DOI 10.1002/mnfr.200800552 Mol. Nutr. Food Res. 2009, *53*, 1084 – 1097

Review

Efficacy of isoflavones in relieving vasomotor menopausal symptoms – A systematic review

Anja Jacobs[1], Uta Wegewitz[1], Christine Sommerfeld[2], Rolf Grossklaus[1], and Alfonso Lampen[1]

[1] Department of Food Safety, Federal Institute for Risk Assessment, Berlin, Germany
[2] Department of Scientific Service, Federal Institute for Risk Assessment, Berlin, Germany

This review assessed the efficacy of isoflavone supplements to reduce vasomotor symptoms in menopausal women by reviewing all published randomized controlled trials. Systematic literature searches were carried out in 70 databases. Randomized and placebo controlled studies were included if they investigated the treatment of isoflavone supplements derived from soy or red clover on vasomotor symptoms in peri- or postmenopausal women for at least 12 wks. Data were analyzed concerning outcome and methodological quality of the study. Twenty-three trials met the inclusion criteria, thereof 17 investigated soy isoflavones and 6 red clover isoflavones. Without exception, selected trials examining the effect of red clover isoflavones were already assessed in several meta-analyses and were therefore excluded from this evaluation. As the soy isoflavone studies were very heterogeneous concerning interventions and outcome measures, meta-analysis could not be performed and trials were systematically assessed in a structured approach. Included soy isoflavone studies had numerous quality deficiencies and did not consistently show a reduction of flushes after treatment with soy isoflavones. Therefore, there is no conclusive evidence, but only some indication of a benefit of soy isoflavones on hot flush frequency or severity.

Keywords: Efficacy / Hot flushes / Isoflavone / Menopause / Soy

Received: December 5, 2008; revised: January 27, 2009; accepted: January 29, 2009

1 Introduction

Phyto-estrogens are estrogen-like substances produced by plants. These phyto-chemicals bind to estrogen receptors (ERs) and exert various estrogenic or anti-estrogenic effects. The three main classes of phyto-estrogens are isoflavones, lignans, and coumestans, which are found in fruits, vegetables, and whole grains. The most extensively studied class of phyto-estrogens, the isoflavones, occurs largely in soybeans [1, 2]. Soybeans contain three primary isoflavones in their glycoside form: genistin, daidzin, and glycitin. The sugar moiety is cleaved during digestion by β-glycosidases, resulting in the formation of their respective aglycones, genistein, daidzein, and glycitein [3]. Soy isoflavones act *via* estrogen receptor binding activities, with a higher affinity to ER-β in comparison to ER-α [4].

Correspondence: Anja Jacobs, Federal Institute for Risk Assessment, Thielallee 88 – 92, 14195 Berlin
E-mail: anja.jacobs@bfr.bund.de
Fax: +49 30 8412 37 15

Abbreviations: ER, estrogen receptors; **HF,** hot flushes; **MHT,** menopausal hormone therapy; **RCT,** randomized controlled trial

Red clover (*Trifolium pratense*), an isoflavone rich medicinal herb traditionally used by Native Americans, mainly contains the four isoflavones formononetin, biochanin A, daidzein, and genistein. Formononetin does not bind to the ER at physiological concentrations, but it is metabolized to daidzein and then by intestinal bacteria to equol, both of which have been shown to be estrogenic [5, 6].

Thermoregulatory disturbances like hot flushes (HF) and night sweats, also referred to as vasomotor symptoms, are the most common menopausal symptoms and occur in about 75% of peri- and postmenopausal women in the United States [7]. In Europe prevalence of HF is up to 73%, with variations in different populations [8]. HF arise as a sudden feeling of heat in the face, neck, and chest. Night sweats are the night-time manifestation of HF [9].

Menopausal hormone therapy (MHT) is the most consistently effective therapy for vasomotor symptoms [10]. However, current data have indicated adverse effects of MHT by increasing the risk of *e.g.* stroke, breast cancer (estrogen-progestin therapy only), and gallbladder disease [11, 12]. Dietary soy has gained much attention since reports of reduced menopausal discomfort and reduced morbidity incidence of several hormone-dependent dis-

eases in Asian compared with Western populations [2]. Epidemiological studies in Japanese women suggest that consumption of soy products has a protective effect against HF [13]. In Japan, the total isoflavone intake from soy food averages from 25 to 50 mg *per* day. A small proportion of the Asian population (≤10%) seems to consume more than 100 mg isoflavones [14]. In Western countries average daily intakes are less than 2 mg isoflavones [15].

Currently, numerous isoflavone preparations derived from soy or red clover are available on the market as dietary supplement and are used by many women to treat their menopausal disorders. It has been shown that about 62% of women believe that "natural" (plant based) hormones are at least as effective for managing menopausal symptoms as MHT [16]. However, up to now the efficacy of isoflavones in the treatment of HF or night sweats has not been scientifically substantiated. Results from meta-analyses and reviews analyzing the efficacy of isoflavones are inconsistent [17–22]. Recently several studies were published which still have to be assessed.

In this systematic review the efficacy of isoflavone supplements derived from soy in the treatment of menopausal vasomotor symptoms was critically assessed by reviewing all published randomized controlled trials (RCTs).

2 Methods

2.1 Data sources/Identification of clinical trials

A systematic literature search was conducted in March 2008 with no restriction on date. Relevant trials were identified from searches with the German institute of medical documentation and information (DIMDI; http://www.dimdi.de/static/en/index.html), containing 70 databases, covering the entire spectrum of medicine. The main search terms used were isoflavones, soy, red clover, vasomotor symptoms, HF, and night sweats. The detailed search strategy is available as Supporting Information 1. Additionally, hand searching was performed in reference lists of relevant reviews. There was no restriction on the language of publication. Abstracts and unpublished studies were not included in the analysis.

2.2 Study selection

Due to the high potential for placebo effect [23], it is required to compare isoflavones with placebo control and not only with standard therapy (MHT for vasomotor symptoms). So the efficacy of isoflavones was evaluated with analyses of all published randomized placebo-controlled trials of dietary supplements containing extracted or purified soy and red clover isoflavones for treatment of vasomotor menopausal symptoms.

2.3 Types of participants

2.3.1 Inclusion

(i) Perimenopausal women (no regular, but at least one menstrual period within the last 12 months) suffering from vasomotor symptoms at baseline
(ii) Postmenopausal women (no menstruation within the last 12 months) suffering from vasomotor symptoms at baseline
(iii) Women reporting spontaneous or surgical menopause

2.3.2 Exclusion

(i) Patients with cancer or history of cancer
(ii) Use of tamoxifen
(iii) Previous MHT within the last month before intervention
(iv) Any major disease
(v) Peri- and postmenopausal women with no vasomotor symptoms at baseline

2.4 Types of intervention

Studies were selected for analysis if the intervention of soy or red clover isoflavones in a defined dose *versus* placebo control was investigated as a monotherapy for menopausal vasomotor symptoms. The isoflavones should have been administered as supplement or as isoflavone-enriched food. Interventions of dietary soy were not considered in this analysis because it is assumed that matrix effects can influence the bioavailability of isoflavones [24]. Studies using a combination of soy or red clover isoflavones with other therapies were excluded. The treatment duration should be at least 12 wks, in line with recommendations for MHT [25].

2.5 Types of outcome measures

At least one of the following outcome measures should be presented in the studies:
(i) Individual vasomotor symptoms (frequency and severity of HF, night sweats)
(ii) Vasomotor scales or sub-scales of scores
(iii) Composite scores of vasomotor symptoms

Studies reporting general menopausal symptoms questionnaires lacking distinct data about HF and night sweats were excluded.

2.6 Data extraction and study quality assessment

Data extraction and quality assessment were performed independently by two reviewers A. J. and U. W. The assessments were performed under unmasked conditions since it was suggested that masked condition did not affect the overall results of systematic reviews [26]. Disagreements were resolved by consensus or by discussion with a third reviewer R. G.

Table 1. Criteria for evaluation of study quality. Study quality of the included studies were analyzed according randomization, treatment blinding, allocation concealment, baseline comparability of treatment groups, number of analyzed participants per group, drop outs, basis for analysis and presentation of results. The criteria were graded with A, B or C as described in the table.

Assessment	A	B	C
Randomization	concept of generation of an allocation sequence (simple, restricted or stratified randomization, *e.g.* by centre)	not reported/unclear	inadequate
Treatment blinding	containers were identical, supplements were identical in appearance, taste etc.	not reported/unclear	supplement and placebo were not identical
Allocation concealment	(1) sequentially numbered, opaque, sealed envelopes; (2) sequentially numbered containers; (3) pharmacy controlled; (4) central randomization	not reported/unclear	inadequate
Baseline comparability of treatment groups	assessment in randomized patients, comparability of age, menopausal status (FSH-level, or menopausal age etc) and vasomotor symptoms	balance not reported or only stated for analyzed patients	groups not balanced
Number of analyzed participants per group	≥ 50	25 – 50	< 25
Drop outs	withdrawals of 20% or less	not reported/unclear	withdrawals of more than 20%
Basis for analysis	intention-to-treat	unclear	no intention-to-treat
Presentation of results	data about frequency or intensity of HF for each group with a measure of variability, number of patients analyzed	unclear (number of analyzed patients, modality of calculation of the scores)	no absolute values, no measure of variability

2.6.1 Data extraction

Included studies were analyzed according to the following methodological characteristics:

(i) Randomization method, blinding method, concealment of allocation
(ii) Study design (parallel/cross over, duration of treatment)
(iii) Flow chart
(iv) Power calculation
(v) Number of randomized and analyzed patients
(vi) Drop outs, reasons for drop out
(vii) Method of analysis
(viii) Baseline comparability of the groups
(ix) Presentation of results
(x) Location and timing of the study
(xi) Funding

Concerning outcome, data were extracted for vasomotor symptoms presented as frequency, severity, subscales, or composition scores. The study outcome was filtered out as the difference in reduction of vasomotor symptoms from baseline compared to placebo. In case of missing data the percentage change of hot flush frequency or severity between intervention and placebo group were stated.

Initial hot flush frequency as well as time since last menstrual period was filtered out to characterize the study population. The intervention was described regarding application type and reported isoflavone dose.

Beside efficacy, outcome data were extracted for adverse effects for all included soy isoflavone studies.

2.6.2 Study quality assessment

In the literature, there are numerous scales used to assess the methodological quality of RCT, of which most have not been tested for validity and reliability in the area of application [27]. Therefore, a catalog of quality criteria was developed based on the consolidated standards of reporting trials [28].

The quality of the included studies was assessed according the criteria of selected methodological issues presented in Table 1.

Description of randomization and blinding method is very important since it was shown that trials excluding features such as blinding and allocation concealment tended to report an exaggerated treatment effect compared with trials that did include these features [29 – 31]. In view of baseline comparability of the study groups, patient age and menopausal transition stage or any measure of menstrual/hormonal status were focused upon, since the prevalence of HF could be influenced by these factors (National Institutes of Health, assessing and improving measures of hot flashes. Summary of an NIH Workshop, 2004, http://nccam.nih.gov/health/hotflashes/pdf/hotflashessumm.pdf.). Additionally, baseline values for HF should be stated by the authors and should be comparable between the groups.

A certain number of participants are required to obtain reproducible and significant results. Sloan *et al*. outlined a number of 50 patients *per* arm to be appropriate for understanding the effect an agent has on HF in a patient population [23]. In pilot studies, a reduction of symptoms has to be measured in 25 patients to further study a particular agent.

Schulz and Grimes summarized that validity of study results are compromised by drop out rates greater than 20% [32]. Fewer than 5% lead to little bias, whereas 5–20% leads to intermediate levels of problems. Therefore, in this review dropout rates were checked for greater than 20%. Of more importance than absolute overall loss to follow-up rates are comparative loss rates in the groups. If there were differences between the groups, the evaluation was based on the higher drop out rate.

The intention-to treat-principle is the most appropriate criterion for the assessment of the utility of a new therapy and provides the most realistic and unbiased answer to the relevant question of clinical effectiveness [33]. Finally, it is important for comparison of study results to provide absolute data of outcome including a measure of variability.

2.7 Statistical analyses

Potential influences of initial HF as well as isoflavone doses on the study outcome were investigated with correlation analyses (Spearman rank correlation) using the Statistical package for the Social Science Software (SPSS). For correlation analyses, study outcome was defined as percent reduction from baseline compared to placebo. Analyzing the correlation between initial HF and outcome initial hot flush values of the active treatment group were used as a mean for all patients. The correlation of the administered doses was carried out with isoflavone aglycone equivalents. For studies with an imprecise isoflavone specification, it was assumed that isoflavones were present as aglycones. Correlation analysis was performed firstly for all studies together, but also separately for studies with known as well as unknown aglycone content. Analyzing cross-over studies only the first phase was considered.

3 Results

266 abstracts were identified after systematic literature searches of DIMDI, precisely within the following databases: Cochrane Library, MEDLINE databases, CAB Abstracts ® CAB, ISTPB+ISTP/ISSHP ® The Thomson Corporation and SciSearch ® The Thomson Corporation. Four additional articles were found by manual search. 48 of 269 abstracts were considered as relevant to the topic (Fig. 1). Twenty-five articles were excluded from the systematic review. Reasons for exclusion are presented in Supporting Information 2. Additionally, one selected article was not

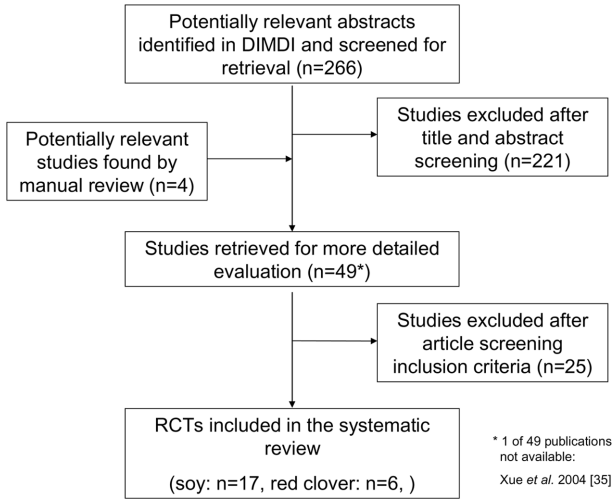

Figure 1. Systematic review flowchart summary.

available from several libraries, and the author did not respond to our query [34].

Twenty-three studies met inclusion criteria. Seventeen RCT dealt with soy and six with red clover isoflavones. Twenty-one papers were published in English; one was available in Polish and one in Portuguese.

After screening the included RCT, it became obvious that all the six trials of red clover isoflavones [35–40] were already considered in several meta-analyses [17–20]. Therefore, they were excluded from a detailed description in the present study which focuses specifically on RCT of soy isoflavones.

3.1 Study quality

The methodological characteristics of the included soy isoflavone studies are summarized in tabular form and available as Supporting Information 3. Except for one trial [41] all studies were performed in a parallel design.

The summary of the study quality assessment is presented in Table 2. Five studies specified their randomization methods in an adequate manner. Eleven authors did not provide further details for the randomization methods. Information about the study design of D'Anna *et al*. were received from the publication of Marini *et al*. [42]: D'Anna *et al*. performed a subgroup analysis and the randomization was carried out for the parent study.

Ten studies gave information about their method of treatment blinding, whereas in seven publications the methods were not stated.

Three trials provided details for the allocation concealment of the treatment in the studies. In all the other publications, it was ambiguously described or not mentioned.

Five studies described baseline comparability of randomized patients of the intervention and control groups according to age, menopausal status and initial vasomotor symp-

Table 2. Quality assessment of the studies. Each study was assessed according the criteria of table 1. Studies were sorted by number of A counts.

Study	Randomization	Treatment Blinding	Allocation concealment	Baseline comparability	N Participants	Drop outs	Intention-to-treat analysis	Presentation of results	A counts (of 8)
D'Anna *et al.* 2007 [48]	B[a)]	A	B	A	A	A	B	A	5
Crisafulli *et al.* 2004 [47]	B	A	B	A	B	A	A	C	4
de Sousa *et al.* 2006 [52]	A	B	B	A	B	A	C	A	4
Knight *et al.* 2001 [46]	A	A	A	B	C	C	C	A	4
Penotti *et al.* 2003 [58]	A	A	B	A	C	C	C	A	4
Campagnoli *et al.* 2005 [41]	B	A	A	B	C	A	B	C	3
Duffy *et al.* 2003 [55]	B	A	B	B	C	A	C	A	3
Fauré *et al.* 2002 [59]	B	A	B	B	B	C	A	A	3
Han *et al.* 2002 [57]	B	B	A	B	B	A	B	A	3
Nahas *et al.* 2007 [50]	B	A	B	B	B	A	C	A	3
Burke *et al.* 2003 [54]	B	B	B	B	A	A	C	A	3
Albertazzi *et al.* 1998 [51]	A	B	B	A	B	C	B	C	2
Cheng *et al.* 2007 [45]	B	B	B	B	B	A	C	A	2
Khaodhiar *et al.* 2008 [49]	A	B	B	B	B	C	C	A	2
St. Germain *et al.* 2001 [44]	B	A	B	B	C	B	B	A	2
Stanosz *et al.* 2006 [43]	B	A	B	B	C	B	B	C	1
Upmalis *et al.* 2000 [53]	B	B	B	B	A	C	C	C	1

a) Subgroup analysis of a parent study [42], which was adequate randomized

toms. The remaining studies provided information only for analyzed patients or at least one of the three points was missing or unclear.

Three studies involved more than 50 women *per* treatment arm in the analysis. Eight trials had, due to their number of participants, the character of a pilot study. Six trials analyzed less than 25 women *per* group.

Ten studies had a drop out rate of 20% or less. More than 20% drop outs emerged in five studies. In one study, drop out rate was not reported [43]. The number of randomized patients was unclear in one study [44], so a drop out rate could not be stated.

Two studies performed intention-to-treat analyses. Nine studies were analyzed *per* protocol. In six studies, the method of analysis was unclear or not stated.

Twelve studies presented their results about frequency or intensity of HF with baseline values as well as after treatment values, with a measure of variability and number of patients analyzed. Five studies were lacking measures of variability or exhibited the results only as percent changes from baseline compared to placebo or in a diagram.

None of the studies met all quality criteria although the criterion most frequently satisfied was presentation of results, whereas allocation concealment was the issue met by the fewest trials.

Compliance, a further study characteristic, was biochemically measured in seven of 17 studies, with three trials measuring urinary isoflavone excretion [44–46] and four measuring plasma isoflavone concentration [47–50]. In four trials compliance was assessed by counts of returned supplements [41, 51–53], in one trial compliance was self-reported [54], and another trial assessed it indirectly by individual food diaries [55]. In the remaining four trials assessment of compliance was not stated. Overall, compliance with soy supplements was good.

3.2 Efficacy outcomes

Included studies are very heterogeneous concerning intervention and outcome. Therefore, the trials were divided into three subgroups: (1) isolated genistein group, (2) soy extract group and (3) soy protein powder group (Table 3). Nevertheless, it was not practicable to perform a meta-analysis of the subgroups because not all articles provided adequate outcome data. So the trials were described in detailed tabular form and systematically assessed in a structured approach.

The outcome of hot flush frequency was given in 14 of the included soy isoflavone studies. Hot flush severity was presented in 12 trials. Six publications measured hot flush frequency as well as hot flush severity.

Looking at hot flush frequency, seven of 14 studies presented absolute data with measure of variability. In six studies results were given as reduction in percent from baseline. One study outlined results of frequency in a diagram but also as percent change of women who experienced a decrease of HF.

The determination of hot flush severity varied among the 12 trial, using all sorts of severity scores:

(i) a composite score of frequency and severity,
(ii) a severity scale of 0–3 (none to severe),
(iii) a severity scale of 1–3,
(iv) 4 × weighted severity scale of 0–3,
(v) five-point self-rating scale,

Table 3. Summary of the soy isoflavone studies included in the systematic review. Trials were classified according their type of intervention into studies investigating isolated genistein, soy extract or soy protein powder. Each trial was described by data of randomized/analyzed patients, menopausal status of patients, dose of isoflavones per day, duration of intervention and main results.

	Study	N randomized/ analyzed	Patients		Intervention/day	Control	Duration of intervention	Main results
			Basal HF frequency/ day	Time since last menstrual period (months)				
Isolated genistein improvement of HF a) frequency: in 2 of 2 trials b) severity: in 1 of 1 trial	Crisafulli *et al.* 2004 [47]	90/90 participants of a prior bone loss trial [60]	genistein: 4.6 ± 0.6; placebo: 4.7 ± 0.6 (mean ± SEM)	≥12	54 mg genistein; 1 mg estradiol combined with norethisterone (EPT)	placebo	1 year	significant difference in percent change from baseline of HF between the groups: 24% reduction in the isoflavone group compared with placebo ($p<0.01$); 54% reduction in EPT group compared with placebo ($p<0.001$); 30% reduction in EPT group compared with genistein ($p<0.05$) [a]
	D'Anna *et al.* 2007 [48] (Subgroup analysis of the bone loss trial) [42]	389 (thereof 247 suffered from HF)/ 247	genistein: 4.4 ± 0.3; placebo: 4.2 ± 0.4 (mean ± SEM)	≥12	54 mg genistein	placebo	1 year	significant differences in HF frequency and severity between genistein and placebo ($p<0.001$) [a]
Soy extract improvement of HF a) frequency: in three of eight trials additionally, improvement in two trials: (1) for percent change from baseline to the end of treatment between soy and placebo, but NS significance in reduction between both groups; (2) after pooling of 40 mg and 60 mg group b) severity: in 5 of 8 trials	Campagnoli *et al.* 2005 [41]	36/29	38/week	>6 or surgical menopause for ≥3 months	60 mg soy isoflavones	placebo (soy oil without isoflavones)	12 wks × 12 wks (cross over)	NS difference in the reduction of weekly HF between both groups
	Cheng *et al.* 2007 [45, 75]	60/51	*HF score:* isoflavones: 1.4 ± 1.3 placebo: 1.3 ± 1.1 (mean ± SD)	≥12	60 mg soy isoflavones (in a fruit drink)	placebo: oatmeal drink	12 wks	significant difference in HF score between isoflavones and placebo ($p<0.01$); NS differences in night sweats score between the groups
	de Sousa *et al.* 2006 [52]	84/79	soy: 7.0 ± 3.6 placebo: 6.5 ± 2.8 (mean ± SD)	≥12	120 mg soy isoflavones	placebo	16 wks	HF frequency: NS difference after treatment between the groups (absolute values); significant difference of % change from baseline to week 16 between isoflavones and placebo ($p = 0.01$); HF severity score: NS difference between isoflavones and placebo (absolute values)[a]
	Duffy *et al.* 2003 [55]	36/33	*Greene Scale*: soy: 1.2 ± 0.4 placebo: 1.5 ± 0.4 (mean ± SEM)	≥12	60 mg soy isoflavones	placebo (lactose)	12 wks	NS difference in Greene Scale for vasomotor symptoms between the groups after treatment[a]

Table 3. Continued.

	Study	N randomized/ analyzed	Patients		Intervention/day	Control	Duration of intervention	Main results
			Basal HF frequency/ day	Time since last menstrual period (months)				
	Fauré *et al.* 2002 [59]	75/73	soy: 10.1 ± 6.4 placebo: 9.4 ± 3.4 (mean ± SEM)	≥6	70 mg soy isoflavones	placebo	16 wks	61% reduction of HF frequency with soy versus placebo (21%), p not stated
	Han *et al.* 2002 [57]	82/80	*KI for vasomotor symptoms*: soy: 11.3 ± 0.2 placebo: 10.0 ± 0.4 (mean - ± SEM)	≥12	100 mg soy isoflavones	placebo	4 months	significant difference in KI for vasomotor symptoms between isoflavones and placebo ($p<0.01$)
	Khaodhiar *et al.* 2008 [49]	191/142	40 mg DRI: 8.5 ± 3.5 60 mg DRI: 7.6 ± 2.6 placebo: 7.3 ± 2.5 (mean ± SD)	≥6	40 mg or 60 mg daidzein rich isoflavones (DRI)	placebo	12 wks	NS difference in % change of HF frequency from baseline at 12 wks between the groups: 40 mg: 52% 60 mg: 51% placebo: 39%; after pooling of 40 mg and 60 mg groups: significant difference in reduction of HF frequency between isoflavones and placebo (p = 0.048); reduction of HF severity in all groups (no p value)
	Nahas *et al.* 2007 [50]	80/76	soy: 9.6 ± 3.9 placebo: 10.1 ± 4.9 (mean ± SD)	≥12	100 mg soy isoflavones	placebo (lactose)	10 months	significant difference in HF frequency ($p<0.001$) and severity ($p<0.05$) between isoflavones and placebo
	Penotti *et al.* 2003 [58]	62/49	soy: 9.9 ± 4.5 placebo: 8.6 ± 2.9 (mean ± SD)	≥6	39 mg soy isoflavones	placebo	6 months	NS difference in HF frequency between the groups
	Stanosz *et al.* 2006 [43]	71/71	*KI for HF*: group 1: 7.3 ± 3.6 group 2: 6.9 ± 2.3 group 3: 7.3 ± 3.4 (mean ± SD[b)])	not stated	52 mg or 104 mg soy isoflavones	placebo	1 year	significant differences in % change from baseline of HF frequency and in KI for HF and night sweats between isoflavones and placebo ($p<0.001$)
	Upmalis *et al.* 2000 [53]	177/122	soy: 8.8 ± 6.2 placebo: 9.4 ± 6.0 (mean ± SD)	≥6	50 mg soy isoflavones	placebo	12 wks	NS difference in % change from baseline of HF frequency between the groups; significant difference in % change from baseline of HF severity score between isoflavones and placebo, (p = 0.01); NS difference in reduction of night sweats between the groups
Soy protein powder improvement of HF a) frequency: in 1 of 4 trials b) severity: in 0 of 3 trials	Albertazzi *et al.* 1998 [51]	104/79	soy: 11.4 (10.7–12.7) placebo: 10.9 (10.2–11.8) (mean (range))	≥6 or surgical menopause for ≥6 wks	76 mg soy isoflavones (60 g isolated soy protein in powder form)	placebo: 60 g of casein in powder form	12 wks	significant difference in % change from baseline in HF frequency between soy and placebo ($p<0.01$)

Table 3. Continued.

Study	N randomized/ analyzed	Patients		Intervention/day	Control	Duration of intervention	Main results
		Basal HF frequency/ day	Time since last menstrual period (months)				
Burke *et al.* 2003 [54]	241/211	42 mg: 2.6 ± 0.31 58 mg: 3.2 ± 0.38 placebo: 3.5 ± 0.38 (mean ± SEM)	≥1 menstrual period/ 3 months (perimenopausal) or surgical menopause	42 or 58 mg soy isoflavones (25 g ready-to-drink soy protein beverage)	placebo: 25 g ready-to-drink soy protein beverage with =4 mg isoflavones	12 wks	NS differences in HF frequency or severity between the groups
Knight *et al.* 2001 [46]	24/20	HF/week soy: 50.2 ± 13.6 placebo: 56.2 ± 26.5 (mean ± SD)	≥6	77.4 mg soy isoflavones (60 mg powder to be made into a beverage)	placebo: 60 g casein-based powder	12 wks	NS difference in % change of HF frequency between the groups ($p = 0.32$), NS difference in vasomotor Greene Scale between the groups
St. Germain *et al.* 2001 [44]	69[b]/69	soy: 36/week placebo: 32/week	median: 4 (perimenopausal)	80.4 mg isoflavones (40 g isoflavone-rich soy protein in muffins and for addition in foods) or 4.4 mg isoflavones (40 g isoflavone-poor soy protein)	placebo: 40 g whey protein in muffins and for addition in foods	24 wks	NS difference in reduction of frequency of HF and night sweats between the groups, NS difference in percent of women who experienced a decrease in frequency, duration and severity of HF and night sweats between the groups

a) vasomotor symptoms = secondary outcome
b) unclear, see Supporting Information 3

(vi) a ten-point scale, which finally was translated into a four-point scale,
(vii) Kupperman index for HF and night sweats, without further description of calculation of the score.

Among the included studies, there were differences regarding the menopausal status of the study population. The effect of isoflavones in only perimenopausal women was investigated in one trial. Seven trials included postmenopausal participants with amenorrhea of at least 12 months. One trial defined the study population as peri- and postmenopausal. A further eight trials described participants as postmenopausal, but time since last menstruation was at least six months as defined as their inclusion criterion. So these trials involved peri- and postmenopausal women according to the definition of the stages of reproductive aging workshop in 2001 [56]. One trial gave no information.

3.3 Efficacy outcome in genistein studies

Both genistein studies found an improvement of hot flush frequency in women suffering from about four daily HF after one year treatment with 54 mg genistein [47, 48]. One of both studies showed additionally an improvement of severity. The results of this study display a subgroup analysis of participants of a bone loss trial which suffered from HF. Severity score values were not analyzed as ordinal measurements, but as metric data [48]. In both studies vasomotor symptoms were secondary outcomes.

3.4 Efficacy outcome in soy extract studies

Eleven studies investigated the effect of isoflavones applied as soy extracts on vasomotor symptoms, two of them only as a secondary outcome [52, 55].

The isoflavones were administrated as capsules, tablets or as a ready-to-drink beverage enriched with isoflavones [45]. The isoflavone composition of the soy extracts differed between the included trials. The reported daily doses varied from 39 to 100 mg and 50 to 60 mg isoflavones calculated as aglycones [49, 55, 57] and as glycosides [41, 45, 53], respectively. A study reporting an intervention with 120 mg isoflavones referred to a composition of glycosides and aglycones [52]. The isoflavone specification was unclear in three studies [43, 50, 59].

Eight trials evaluated the effect on hot flush frequency, of which three [43, 50, 59] found an improvement after treatment with isoflavones compared to placebo. Additionally, one trial showed a significant effect of isoflavones after pooling two active arms to one active arm compared to placebo ($p = 0.048$) [49]. A further trial [52] found a reduction of HF with isoflavones after calculation of percent change from baseline to the end of the treatment whereas the difference between both groups after treatment was not significant.

Eight trials measured hot flush severity, of which five observed a significant improvement after consumption of soy supplements [43, 45, 50, 53, 57]. Except for three studies [53, 55, 57], all authors measuring severity analyzed the score data as metric data.

3.5 Efficacy outcome in soy protein powder studies

Four studies were identified using soy protein powder [44, 46, 50, 54]. The isoflavones were administered in doses of 76 – 80.4 mg *per* day calculated as aglycones [44, 46, 51]. In one study, the isoflavones with reported doses of 42/58 mg were not specified concerning aglycone or glycoside [54].

All studies measured hot flush frequency. A reduction of flushes was shown in one trial [51]. Additionally, the effect of isoflavones on hot flush severity was also evaluated in three studies [44, 46, 54], but no significant reduction was shown.

3.6 Correlation of initial HF and isoflavone doses with outcome

Analyses yielded no correlation between initial HF and outcome, neither weighted by number of participants nor unweighted. The outcome, stated as the difference in reduction from baseline compared to placebo, could only be estimated for some studies because of missing data. Four studies did not specify the isoflavone form (aglycone or glycoside) or report precise content of aglycone equivalents. No correlation could be detected. Again weighting by number of participants had no influence on the results.

3.7 Adverse effects

As summarized in Supporting Information 4, no proliferative effects of soy isoflavones on the endometrium, vagina or breast tissue could be detected in the analyzed studies. In several studies gastrointestinal disorders occurred after treatment, but a significant difference between soy and placebo was found in only one trial [53].

4 Discussion

This systematic review of the efficacy of isoflavone in the treatment of vasomotor symptoms focused on randomized placebo controlled trials investigating soy isoflavone supplements, published before March 2008. It was not appropriate to pool data from soy isoflavone studies because of the heterogeneity concerning intervention (isoflavone doses, composition of products, types of application) as well as measure of outcome. Overall, because of inconsistent study results, there is no conclusive evidence of a bene-

fit of soy isoflavones on hot flush frequency or severity. However, due to the results of the genistein studies there are some indications of the efficacy of isolated genistein.

In general, the quality of the included soy isoflavone studies showed numerous deficiencies, with limited numbers of patients, inadequate description of randomization, treatment allocation and blinding methods, as well as incomplete presentation of results. Furthermore, high drop-out rates, failure to include all randomized patients in the final analysis, and the marked placebo effect may question the validity of findings reported in several trials.

A clear classification according to the type of intervention was difficult in some cases because of the variability of soy isoflavones in supplements and the type of application, as well as limited information about the isoflavone form and purity.

Analyzing the studies achieved no clear result of the efficacy of isoflavones for the treatment of vasomotor symptoms. Although two studies examining the effect of isolated genistein on vasomotor symptoms [47, 48] found a benefit on HF and were of fair quality, the results have to be interpreted with caution. In both trials, the measurement of HF was a secondary endpoint of the parent studies [42, 60], and in one of both data were collected in a subgroup of participants suffering from HF. Overall, results of secondary endpoints are only suggestive and results of subgroup analyses have a high likelihood of either achieving a positive result by chance or missing a true benefit [61, 62]. Therefore, the effect of genistein on vasomotor symptoms should be verified in further studies.

However, conclusions of a recent review are in line with the assumption of the efficacy of genistein. Williamson-Hughes *et al*. [63] stratified studies according to the amount of genistein in several soy extracts and arrived to the conclusion that isoflavone extracts providing a minimum of 15 mg genistein *per* day can be effective in reducing severity and frequency of HF. Although, this hypothesis is not supported by the longitudinal study of women's health across the nation, this assumption has to be further investigated [19, 64]. Due to the imprecise specification of the intervention, it was not possible to examine the effect of genistein across the studies.

The only study, which found a significant improvement of vasomotor symptoms in the isoflavone soy protein powder group, stated that tamoxifen was not considered to be contraindicated for investigation of isoflavones [51]. In this review, tamoxifen use was a reason for exclusion, because HF occur as side effects of tamoxifen. Nevertheless, this trial was included because it was not clear whether women were actually treated with tamoxifen. However, it cannot be excluded that results were affected by tamoxifen.

It is discussed that in studies including participants with mild symptoms such as Burke *et al*., an effect of isoflavones on HF could not be measured [18]. However, a subgroup analysis of women with more than four HF failed to find differences between soy and placebo [54]. In this review, correlation analyses revealed no pattern to suggest that trials with a higher number of initial HF were more likely to find a treatment benefit. This finding agrees with the sensitivity analyses of Lethaby *et al*., who compared the overall pattern of results with those involving women with at least five HF baseline [19].

As indicated also by correlation analysis, the use of higher isoflavone doses did not seem to coincide with a benefit in vasomotor symptoms. However, aglycone values were estimated, because in some studies no information was given about isoflavone aglycone and glycoside content. Therefore, the comparison of the intervention doses must be considered with caution. Additionally, in correlation analyses it was not distinguished between different types of application resulting in limited conclusion.

It has to be pointed out, that only greater treatment effects could be significantly measured with a study design such as in Knight *et al*. which analyzed only 20 patients [46]. In contrast Cheng *et al*. showed a benefit of isoflavones on the hot flush score with 51 participants which had also only mild symptoms with initial hot flush score values of 1.4 on a five-point scale [45]. With 25 and 26 patients *per* group this trial exhibit a pilot study [23].

The comparison of severity outcomes leads to a limited conclusion because of the diversity of severity scores used. Whereas the Greene Vasomotor Scale comprises HF as well as night sweats, this is not the case for the Kupperman Scale. However, the Kupperman Index is widely used, despite there is no formal evaluation of the index and a lack of statistical justification for weights (National Institutes of Health, assessing and improving measures of hot flashes. Summary of an NIH Workshop, 2004, http://nccam.nih.gov/health/hotflashes/pdf/hotflashessumm.pdf.)[65]. Furthermore, the composition scores used by de Sousa *et al*. [52] and Upmalis *et al*. [53] included hot flush frequency and severity and are hardly comparable with the other scales. Additionally, severity data were often evaluated statistically with inappropriate tests analyzing score values as metric measurements, which could introduce bias and therefore have to beconsidered with caution.

With the trial of de Sousa *et al*. [52], it becomes apparent that the presentation of results (absolute values after treatment or percent reduction from baseline) can influence the outcome. In this study, significant positive results for frequency were yielded only after calculating the percentage changes from baseline, but no significant difference of hot flush values between the groups after treatment could be shown. This therefore necessitates the standardized description of results, such as reporting absolute values and percent reduction from baseline of all groups, when comparing studies and evaluating the reported effect.

The estimated prevalence of vasomotor symptoms varies from 35 to 50% in perimenopause and from 30 to 80% in post-menopause [10]. It was not possible to examine the

response to isoflavones according to menopausal status, because many trials included, but did not distinguish, peri- and post-menopausal women in the analysis, or defined the postmenopausal status as at least six months of amenorrhea.

Perimenopausal women may have contributed to a higher placebo response than postmenopausal women [66]. The mean placebo effect in all included trials dealing with soy supplements was 27%, which is in line with reported average values by Sloan *et al*. of 20 to 30% after 4 wks [23]. However, due to different definitions of menopausal status, correlation between menopausal status and placebo response could not be evaluated. The possibility that the placebo effect may derive from dietary isoflavone intake by women of control groups could be to a great extent excluded, since the placebo effect in studies measuring compliance by urinary or plasma isoflavone concentrations also averages 25%.

Poor information about induced or natural menopausal status of women was also often given. Only four trials stated directly that postmenopausal women with intact uterus and ovaries were investigated. The remaining trials involved both women with natural or with induced menopause or did not further described the participants, so a potential influence could not be evaluated.

The FDA proposed a washout period for prior estrogen and/or progestin therapy of at least 8 wks for oral therapy and at least 4 wks for transdermal application. Several studies did not confirm to this recommendation: some studies defined inclusion criteria with last MHT > 6 wks [46, 49, 51, 59] or did not provide information about past MHT [43, 48, 58]. So it is not sure whether the outcome was affected by MHT.

Besides methodological weakness of the studies, the variability of phytoestrogen compositions are discussed for the inconsistent results [3, 67]. On the one hand, it may be important which isoflavones are contained in the supplement, and on the other hand whether the isoflavones occur as glycosides or aglycones. This emphasizes the importance of clear specification of intervention. In the literature, there are conflicting data concerning the bioavailability of isoflavone glycosides and aglycones, where both forms have been shown as having higher bioavailability. Other studies found no differences in the absorption efficiency of glycosides and aglycones, thus no clear conclusion could be drawn [68].

The half-life of daidzein and genistein is nearly 8 h, thus steady state plasma concentrations would be better achieved by repeated ingestion of isoflavones throughout the day than by ingestion only once a day. Therefore, variations of isoflavone administration may also account for differences of study results [69]. Plasma genistein concentrations are consistently higher than daidzein concentrations when equal amounts of the two isoflavones were ingested, indicating a higher bioavailability of genistein than of daidzein. Furthermore, urine has higher concentrations of daidzein than of genistein because of the greater plasma clearance rate of daidzein, thus limiting the use of urine measurement as a predictor of systemic bioavailability [69, 70]. In addition, it is assumed that the composition of diet may influence the metabolism since it was shown that a diet rich in carbohydrates and dietary fiber was associated with a greater equol production [67].

Equol production by intestinal bacteria may enhance the action of isoflavones, but its effect on menopausal symptoms has not been clarified. It is assumed that 30 to 50% of the population are equol producers [71, 72]. Studies included in this review did not control for equol production. A recently published trial investigated the effect of intestinal production of equol on menopausal symptoms in women treated with 135 mg soy isoflavones. The results revealed that isoflavones had no beneficial effects in women who did not produce equol. Kupperman score for HF and excessive sweating showed a significantly greater reduction after isoflavone intake compared to placebo in women producing equol [73]. The results of this pilot study should encourage investigators to establish whether efficacy is influenced by equol production. Differences in individual intestinal flora may in large part explain the different results of intervention studies concerning the effect of isoflavones.

Overall, the outcome of this review is in line with previous reviews, where authors concluded that soy isoflavone studies are very heterogeneous and yielded inconsistent results. Isoflavones are often not superior to placebo for relief of vasomotor symptom frequency or severity [19–22].

In contrast, Howes *et al*. [18] found in their meta-analysis a clinically small – modest benefit of isoflavones on hot flush frequency. The trials were collectively evaluated and no subgroup analyses were performed to distinguish between different soy isoflavone composition and application. However, there are some differences in the evaluation of the individual studies. For example, in the study of Han *et al*. the Kupperman vasomotor symptom score was assessed, reflecting the severity of HF [57]. Combining different outcomes like frequency and severity in the calculation of efficacy may be misleading. A further study was included in which the control group was not evidently randomized [74]. The apparent heterogeneity of analyzed studies indicates that it is not appropriate to estimate the total effect size of all studies and to draw a clear conclusion. Lethaby *et al*. pointed out that pooling highly variable studies causes heterogeneity and affects the credibility of effects and concluded that there is no evidence of effectiveness in the alleviation of menopausal symptoms with the use of phytoestrogens [19].

However, whereas there is a long history of safe consumption for soy, this is not valid for plant extracts or for purified compounds like genistein. Because this review was

not focused on safety aspects, no conclusion could be drawn. In the analyzed studies, no side effects were detected but none of the studies have been of adequate size or duration to document the safety of soy supplements. Long-term studies investigating the safety of soy isoflavone supplements have to be reviewed systematically to determine adverse effects.

5 Limitations of this review

In this review only published articles were included, sole abstracts were not considered. No attempts were made to obtain missing data from authors of primary studies. Therefore, no access to original data existed. The given statistical methods in each trial were dealt with, even where such statistical analysis was considered by the authors to be inappropriate.

The literature search was comprehensive with no restriction of publication language to avoid publication bias. However, two studies are currently awaiting assessment:

(i) Xue *et al*. 2004 [34], which was not available and

(ii) Jou *et al*. 2008 [73], which was published after the performance of systematic literature search.

6 Conclusion

This systematic review reveals no conclusive evidence that treatment with soy isoflavone supplementation improves vasomotor symptoms in peri- and postmenopausal women. According to the lack of scientific substantiation clinicians and patients should seek for alternatives for treatment of vasomotor menopausal symptoms.

The present finding of inconclusive evidence was largely due to inconsistent study results and deficiencies in study quality. However, there is an indication for positive effects derived from pilot studies investigating genistein which have to be examined in the future. Further trials should pay attention to clear specification of the isoflavone supplement as well as to clear classification of participants concerning menopausal status and initial vasomotor symptoms with regard to ideally both severity and frequency of flushes. As recommended by the FDA HRT working group, efficacy has to be evaluated as a primary endpoint with a significant decrease in frequency and severity of HF.

The authors would like to thank Antje Dörendahl for the assistance in screening the databases and Dr. Birgit Niemann and Anke Weissenborn for their helpful comments on earlier versions of the manuscript.

The authors have declared no conflict of interest.

7 References

[1] Usui, T., Pharmaceutical prospects of phytoestrogens, *Endocr. J.* 2006, *53*, 7 – 20.

[2] Adlercreutz, H., Mazur, W., Phyto-oestrogens and Western diseases, *Ann. Med.* 1997, *29*, 95 – 120.

[3] Hulem, R., Blair, R. M., Soy isoflavones for postmenopausal symptoms. An examination of evidence, *Adv. Nurse Pract.* 2006, *14*, 32 – 38.

[4] Kuiper, G. G., Lemmen, J. G., Carlsson, B., Corton, J. C., *et al*., Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta, *Endocrinology* 1998, *139*, 4252 – 4263.

[5] Booth, N. L., Overk, C. R., Yao, P., Burdette, J. E., *et al*., The chemical and biologic profile of a red clover (Trifolium pratense L.) phase II clinical extract, *J Altern. Complement Med.* 2006, *12*, 133 – 139.

[6] Fugh-Berman, A., Kronenberg, F., Red clover (Trifolium pratense) for menopausal women: Current state of knowledge, *Menopause* 2001, *8*, 333 – 337.

[7] Freedman, R. R., Hot flashes: Behavioral treatments, mechanisms, and relation to sleep, *Am. J. Med.* 2005, *118 (12B)*, 124 – 130.

[8] Freeman, E. W., Sherif, K., Prevalence of hot flushes and night sweats around the world: A systematic review, *Climacteric* 2007, *10*, 197 – 214.

[9] WHO, Research on the menopause in the 1990s. Report of a WHO scientific group, *World Health Organ Tech Rep Ser.* 1996, *866*, 1 – 107.

[10] National Institutes of Health, State-of-the-Science Conference statement: Management of menopause-related symptoms, *Ann. Intern. Med.* 2005, *142*, 1003 – 1013.

[11] Farquhar, C. M., Marjoribanks, J., Lethaby, A., Lamberts, Q., Suckling, J. A., Long term hormone therapy for perimenopausal and postmenopausal women, *Cochrane Database Syst. Rev.* 2005, CD004143. DOI: 10.1002/14651858.CD004143.pub2.

[12] Flesch-Janys, D., Slanger, T., Mutschelknauss, E., Kropp, S. *et al*., Risk of different histological types of postmenopausal breast cancer by type and regimen of menopausal hormone therapy, *Int. J. Cancer* 2008, *123*, 933 – 941.

[13] Nagata, C., Shimizu, H., Takami, R., Hayashi, M., *et al*., Hot flushes and other menopausal symptoms in relation to soy product intake in Japanese women, *Climacteric* 1999, *2*, 6 – 12.

[14] Messina, M., Nagata, C., Wu, A. H., Estimated Asian adult soy protein and isoflavone intakes, *Nutr. Cancer* 2006, *55*, 1 – 12.

[15] Munro, I. C., Harwood, M., Hlywka, J. J., Stephen, A. M., *et al*., Soy isoflavones: A safety review, *Nutr. Rev.* 2003, *61*, 1 – 33.

[16] Adams, C., Cannell, S., Women's beliefs about“natural” hormones and natural hormone replacement therapy, *Menopause* 2001, *8*, 433 – 440.

[17] Coon, J. T., Pittler, M. H., Ernst, E., Trifolium pratense isoflavones in the treatment of menopausal hot flushes: A systematic review and meta-analysis, *Phytomedicine* 2007, *14*, 153 – 159.

[18] Howes, L. G., Howes, J. B., Knight, D. C., Isoflavone therapy for menopausal flushes: A systematic review and meta-analysis, *Maturitas* 2006, *55*, 203 – 211.

[19] Lethaby, A. E., Brown, J., Marjoribanks, J., Kronenberg, F., *et al*., Phytoestrogens for vasomotor menopausal symptoms, *Cochrane Database Syst. Rev.* 2007, CD001395.

[20] Nelson, H. D., Vesco, K. K., Haney, E., Fu, R., *et al*., Nonhormonal therapies for menopausal hot flashes: Systematic review and meta-analysis, *JAMA* 2006, *295*, 2057 – 2071.

[21] Krebs, E. E., Ensrud, K. E., MacDonald, R., Wilt, T. J., Phytoestrogens for treatment of menopausal symptoms: A systematic review, *Obstet. Gynecol.* 2004, *104*, 824 – 836.

[22] Balk, E., Chung, M., Chew, P., Ip, S. *et al*., Effects of soy on health outcomes, *Evid. Rep. Technol. Assess. (Summ.)* 2005, 1 – 8.

[23] Sloan, J. A., Loprinzi, C. L., Novotny, P. J., Barton, D. L., *et al*., Methodologic lessons learned from hot flash studies, *J. Clin. Oncol.* 2001, *19*, 4280 – 4290.

[24] Setchell, K. D., Absorption and metabolism of soy isoflavones-from food to dietary supplements and adults to infants, *J. Nutr.* 2000, *130*, 654 – 655.

[25] FDA HRT Working Group, Guidance for Clinical Evaluation of Combination Estrogen/Progestin-Containing Drug Products Used for Hormone Replacement Therapy of Postmenopausal Women, *Menopause* 1995, *2*, 131 – 136.

[26] Jadad, A. R., Moher, D., Klassen, T. P., Guides for reading and interpreting systematic reviews: II. How did the authors find the studies and assess their quality?, *Arch Pediatr. Adolesc. Med.* 1998, *152*, 812 – 817.

[27] Olivo, S. A., Macedo, L. G., Gadotti, I. C., Fuentes, J., *et al*., Scales to assess the quality of randomized controlled trials: A systematic review, *Phys. Ther.* 2008, *88*, 156 – 175.

[28] Moher, D., Schulz, K. F., Altman, D. G., The CONSORT statement: Revised recommendations for improving the quality of reports of parallel-group randomised trials, *Clin. Oral Investig.* 2003, *7*, 2 – 7.

[29] Kunz, R., Vist, G., Oxman, A. D., Randomisation to protect against selection bias in healthcare trials, *Cochrane Database Syst. Rev.* 2007, MR000012.

[30] Schulz, K. F., Grimes, D. A., Allocation concealment in randomised trials: Defending against deciphering, *Lancet* 2002, *359*, 614 – 618.

[31] Schulz, K. F., Grimes, D. A., Blinding in randomised trials: Hiding who got what, *Lancet* 2002, *359*, 696 – 700.

[32] Schulz, K. F., Grimes, D. A., Sample size slippages in randomised trials: Exclusions and the lost and wayward, *Lancet* 2002, *359*, 781 – 785.

[33] Lachin, J. M., Statistical considerations in the intent-to-treat principle, *Control Clin. Trials* 2000, *21*, 167 – 189.

[34] Xue, X., Niu, J., Wang, J., Ai, H., A prospective controlled study on the clinical therapeutic effect of treatment of menstrual syndrome by isoflavone of soybean, *Zhongguo Zhong Xi Yi Jie He Za Zhi* 2004, *24*, 835 – 836.

[35] Baber, R. J., Templeman, C., Morton, T., Kelly, G. E., West, L., Randomized placebo-controlled trial of an isoflavone supplement and menopausal symptoms in women, *Climacteric* 1999, *2*, 85 – 92.

[36] Hidalgo, L. A., Chedraui, P. A., Morocho, N., Ross, S., San Miguel, G., The effect of red clover isoflavones on menopausal symptoms, lipids and vaginal cytology in menopausal women: A randomized, double-blind, placebo-controlled study, *Gynecol. Endocrinol.* 2005, *21*, 257 – 264.

[37] Jeri, A., The use of an isoflavone supplement to relieve hot flushes, *The Female Patients* 2002, *27*, 35 – 37.

[38] Knight, D. C., Howes, J. B., Eden, J. A., The effect of Promensil, an isoflavone extract, on menopausal symptoms, *Climacteric* 1999, *2*, 79 – 84.

[39] Tice, J. A., Ettinger, B., Ensrud, K., Wallace, R., *et al*., Phytoestrogen supplements for the treatment of hot flashes: The Isoflavone Clover Extract (ICE) Study: A randomized controlled trial, *JAMA* 2003, *290*, 207 – 214.

[40] van de Weijer, P. H., Barentsen, R., Isoflavones from red clover (Promensil) significantly reduce menopausal hot flush symptoms compared with placebo, *Maturitas* 2002, *42*, 187 – 193.

[41] Campagnoli, C., Abba, C., Ambroggio, S., Peris, C., *et al*., PUFAs (PUFAs) might reduce hot flushes: An indication from two controlled trials on soy isoflavones alone and with a PUFA supplement, *Maturitas* 2005, *51*, 127 – 134.

[42] Marini, H., Minutoli, L., Polito, F., Bitto, A., *et al*., Effects of the phytoestrogen genistein on bone metabolism in osteopenic postmenopausal women: A randomized trial, *Ann. Intern. Med.* 2007, *146*, 839 – 847.

[43] Stanosz, S., Puk, E., Grobelny, W., Stanosz, M., Kazikowska, A., Ocena dzialania i tolerancji preparatu Soyfem u kobiet we wczesnym okresie pomenopauzalnym [Evaluation of the efficacy and tolerance of Soyfem in women in the early menopausal period], *Przew Lek.* 2006, *5*, 103 – 114.

[44] St Germain, A., Peterson, C. T., Robinson, J. G., Alekel, D. L., Isoflavone-rich or isoflavone-poor soy protein does not reduce menopausal symptoms during 24 wks of treatment, *Menopause* 2001, *8*, 17 – 26.

[45] Cheng, G., Wilczek, B., Warner, M., Gustafsson, J. A., Landgren, B. M., Isoflavone treatment for acute menopausal symptoms, *Menopause* 2007, *14*, 468 – 473.

[46] Knight, D. C., Howes, J. B., Eden, J. A., Howes, L. G., Effects on menopausal symptoms and acceptability of isoflavone-containing soy powder dietary supplementation, *Climacteric* 2001, *4*, 13 – 18.

[47] Crisafulli, A., Marini, H., Bitto, A., Altavilla, D., *et al*., Effects of genistein on hot flushes in early postmenopausal women: A randomized, double-blind EPT- and placebo-controlled study, *Menopause* 2004, *11*, 400 – 404.

[48] D'Anna, R., Cannata, M. L., Atteritano, M., Cancellieri, F., *et al*., Effects of the phytoestrogen genistein on hot flushes, endometrium, and vaginal epithelium in postmenopausal women: A 1-year randomized, double-blind, placebo-controlled study, *Menopause* 2007, *14*, 648 – 655.

[49] Khaodhiar, L., Ricciotti, H. A., Li, L., Pan, W., *et al*., Daidzein-rich isoflavone aglycones are potentially effective in reducing hot flashes in menopausal women, *Menopause* 2008, *15*, 125 – 132.

[50] Nahas, E. A., Nahas-Neto, J., Orsatti, F. L., Carvalho, E. P., *et al*., Efficacy and safety of a soy isoflavone extract in postmenopausal women: A randomized, double-blind, and placebo-controlled study, *Maturitas* 2007, *58*, 249 – 258.

[51] Albertazzi, P., Pansini, F., Bonaccorsi, G., Zanotti, L., *et al*., The effect of dietary soy supplementation on hot flushes, *Obstet. Gynecol.* 1998, *91*, 6 – 11.

[52] de Sousa, R. L., Filizola, R. G., Diniz, M. d. F., E. S. S., S., de Moraes, J. L. R., Ensaio cl¡nico placebo-controlado com isoflavonas da soja para sintomas depressivos em mulheres no climat,rio [Placebo-controlled clinical trial with soy isoflavones for depressive symptoms in climacteric women], *Rev. Bras. Ginecol. Obstet.* 2006, *28*, 91 – 100.

[53] Upmalis, D. H., Lobo, R., Bradley, L., Warren, M., *et al*., Vasomotor symptom relief by soy isoflavone extract tablets in postmenopausal women: A multicenter, double-blind, randomized, placebo-controlled study, *Menopause* 2000, *7*, 236 – 242.

[54] Burke, G. L., Legault, C., Anthony, M., Bland, D. R. *et al*., Soy protein and isoflavone effects on vasomotor symptoms in peri- and postmenopausal women: The soy estrogen alternative study, *Menopause* 2003, *10*, 147 – 153.

[55] Duffy, R., Wiseman, H., File, S. E., Improved cognitive function in postmenopausal women after 12 wks of consumption of a soya extract containing isoflavones, *Pharmacol. Biochem. Behav.* 2003, *75*, 721 – 729.

[56] Soules, M. R., Sherman, S., Parrott, E., Rebar, R., *et al*., Executive summary: Stages of reproductive aging workshop (STRAW), *Climacteric* 2001, *4*, 267 – 272.

[57] Han, K. K., Soares, J. M., Jr., Haidar, M. A., de Lima, G. R., Baracat, E. C., Benefits of soy isoflavone therapeutic regimen on menopausal symptoms, *Obstet. Gynecol.* 2002, *99*, 389 – 394.

[58] Penotti, M., Fabio, E., Modena, A. B., Rinaldi, M., *et al*., Effect of soy-derived isoflavones on hot flushes, endometrial thickness, and the pulsatility index of the uterine and cerebral arteries, *Fertil. Steril.* 2003, *79*, 1112 – 1117.

[59] Faure, E. D., Chantre, P., Mares, P., Effects of a standardized soy extract on hot flushes: A multicenter, double-blind, randomized, placebo-controlled study, *Menopause* 2002, *9*, 329 – 334.

[60] Morabito, N., Crisafulli, A., Vergara, C., Gaudio, A., *et al*., Effects of genistein and hormone-replacement therapy on bone loss in early postmenopausal women: A randomized double-blind placebo-controlled study, *J. Bone Miner Res.* 2002, *17*, 1904 – 1912.

[61] Jacobe, H. T., Leitenberger, J. J., Bergstresser, P. R., Understanding clinical trial outcomes: Design, analysis, and interpretation, *Dermatol. Ther.* 2007, *20*, 77 – 85.

[62] Schulz, K. F., Grimes, D. A., Multiplicity in randomised trials II: Subgroup and interim analyses, *Lancet* 2005, *365*, 1657 – 1661.

[63] Williamson-Hughes, P. S., Flickinger, B. D., Messina, M. J., Empie, M. W., Isoflavone supplements containing predominantly genistein reduce hot flash symptoms: A critical review of published studies, *Menopause* 2006, *13*, 831 – 839.

[64] Gold, E. B., Colvin, A., Avis, N., Bromberger, J., *et al*., Longitudinal analysis of the association between vasomotor symptoms and race/ethnicity across the menopausal transition: Study of women's health across the nation, *Am. J. Public Health* 2006, *96*, 1226 – 1235.

[65] Alder, E., The Blatt-Kupperman menopausal index: A critique, *Maturitas* 1998, *29*, 19 – 24.

[66] Simon, J. A., Stevens, R. E., Ayres, S. A., Phelps, K. V., Perimenopausal women in estrogen vasomotor trials: Contribution to placebo effect and efficacy outcome, *Climacteric* 2001, *4*, 19 – 27.

[67] Cassidy, A., Albertazzi, P., Lise, N. I., Hall, W., *et al*., Critical review of health effects of soyabean phyto-oestrogens in postmenopausal women, *Proc. Nutr. Soc.* 2006, *65*, 76 – 92.

[68] Manach, C., Williamson, G., Morand, C., Scalbert, A., Remesy, C., Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies, *Am. J. Clin. Nutr.* 2005, *81*, 230S – 242S.

[69] Setchell, K. D., Faughnan, M. S., Avades, T., Zimmer-Nechemias, L. *et al*., Comparing the pharmacokinetics of daidzein and genistein with the use of 13C-labeled tracers in premenopausal women, *Am. J. Clin. Nutr.* 2003, *77*, 411 – 419.

[70] Setchell, K. D., Brown, N. M., Desai, P., Zimmer-Nechemias, L., *et al*., Bioavailability of pure isoflavones in healthy humans and analysis of commercial soy isoflavone supplements, *J. Nutr.* 2001, *131*, 1362 – 1375.

[71] Setchell, K. D., Brown, N. M., Lydeking-Olsen, E., The clinical importance of the metabolite equol-a clue to the effectiveness of soy and its isoflavones, *J. Nutr.* 2002, *132*, 3577 – 3584.

[72] Yuan, J. P., Wang, J. H., Liu, X., Metabolism of dietary soy isoflavones to equol by human intestinal microflora – implications for health, *Mol. Nutr. Food Res.* 2007, *51*, 765 – 781.

[73] Jou, H. J., Wu, S. C., Chang, F. W., Ling, P. Y., *et al*., Effect of intestinal production of equol on menopausal symptoms in women treated with soy isoflavones, *Int. J. Gynaecol. Obstet.* 2008, *102*, 44 – 49.

[74] Colacurci, N., Zarcone, R., Borrelli, A., De Franciscis, P., *et al*., Effects of soy isoflavones on menopausal neurovegetative symptoms, *Minerva Ginecol.* 2004, *56*, 407 – 412.

[75] Messina, M., The safety and benefits of soybean isoflavones. A natural alternative to conventional hormone therapy?, *Menopause* 2007, *14*, 958; author reply 958 – 959.

