dyspareunia or external dysuria of the climacteric syndrome (Palacios 2002; Yildiz 2006).

38.2 Clinical Evidence

The number of studies published on the efficacy of isoflavones to manage climacteric hot flushes has increased significantly since the 1990s (Usui 2006). Synthesis evidence currently reveals a clear tendency in favor of the effect of isoflavones on menopausal vasomotor syndrome, despite the existence of studies that have not proven a significant difference from placebo. Various hypotheses have been proposed to explain this difference in results, such as the

capacity for metabolizing equol, the type of isoflavones or the critical quantity of some of them; however, new publications with better standardized sources of these compounds are awaited to homogenize the combined results of the studies. For instance, the meta-analysis by Williamson-Hughes *et al.* (2006) established a critical threshold of genistein to predict the efficacy of these phytoestrogens by observing that the trials which had found a significant clinical response associated to the use of phytoestrogens contained an intake of genistein above 15 mg/day in comparison with those studies in which the intake of isoflavones was smaller (Figure 38.3). However, this hypothesis was not strengthened by the observations of the Study of Women's Health Across the Nation (SWAN), which evaluated the relationship between vasomotor symptoms and race during menopausal transition in 3198 women, and where no relationship was found between the consumption of genistein and vasomotor symptoms (Gold *et al.* 2006).

Initially, the few trials that used *T. pratense* as sn isoflavone source did not yield convincing results about the efficacy of isoflavones (Knight *et al.* 1999; Tice *et al.* 2003); however, it is clear that the sources of these compounds may greatly differ in their composition. Thus, for instance a standardized extract of *T. pratense* (Promensil®) has a very different isoflavone composition from a standardized soy extract (the most currently used source in clinical trials). More than 80% of the composition of the first one is based on formononetin and

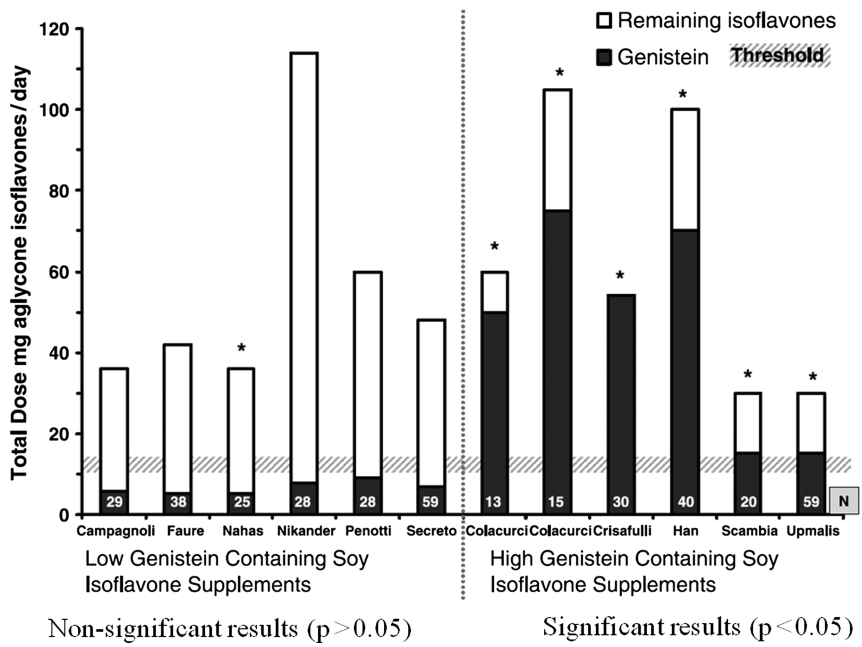


Figure 38.3 Meta-analysis by Williamson-Hughes *et al.* (2006).

biochanin A, whereas more than 90% of the composition of the latter is based on genistein and daidzein. These differences in composition generate part of the heterogeneity in published meta-analyses.

The importance and need to provide an appropriate treatment for menopausal vasomotor syndrome has been well proven, not only for the deterioration it causes to the quality of life in women but also for its probable association with other clinical conditions such as osteoporosis (Lee and Kanis 1994). The cumulative evidence proves the usefulness of isoflavones and their role in the management of menopausal vasomotor syndrome. A brief review of the main synthesis studies published in this regard is shown below.

The review of the Cochrane collaboration, in charge of Lethaby *et al*. (2007), grouped the studies conducted with isoflavones according to the isoflavone sources used. Thus, four groups of studies were analyzed: soy extract, dietary soy, *T. pratense* (red clover), and "other phytoestrogens" (*C. racemosa*, *A. sinensis*, *etc*.). A relevant aspect of this meta-analysis was the exclusion of studies conducted with women with breast cancer background, under the hypothesis that such background would alter hormonal physiology in some way. The studies that compose the analysis of each one of these groups are shown in Table 38.1**,** and those studies excluded from the analysis because of the participants' breast cancer background have been identified (Nahas *et al*. 2004; Nikander *et al*. 2005; Quella *et al*. 2000; Secreto *et al*. 2004; Van Patten *et al*. 2002).

Due to the presence of an important heterogeneity in the studies with "soy extract" (Bicca *et al*. 2004; Campagnolli *et al*. 2005; Duffy *et al*. 2003; Faure *et al*. 2002; Han *et al*. 2002; Kaari *et al*. 2006; Khaodhiar *et al*. 2008; Penotti *et al*. 2003; Upmalis *et al*. 2000), "dietary supplement" (Albertazzi *et al*. 1998; Balk *et al*. 2002; Brzezinski *et al*. 1997; Burke *et al*. 2003; Colacurci *et al*. 2004; Dalais *et al*. 1998; Knight *et al*. 2001; Kotsopoulos *et al*. 2000; Lewis *et al*. 2006; Murkies *et al*. 1995; St Germain *et al*. 2002) and "other phytoestrogens" (Crisafulli *et al*. 2004; Dalais *et al*. 1998; Dodin *et al*. 2005; Heyerick *et al*. 2006; Lewis *et al*. 2006; Woo *et al*. 2003), the Cochrane review only carries out the meta-analysis for the group of studies developed with *T. pratense* (Baber *et al*. 1999; Jeri 2002; Knight *et al*. 1999; Tice *et al*. 2003; Van der Weijer and Barentsen 2002), which shows a discrete tendency in favor of this isoflavone source *versus* placebo in the efficacy to treat the menopausal vasomotor syndrome (Figure 38.4). This result coincides with those of other published meta-analyses (Howes *et al*. 2006; Nelson *et al*. 2006; Thompson *et al*. 2007). Nevertheless, in this group of studies the heterogeneity reached 59.9% ($I^2 = 0.599$), and in the subgroup analysis according to the isoflavone dose ($40\,mg\,day^{-1}$ *vs*. $80\,mg\,day^{-1}$) it showed a reduction in the statistical tendency and an increase in heterogeneity (68.3% in the $40\,mg\,day^{-1}$ subgroup). The trial carried out by Atkinson *et al*. (2004) was not included in this analysis; although nearly half of the women in each group of this study suffered from hot flushes, this was not a requirement to participate in the trial. The authors also excluded the trial by Hidalgo *et al*. (2005) from this analysis because they did not present sufficient statistical data (did not report *P*-values or measures of variation).

Table 38.1 Meta-analysis by Lethaby *et al.* (2007). Grouping of studies according to isoflavone source.

Trifolium pratense	*Soy extract*	*Dietary soy*	*Other phytoestrogens*	*Excluded studies*[c]
Baber *et al.* (1999)	Bicca *et al.* (2004)	Albertazzi *et al.* (1998)	Crisafulli *et al.* (2004)[b]	Nikander *et al.* (2005)
Jeri (2002)	Campagnolli *et al.* (2005)	Balk *et al.* (2002)	Dodin *et al.* (2005)	Van Patten *et al.* (2002)
Knight *et al.* (1999)	Duffy *et al.* (2003)	Brzezinski *et al.* (1997)	Heyerick *et al.* (2006)	Nahas *et al.* (2004)
Tice *et al.* (2003)	Faure *et al.* (2002)	Burke *et al.* (2003)	Woo *et al.* (2003)	Secreto (2004)
Van der Weijer and Barentsen (2002)	Han *et al.* (2002)	Dalais *et al.* (1998)	Dalais *et al.* (1998)	Quella *et al.* (2000)
Atkinson *et al.* (2004)[a]	Khaodhiar (2008)	Knight *et al.* (2001)	Lewis *et al.* (2006)	
Hidalgo *et al.* (2005)[a]	Penotti *et al.* (2003)	Kotsopoulos *et al.* (2000)		
	Upmalis *et al.* (2000)	Lewis *et al.* (2006)		
	Kaari *et al.* (2006)[a]	St Germain *et al.* (2001)		
		Colacurci *et al.* (2004)[a]		
		Murkies *et al.* (1995)[a]		

[a]Not considered in the analysis because of some exclusion criteria established by the authors.
[b]Genistein concentrate was used as the isoflavone source.
[c]Women had breast cancer antecedent.

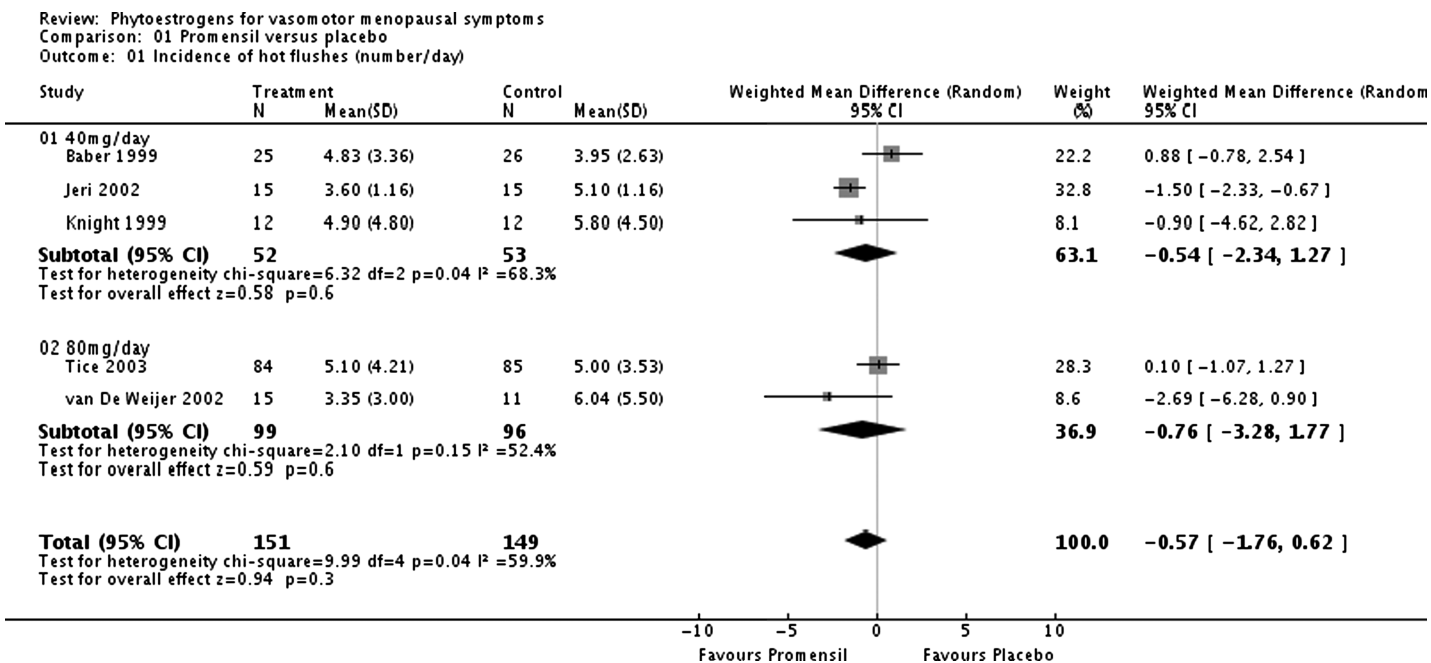


Figure 38.4 Meta-analysis of *T. pretense* by Lethaby *et al.* (2007).

The prior meta-analysis by Nelson *et al*. (2006), which also grouped studies by isoflavone source and administered dose, did not find statistically relevant results in the group of studies that used *T. pratense*.

In the Cochrane review, five out of the eight studies that compared soy extract with placebo reported significant differences with respect to the frequency and severity of hot flushes (Bicca *et al*. 2004; Faure *et al*. 2002; Han *et al*. 2002; Khaodhiar *et al*. 2008; Upmalis *et al*. 2000); three studies found a reduction in the frequency of hot flushes (Bicca *et al*. 2004; Faure *et al*. 2002; Khaodhiar *et al*. 2008); and two studies observed a reduction in the severity of hot flushes (Han *et al*. 2002; Upmalis *et al*. 2000). The trial by Kaari *et al*. (2006) was not analyzed within this group of studies because it had a comparative design between soy extract and hormonal therapy and did not report significant differences between soy and estrogens in the decrease of hot flushes (at 6 months, $P = 0.74$, t test). The trial by Crisafulli *et al*. (2004) was not analyzed within this group as well, although the source used was a genistein concentrate; however, this decision from the authors contrasts with the matter of including the study by Khaodhiar *et al*. (2008) within the group that used soy extract, despite it used a daidzein concentrate (Table 38.1).

Crisafulli *et al*. (2004) had a parallel 3-arm design and contrasted the efficacy of genistein and hormonal therapy *versus* placebo. Although both alternatives surpassed the placebo, the effect of hormonal therapy was significantly better than that of genistein. These results contrast with Kaari *et al*. (2006), who did not find significant differences between soy and estrogen therapy. By combining direct and indirect evidence, it was recently proven in a meta-analysis that the effect of hormonal therapy is significantly distinguishable from that of soy when the purpose of the study corresponds to menopausal vasomotor syndrome (Bolaños *et al*. 2011). However, it is possible that the differences between both interventions shorten, depending on the effect studied; thus, for instance, this same difference was not found in a recent indirect meta-analysis when the effect evaluated was the risk of osteoporotic fracture (Bolaños *et al*. 2010-a).

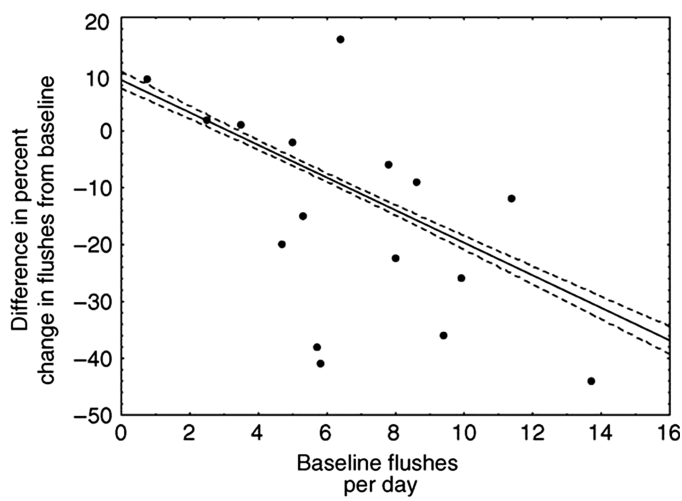
Nelson *et al*. (2006) evaluated the studies conducted with soy under the perspective of the time of duration of the intervention. The authors of this meta-analysis made a separate evaluation of studies with durations of 4–6 weeks, 12–16 weeks and 24 weeks, but did not observe special changes in the combined result favorable to soy in these subgroups. Without making a special subgroup analysis, the meta-analysis by Thompson *et al*. (2007) found a marginal benefit in favor of a short-term use of *T. pratense*.

Other meta-analyses have been subsequently conducted with the same purpose of evaluating the efficacy of isoflavones *versus* placebo; however, the perspective of each one of these meta-analyses is varied, for the purpose of optimizing the analysis and not just updating the studies. Thus, the meta-analysis by Howes *et al*. (2006) only includes published studies and does not include those studies in which women with a breast cancer background have participated. The meta-analysis by Bolaños *et al*. (2010-b) only focused on published studies with soy or its by-products, regrouping them by the type of by-product used: soy extract, dietary isoflavone intake or soy isoflavone

concentrate. As well as the Cochrane study, this meta-analysis did not include studies on women with a breast cancer background.

It is important to observe various studies included in each one of these meta-analyses to explain the change in the trend of the results. For example, unlike the Cochrane study, besides including studies on women with breast cancer background (Nahas *et al*. 2004; Nikander *et al*. 2005; Quella *et al*. 2000; Secreto *et al*. 2004; Van Patten *et al*. 2002), Howes *et al*. (2006) carries out subgroup analysis according to the source used: *T. pratense* and soy by-products; and it considers Atkinson *et al*. (2004), which was not included in the Cochrane meta-analysis, whereas it does not include Baber *et al*. (1999), which was included in the Cochrane meta-analysis. However, despite these modifications, the results obtained the same trend in both meta-analyses (Figure 38.5). As it was to be expected, Howes *et al*. (2006) includes a larger number of studies and their combined results show an increase in the tendency observed in the review of the Cochrane study for the group of studies with soy by-products. Another result to be highlighted in the analysis by Howes *et al*. (2006) is the following relationship that exists between the frequency of hot flushes and the clinical result, finding in the corresponding regression a higher clinical response in women with ≥ 4 hot flushes day^{-1} (Figure 38.6).

On the other hand, the meta-analysis by Bolaños *et al*. (2010-b) not only includes two studies in addition (D'Anna *et al*. 2007; Cheng *et al*. 2007) to the previously described studies, but it also has some peculiarities that distinguish it from previous analyses. The authors only focus on studies conducted with soy and its by-products and categorized these studies according to the isoflavone



* The relationship was significant on multiple regression analysis. ($\beta = -0.48$, $P < 0.0001$).

Figure 38.5 Meta-analysis by Howes *et al*. (2006). Weighted regression analysis plot for the number of baseline flushes as a predictor of the percentage fall from baseline of flushes.

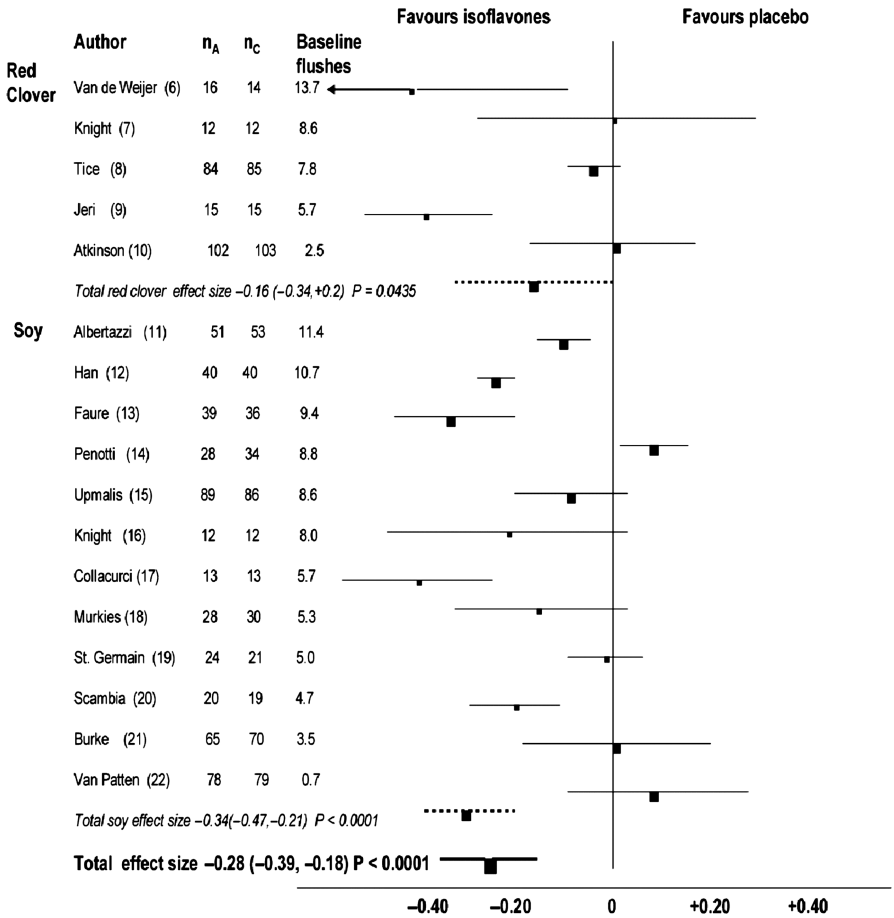
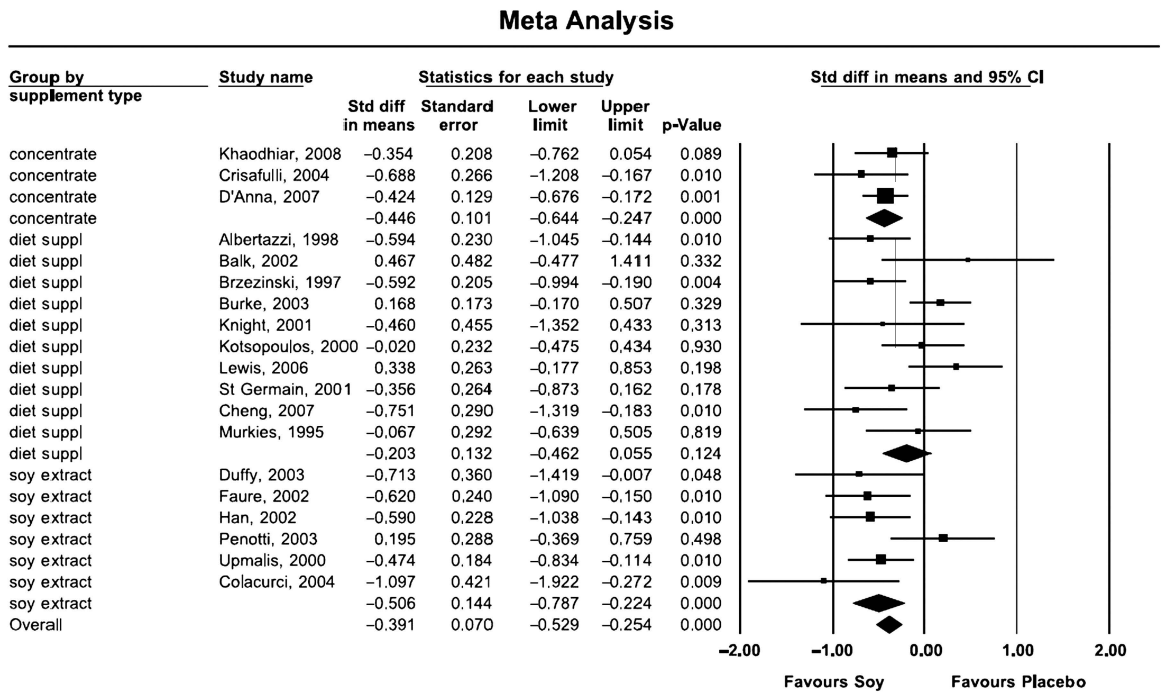


Figure 38.6 Meta-analysis by Howes *et al.* (2006).

source: soy extract, dietary isoflavone intake or soy isoflavone concentrate (Figure 38.7). The results from Crisafulli *et al.* (2004) are also included in the analysis (as this study used genistein concentrate), together with the trials by Khaodhiar *et al.* (2008) and D'Anna *et al.* (2007), which used daidzein and genistein concentrates, respectively. Similar to Howes *et al.* (2006), the authors of this work also considered the studies of Colacurci *et al.* (2004) (subgroup with soy extract) and Murkies *et al.* (1995) (subgroup with dietary isoflavone) to be acceptable for the analysis. Both trials were excluded from the Cochrane analysis.

The results of this meta-analysis are consistent with those of previous meta-analyses. The trend of the combined results favors soy and its by-products in the three subgroups analyzed; nevertheless, heterogeneity is important in "soy extract" and "dietary isoflavone intake" subgroups (42% and 56%, respectively). However, the isoflavone concentrate subgroup, made up by three



Meta Analysis

Random Effects Model:
Overall heterogeneity: $I^2 = 53.5\%$
Sub-group heterogeneity: Concentrate: $I^2 = 0\%$; Diet suppl: $I^2 = 60\%$; Soy extract: $I^2 = 42\%$

Figure 38.7 Meta-analysis by Bolaños *et al.* (2010-b).

clinical trials (Crisafulli *et al*. 2004; D'Anna *et al*. 2007; Khaodhiar *et al*. 2008), did not show a higher heterogeneity (0%). The global analysis of all the trials reached 53% heterogeneity. The same as found by Howes *et al*. (2006), in this study regression found a significant direct relationship between number of hot flushes and the response to the treatment with isoflavones.

Since the meta-analysis by Bolaños *et al*. (2010-b), new randomized and placebo-controlled trials have been published which have not been evaluated in a new synthesis review (Borges and Salazar 2009; Ferrari 2009). In the near future, this would provide higher statistical forcefulness to the results described in this review. It is important that the new studies for this same purpose standardize the main heterogeneity factors observed, such as isoflavone source, duration of treatment and measurement scales for results. On the other hand and taking into account that the updating of a meta-analysis may significantly increase the type I error (even more than the known publication bias), Bolaños *et al*. (2010-b) verified that including three additional studies to the analysis (not included in previous meta-analyses) would lead to an increase in said error. The *P*-value of the meta-analysis and the previously defined alpha value (0.05) made the updating possible without increasing the type I error (Borm and Donders 2009).

Although some meta-analyses exclude unpublished studies (unlike the Cochrane study), it is important to observe that, in general, such studies do not show the minimum methodological quality to include them in the final analysis. Because of this reason, many experts consider it more worthwhile investing in updating reviews than an exhaustive search for unpublished data. The authors also included studies with small samples and low statistical power, which increases the probability of obtaining non-significant results and reducing the probability of being published; however, this situation does not detract from the results, as the meta-analyses that include low power studies (50% on average) closely agree with the "actual" difference in the effect size of the intervention under study, even in presence of a publication bias. Previous studies have proven that the results of small trial meta-analyses are very similar to those of large trial meta-analyses on the same intervention. According to Van Driel *et al*. (2009), the key question should not focus on the existence of a publication bias but on the true impact of this bias.

It is important to observe some limitations in the synthesis studies published until now, as they limit the interpretation of results. First, in accordance with the current regulatory frame, a definite dose for dietary soy supplements has not been established. This hinders the comparison of studies that use said supplements without an appropriate standardization in their isoflavone composition. Besides, the role of intact soy protein on the effect size is still uncertain, and it has not yet been defined whether the presence of soy protein constitutes a heterogeneity factor across the studies. Second, despite the evidence that suggests that an intestinal metabolic trait could have a significant influence on the bioavailability and activity of isoflavones (a hypothesis that involves the formation of *equol*), most of the studies do not evaluate the participants' intestinal metabolic pattern, which could also be a source of

heterogeneity in the results. Third, the studies analyzed often use different scores to measure the effect size (frequency and/or severity of hot flushes), which could generate another additional heterogeneity factor.

38.3 Conclusions

Studie on isoflavones (a type of phytoestrogen) have accumulated enough evidence to support their alternative use in the management of climacteric vasomotor symptoms, and such a benefit seems to be supported by its isoflavone content. However, the main sources of these compounds (soy, *T. pretense* and C. *racemosa*) differ in isoflavone content, and such differences may explain the heterogeneity in the results of the studies when analyzed as a whole.

Genistein, daidzein and glycitein are the predominant isoflavones in soy (*G. max*), in contrast with the composition of red clover (*T. pratense*) based on formononetin and biochanin A, its main flavonoids. Black cohosh (*C. racemosa*) is popularly used but evidence about its exact isoflavone content is uncertain.

Considering that the proportion of isoflavones in soy may vary in accordance with the preparation administered, this source could be classified into three types: ''dietary isoflavone intake'', ''soy extract'' and ''soy isoflavone concentrate'' (genistein or daidzein), to try to reduce the heterogeneity observed in all the studies.

Summary Points

- Isoflavones show a significantly higher efficacy than placebo on climacteric vasomotor syndrome.
- There are apparent differences in efficacy associated with the evaluated isoflavone source, as different sources vary in their isoflavone composition.
- The evidence does not support an effect equivalent to hormone therapy.
- A better response is obtained with isoflavones when the intensity of the vasomotor syndrome is mild or moderate, and when the number (frequency) of hot flushes is high (≥ 4 hot flushes day^{-1}).
- The use of isoflavones is pertinent when the patient does not wish to or cannot receive hormone therapy.
- Isoflavones show a safety level comparable with placebo.

Key Facts

- There has been some uncertainty about the true effect size of isoflavones. For a long time they were considered to hold a placebo effect.
- The evidence has increased in the last 20 years and several meta-analyses have demonstrated a significant effect size.
- Heterogeneity is still the key-point for the robustness of the conclusions, so it is necessary to standarize some criteria as type of supplement, patients follow-up and clinical scores to measure menopausal symptoms.

Definition of Words and Terms

***Dietary isoflavone intake*:** Total amount of isoflavones that an individual intakes in their diet every day.

***Soy extract*:** A genistein, daidzein and glicitein mixture that is isolated from soy plant.

***Soy isoflavone concentrate*:** Total amount of genistein or daidzein administered as a formula.

List of Abbreviation

ER estrogen receptor

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