Human Reproduction Update, Vol.15, No.4 pp. 423–440, 2009
Advanced Access publication on March 19, 2009 doi:10.1093/humupd/dmp010

human reproduction update

Effects of soy protein and isoflavones on circulating hormone concentrations in pre- and post-menopausal women: a systematic review and meta-analysis

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BACKGROUND: Hormonal effects of soy and isoflavones have been investigated in numerous trials with equivocal findings. We aimed to systematically assess the effects of soy and isoflavones on circulating estrogen and other hormones in pre- and post-menopausal women.

METHODS: The Cochrane Library, MEDLINE and EMBASE (plus reviews and experts) were searched to December 2007. Inclusion of randomized or residential crossover trials of soy or isoflavones for 4 or more weeks on estrogens, SHBG, FSH, LH, progesterone and thyroid hormones in women was assessed independently in duplicate. Six percent of papers assessed were included. Data concerning participants, interventions, outcomes, potential effect modifiers and trial quality characteristics were extracted independently in duplicate.

RESULTS: Forty-seven studies (11 of pre-, 35 of post- and 1 of perimenopausal women) were included. In premenopausal women, meta-analysis suggested that soy or isoflavone consumption did not affect primary outcomes estradiol, estrone or SHBG concentrations, but significantly reduced secondary outcomes FSH and LH [by ~20% using standardized mean difference (SMD), $P = 0.01$ and 0.05, respectively]. Menstrual cycle length was increased by 1.05 days (95% CI 0.13, 1.97, 10 studies). In post-menopausal women, there were no statistically significant effects on estradiol, estrone, SHBG, FSH or LH, although there was a small statistically non-significant increase in total estradiol with soy or isoflavones (~14%, SMD, $P = 0.07$, 21 studies).

CONCLUSIONS: Isoflavone-rich soy products decrease FSH and LH in premenopausal women and may increase estradiol in post-menopausal women. The clinical implications of these modest hormonal changes remain to be determined.

Key words: soy foods / isoflavones / estradiol / sex hormone-binding globulin / gonadotrophins

Introduction

Although interest in the relative importance of isoflavones to human health has increased over the last 15 years, their effects on health are not clearly established. Isoflavones are diphenolic compounds with a range of characterized biological effects from *in vitro* studies. To date much of the interest in their biologic activity relates to estrogen receptor-mediated mechanisms, given their structural similarity to estrogens (Axelson *et al*., 1984; Kuiper *et al*., 1998; Gallo *et al*., 2005; Hwang *et al*., 2006; Messina, 2007), but numerous other biological effects independent of estrogen receptors have also been determined [e.g. antioxidant capacity, antiproliferative and anti-inflammatory effects (Kuiper *et al*., 1998; Setchell and Cassidy, 1999; Gallo *et al*., 2005; Hwang *et al*., 2006; Messina, 2007)].

Following modest soy consumption, typical for East Asians, circulating isoflavone concentrations reach the low micromolar level, 100–1000 times that of endogenous estrogen levels, although they circulate predominantly in the less biologically active conjugated form (Axelson *et al*., 1984; Adlercreutz and Mazur, 1997). These compounds may affect estrogen action by directly binding to estrogen receptors, preferentially estrogen receptor β, thus potentially directly affecting transcription of estrogen-regulated gene products (Kuiper *et al*., 1997; Pike *et al*., 1999; An *et al*., 2001; Lacey *et al*., 2005; Hwang *et al*., 2006), acting as estrogen agonists in some contexts and estrogen antagonists in others, much like the selective estrogen receptor modulators tamoxifen or raloxifene. Isoflavones may also influence estrogen action by virtue of effects on enzymes involved in steroid metabolism, including aromatase (Rice *et al*., 2006), 17β-hydroxysteroid dehydrogenases, steroid sulfatases and sulfotransferases (Lacey *et al*., 2005), potentially resulting in alterations of circulating estrogens.

Data from human clinical trials evaluating possible beneficial effects of isoflavone-rich products on a variety of health outcomes have been mixed—e.g. some studies suggest that isoflavones inhibit bone loss or alleviate hot flushes (Howes *et al*., 2006; Williamson-Hughes *et al*., 2006; Marini *et al*., 2007; Ma *et al*., 2008), whereas others have observed no effect (Balk *et al*., 2005; Nelson *et al*., 2006; Sacks *et al*., 2006). Although evidence on health effects in humans is debatable, endocrine modulation in several animal species has been reported following exposure to high levels of isoflavones from soy and red clover (Bennetts *et al*., 1946; Setchell *et al*., 1987; Adams, 1995). These hormonal effects in animals have raised questions about the safety of soyfoods (the main source of isoflavones in the human diet), despite a long history of soyfood consumption by many East Asian populations (Messina *et al*., 2006b).

There are a number of possible explanations for the variability in results among soy studies. A wide variety of intervention products with markedly varying isoflavone content have been used, including traditional soyfoods, isolated soy protein (ISP), soy extracts and isolated isoflavones, each with a variety of controls (Erdman *et al*., 2004). Other variables include amounts of protein in products, menopausal status of the participants, stage of menstrual cycle in premenopausal women and degree to which dietary intake is controlled.

A systematic evaluation of the literature, ensuring inclusion of the entire set of relevant studies, with greater statistical power to examine the effects of isoflavone-containing soy products on hormonal status, provides improved potential to examine the hormonal effects of soy isoflavones in women. We therefore conducted such a systematic review, and meta-analysis, of the literature to examine the effects of isoflavone-containing soy products on circulating levels of estrogens and other hormones in pre- and post-menopausal women.

Methods

Included studies were required to: be randomized trials or carefully controlled intervention studies (the former had to state that they were randomized or explicitly describe a true randomization method, the latter had to be residential crossover studies that provided and monitored all food and drink intake); be parallel or crossover in design; have an intervention duration ≥4 weeks; be in women ≥16 years old (pre- or post-menopausal, not critically ill, pregnant or breastfeeding); increase intake of soy, soy products or purified soy isoflavones compared with usual diet or usual diet with placebo soy/isoflavone; be unifactorial (so that effects of soy or isoflavones could be separated from those of other interventions); and assess at least one primary or secondary review outcome. Primary outcomes were circulating estradiol, estrone and SHBG in pre- and post-menopausal women. Secondary outcomes were FSH, LH, progesterone, circulating estrone sulfate, circulating free estradiol, thyroid hormones (T_4, T_3 or TSH), urinary estrogens and estrogenic metabolites, menstrual cycle length, luteal and follicular phase lengths, and IGF-1.

Structured electronic searches were carried out on The Cochrane Library, MEDLINE, EMBASE and the Meta-register of controlled trials (http://www.controlled-trials.com/mrct/), from inception to December 2007, in the format: [soy or isoflavones] AND [hormones] AND [randomized controlled trials]. We also checked the reference lists of large non-systematic reviews of trials of soy and isoflavone to ensure studies assessing hormones as secondary outcomes were not missed. Experts were contacted to obtain further (and unpublished) trials. Studies were not limited by publication status (whether fully published, published in abstract form, or unpublished) or language of publication.

Resulting titles and abstracts were assessed independently in duplicate by two reviewers and full text articles collected. Inclusion was assessed independently in duplicate by two reviewers, and disagreements resolved by discussion. Included papers were grouped into studies and data (on participants, interventions, outcomes at the latest time point to 53 weeks, trial quality characteristics and potential effect modifiers) were extracted independently by two reviewers onto a data extraction form refined by the entire review team.

Trial quality characteristics assessed included: masking (separately) of participants and outcome assessors (coded as 'yes' where there was a clear and realistic attempt to mask, 'no' where not, or 'unclear'—success of masking was rarely checked in included studies); industry funding or involvement (any funding, including full funding of the study, co-authorship of a scientist or statistician working for industry or provision of materials to be used during the intervention and/or control, and coded as 'yes, industry funding', 'none reported' or 'unclear'); duration (coded as 'done' for all post-menopausal studies of at least 4 weeks in duration, and premenopausal studies of at least 3 cycles duration or 'not done' for shorter premenopausal studies); assessment and reporting of compliance ('done' when compliance was both assessed and reported, 'partly done' when it was assessed but not reported or reported without any indication of the method used, and 'not done' when neither was addressed adequately); isoflavone content (reported as 'done' when total isoflavone, genistein and daidzein contents reported in both intervention and control, aglycone or glycated form reported, 'partially done' when at some of the above completed, 'not done' when not); isoflavones analyzed ('done' when the intervention dose was checked and reported, or 'unclear' if not carried out or not reported); and dropouts [reported as 'done' when the numbers of participants who were randomized,

completed and analyzed in each arm were all clear, and reasons for dropouts were given (by intervention arm), 'partially done' when some of the above, 'not done' when none of these data were presented]. Trials were considered to be at low risk of bias if participant and outcome assessor blinding were all coded 'yes', industry funding was not reported, duration was done and dropouts 'done'. All other trials were considered at moderate or high risk of bias.

For premenopausal studies, it was not feasible to choose any single phase of the menstrual cycle for data extraction (i.e. data were measured at different points during the cycle and selection of day of sampling within phase varied). Thus, we chose the point in each study with the highest control group baseline measurement for each outcome. This was based on the premise that it is the level and timing of the peak of hormone concentration that is important in determining health effects rather than the baseline levels present at other times of the cycle. This approach, while not ideal, was based on the premise that this was the point at which any changes due to soy or isoflavones might be most easily detected. To check that this assumption did not result in missing effects based on the use of non-peak data, we re-ran the analyses using data from the luteal phase only for estrogens, FSH and LH, and the follicular phase for progesterone.

Isoflavone dose was calculated in aglycone equivalents (glycoside levels were multiplied by 0.6). For primary and secondary outcomes, the number of participants assessed, means and standard deviations of change from baseline (where available, or data at the end of the intervention and control periods where change data were not available) in hormonal concentration in each treatment arm were extracted. For crossover studies, we aimed to use within-participant differences with the variance of these differences (Elbourne *et al*., 2002); however, the information on variance for the within-participant differences were not provided in our included studies, and without at least 2 studies providing such data, it was not possible to impute appropriate variance estimates with any confidence (Follman *et al*., 1992). For this reason, and because exact *P*-values or *t*-statistics were only available for occasional outcomes in a few of the crossover studies (generally being reported as 'significant' or not), mean and variance for the participants while on each treatment were used instead, treating results from the intervention period as if they came from one group of patients and results from the control period as if they came from a second group of patients. This is not ideal as the two groups are not independent (as required for the statistical tests) and will tend to provide a conservative estimate of any association as it ignores the within-patient correlations that give crossover trials their statistical strength (Elbourne *et al*., 2002). We ran a sensitivity analysis omitting crossover data except where an exact *t*-test or *P*-value for the relationship between the two periods was available. Heterogeneity was assessed using I^2 (Higgins *et al*., 2003). Data for pre- and post-menopausal women were analyzed separately.

Owing to varied mean baseline hormone concentrations of many hormones (e.g. for post-menopausal women, where menstrual phase was not relevant, mean total estradiol ranged from <20 to almost 200 pmol/l at baseline), we decided that our primary analyses would combine studies using standardized mean differences (SMD) in random effects meta-analysis, where at least two studies were combined. This was because the studies were effectively using different measurement scales to assess the same effect size. The SMD 'expresses the size of the intervention effect in each study relative to the variability observed in that study' (Anon., 2008; Deeks *et al*., 2008). Interpretation of the effect size is problematic as the units are units of standard deviation rather than the more intuitive pmol/l or equivalent 'real' units. For cycle lengths, studies were combined using mean differences (MD) in random effects meta-analysis. As a check on our results (sensitivity analysis), we also ran the meta-analyses using MD in place of SMD, and assessed robustness of results to trial quality (trials assessed as at moderate or high risk of bias were removed).

Subgroup analyses explored the effects of the following factors on the primary outcomes: intake of soy protein (<10, 10–24, 25–49, 50+ g/day of soy protein); isoflavone dose (<25 mg/day, 25 to <50 mg/day, 50 to <75 mg/day, 75 to <100 mg/day, 100+ mg/day); intervention intensity (dietary advice, supplementation or food provided); equol producers versus non-producers; and isoflavone source. We assessed for evidence of dissemination bias using funnel plots.

Results

Searches identified 1660 titles and abstracts for assessment (Fig. 1), 217 were retrieved in full text, and 47 studies were included and data extracted. Details of the included studies are provided in the appendix.

Eleven included studies were of premenopausal women (579 women analyzed), 35 of post-menopausal women (1165 analyzed), and 1 of perimenopausal women (69 analyzed). Six studies, all of post-menopausal women, provided no useable primary or secondary outcome data, despite requests from authors (data were only provided as least squares means with associated reduction in standard errors, did not separate out women using HRT, stated presence or absence of statistical significance but provided no actual data or did not present variances). However, these studies were included in the review to help us understand the quantity of missing data, and allow us to assess the potential of studies not included in the meta-analyses to alter the results of the pooling (Table I). As outcome reporting may vary according to whether statistically significant effects were seen, we did not want to assume that studies with missing data would be similar to those with presented data. Studies ranged in size from 10 to 304 women analyzed (mean 59). Thirty-two included studies were parallel in design, and 15 crossover. Nineteen studies assessed the effect of isoflavone extract versus control, nine isoflavone-containing ISP versus isoflavone-depleted ISP, 13 ISP versus another control, nine whole soy or soy foods versus control (some studies included more than one comparison). Studies ranged from 4 to 104 weeks long: 29 were 4–12 weeks in duration; nine were 13–26 weeks; seven 27–52 weeks; and two >1 year. One was conducted in metabolic

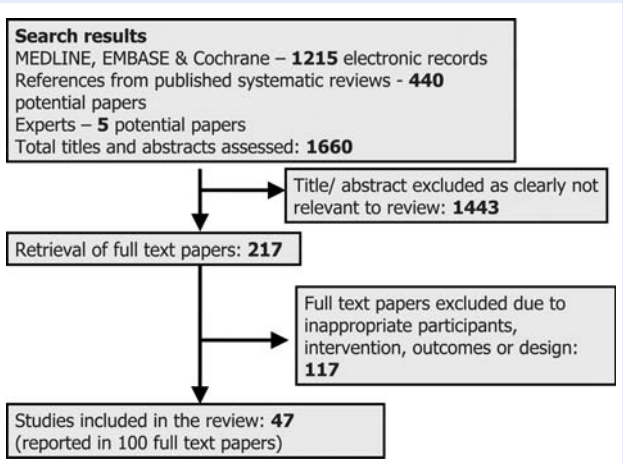

Figure 1 Study flow diagram of the search process and inclusion of studies into the review.

Table I Table of primary and secondary outcomes

	Premenopausal women				Post-menopausal women			
	No. of studies/ participants[a]/ missing studies[b]	SMD (95% CI)	I^2 (%)[c]	MD (95% CI)	No. of studies/ participants[a]/ missing studies[b]	SMD (95% CI)	I^2 (%)[c]	MD (95% CI)
Primary outcomes								
Circulating total E2, pmol/l	11/250/0	−0.05 (−0.23 to 0.12)	0	−7.99 (−48.20 to 32.22)	21/580/6	0.13 (−0.01 to 0.27)	29.3	2.76 (−0.37 to 5.90)
Circulating total E1, pmol/l	6/207/1	−0.09 (−0.29 to 0.10)	0	−12.21 (−36.60 to 12.17)	7/152/1	−0.13 (−0.36 to 0.10)	0	−5.33 (−11.56 to 0.90)
Circulating SHBG, nmol/l	10/233/1	−0.10 (−0.28 to 0.08)	0	−2.19 (−6.37 to 1.99)	17/459/3	−0.06 (−0.19 to 0.07)	0	−0.87 (−3.52 to 1.78)
Secondary outcomes								
Circulating FSH, IU/l	7/73/0	−0.45 (−0.79 to −0.11)	0	−0.52 (−1.15 to 0.11)	23/601/4	−0.08 (−0.26 to 0.10)	59.3	−1.29 (−4.41 to 1.83)
Circulating LH, IU/l	7/73/0	−0.34 (−0.68 to −0.01)	0	−1.26 (−3.30 to 0.78)	13/382/2	0.01 (−0.21 to 0.24)	58.1	0.27 (−2.42 to 2.95)
Circulating progesterone, nmol/l	9/199/0	0.03 (−0.29 to 0.36)	44.0	−1.20 (−6.89 to 4.48)	0/0/0	—	—	—
Circulating E1S, nmol/l	3/44/0	0.09 (−0.34 to 0.52)	0	0.49 (−2.12 to 3.11)	6/125/2	−0.03 (−0.29 to 0.22)	0	−0.03 (−0.28 to 0.22)
Circulating free E2, pmol/L	3/145/0	−0.09 (−0.32 to 0.14)	0	−0.71 (−2.21 to 0.79)	3/77/0	0.07 (−0.25 to 0.39)	0	0.18 (−0.10 to 0.46)
Circulating T3, nmol/l	1/14/0		—	−0.03 (−0.11 to 0.05)	2/41/1	0.29 (−0.14 to 0.72)	0	0.11 (−0.03 to 0.26)
Circulating T4, nmol/l	1/14/0		—	−1.60 (−6.73 to 3.53)	3/59/1	0.31 (−0.05 to 0.67)	0	3.53 (−0.61 to 7.68)
Circulating TSH, mU/l	1/14/0		—	−0.03 (−0.86 to 0.80)	7/192/1	−0.01 (−0.21 to 0.19)	0	0.05 (−0.26 to 0.35)
Circulating IGF-1, nmol/l	3/167/1	0.14 (−0.07 to 0.35)	0	0.75 (−0.60 to 2.11)	3/209/2	0.29 (−0.39 to 0.97)	80.7	1.57 (−1.75 to 4.89)
Urinary E2, nmol/24 h	1/11/3		—	−1.81 (−2.89 to −0.73)	1/18/0		—	0.05 (−0.64 to 0.74)
Urinary E1, nmol/24 h	1/14/3		—	−2.34 (−4.80 to 0.12)	1/18/0		—	−0.57 (−1.68 to 0.54)
Menstrual cycle length, days	10/148/1		0	1.05 (0.13 to 1.97)				
Luteal phase length, days	3/44/0		0	0.54 (−0.32 to 1.40)				
Follicular phase, days	7/92/0		7.6	0.81 (−0.12 to 1.74)				

SMD, standardized mean difference; MD, mean difference; I^2, I^2 test of heterogeneity; 95% CI, 95% confidence interval; E1, estrone; E2, estradiol; E1S, estrone sulfate.
[a]Number of participants in the control arms of all included studies combined.
[b]Number of studies that have data available on this outcome, but where those data are not useable in meta-analysis.
[c]I^2 relates to the SMD except where no SMD data appear (in which case, it relates to MD).

ward conditions, the remainder in the community (three of these provided all food for the intervention period). Twenty-five studies were carried out in North America, nine in Europe, five in Asia and four each in Australia and South America.

Study quality varied, as shown in Supplemental data Table S1. All but one of the included studies was randomized, and the non-randomized study was a closely supervised metabolic crossover study (Cassidy *et al*., 1995). Thirty-five studies masked participants (4 did not and in 8 studies this was unclear) and 31 studies masked outcome assessors (3 did not, 13 unclear). Twenty studies declared a funding source with a commercial interest in the study results; 25 did not report industry funding; and 2 were unclear. Duration of intervention was adequate in 42 studies, not in 5. Compliance was assessed and reported in nine studies, partly done in 24, and not done in 14. Isoflavone dose was well reported in 18 studies, partly in 27, and not in 2. Isoflavones were analyzed in 17 studies, not in one study, unclear in 29. Dropouts were fully reported in 25 studies, partly reported in 20, not in 2. Ten studies were judged at low risk of bias.

Primary outcomes

Summary effect data are presented in Table I, with sensitivity analyses omitting crossover study data unless *t*-test or exact *P*-values were available in Table II. Summary forest plots (for outcomes with at least four studies contributing data) are shown in Figs 2 (for premenopausal women) and 3 (post-menopausal women).

Premenopausal women

Soy and isoflavone consumption had no effect on circulating total estradiol, estrone or SHBG concentrations in premenopausal women (on the basis of 6–11 studies per comparison, each comparison had over 200 women in combined control arms, combining with either SMD or MD, no suggestion of heterogeneity). One further study assessed the effect of soy isoflavones on circulating total estrone and SHBG (data not useable in our meta-analysis), but addition of these results would be unlikely to significantly alter the outcome. Using data only from the luteal phase (rather than the point with the highest baseline measure) for total estradiol and estrone resulted in no suggestions of an effect (circulating estradiol SMD 0.03, 95% CI −0.17 to 0.23, I^2 0%; circulating estrone SMD 0.00, 95% CI −0.23 to 0.23, I^2 0%). Removing data for crossover studies except where exact *t*-test or *P*-values were available resulted in similar results to the main analysis, except that confidence intervals were generally widened slightly due to the loss of some power.

Post-menopausal women

In post-menopausal women, there was a small increase in circulating total estradiol concentrations following soy isoflavone consumption, but this was not statistically significant (on the basis of 21 studies, 580 women in combined control groups, SMD 0.13, 95% CI −0.01 to 0.27, or an increase of 14%, 95% CI −1% to +29%, $P = 0.07$, I^2 29.3%), see Supplementary Fig. 1. Sensitivity analysis using MD was not statistically significant (MD 2.76 pmol/l, 95% CI −0.37 to 5.90), nor was the sensitivity analysis removing studies not assessed as at low risk of bias (SMD 0.17, 95% CI −0.07 to 0.41, eight studies, 231 in control groups, I^2 37.7%), or that removing the underpowered crossover studies (SMD 0.15, 95% CI −0.02 to 0.32, $P = 0.09$, I^2 39.2%). A funnel plot (Fig. 4) suggested that studies finding more extreme increases and decreases of estradiol following soy isoflavone intervention may be missing from the review. A further six studies

Table II Sensitivity analysis of primary and secondary outcomes, removing crossover data except where exact *t*-test statistic given

	Premenopausal women		Post-menopausal women	
	No. of studies/ participants[a]	SMD (95% CI)	No. of studies/ participants[a]	SMD (95% CI)
Primary outcomes				
Circulating total E2, pmol/l	4/347	−0.08 (−0.30 to 0.13)	17/940	0.15 (−0.02 to 0.32)
Circulating total E1, pmol/l	4/347	−0.09 (−0.30 to 0.12)	4/150	−0.14 (−0.59 to 0.30)
Circulating SHBG, nmol/l	4/344	−0.13 (−0.34 to 0.09)	13/750	−0.07 (−0.22 to 0.09)
Secondary outcomes				
Circulating FSH, IU/l	2/40	−0.87 (−1.72 to −0.02)	15/834	−0.05 (−0.32 to 0.22)
Circulating LH, IU/l	2/41	−0.46 (−1.16 to 0.25)	10/605	0.05 (−0.25 to 0.34)
Circulating progesterone, nmol/l	2/219	0.14 (−0.56 to 0.83)	0/0	—
Circulating E1S, nmol/l	1/29	MD: 1.88 (−2.40 to 6.16)	2/77	0.12 (−0.33 to 0.57)
Circulating free E2, pmol/l	3/284	−0.09 (−0.32 to 0.14)	2/85	0.15 (−0.30 to 0.60)
Circulating T3, nmol/l	0/0		2/85	0.29 (−0.14 to 0.72)
Circulating T4, nmol/l	0/0		2/85	0.29 (−0.15 to 0.73)
Circulating TSH, mU/l	0/0		5/289	−0.03 (−0.26 to 0.20)
Circulating IGF-1, nmol/l	3/338	+0.14 (−0.07 to 0.35)	2/387	0.59 (0.38 to 0.79)
Menstrual cycle length, days	3/155	MD: 0.89 (−0.61 to 2.39)		

SMD, standardized mean difference; MD, mean difference; 95% CI, 95% confidence interval; E1, estrone; E2, estradiol; E1S, estrone sulfate.
[a]Number of participants in the intervention and control arms of all included studies combined.

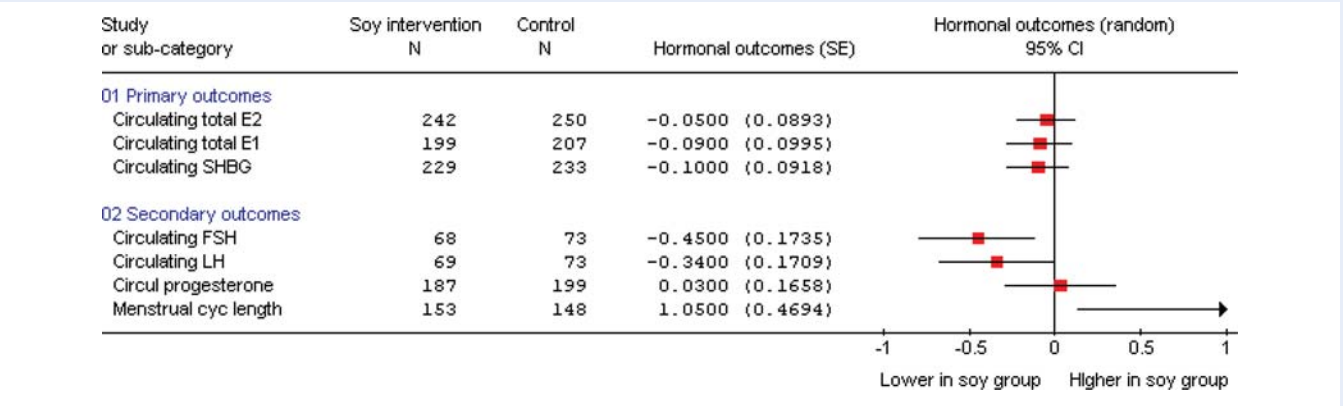

Figure 2 Effects of soy and isoflavones on circulating hormones and menstrual cycle length in premenopausal women (including all outcomes where at least four studies contributed to the data). SMD analysis, all in units of standard deviation, except for menstrual cycle length, which is a MD analysis, where the units are days.

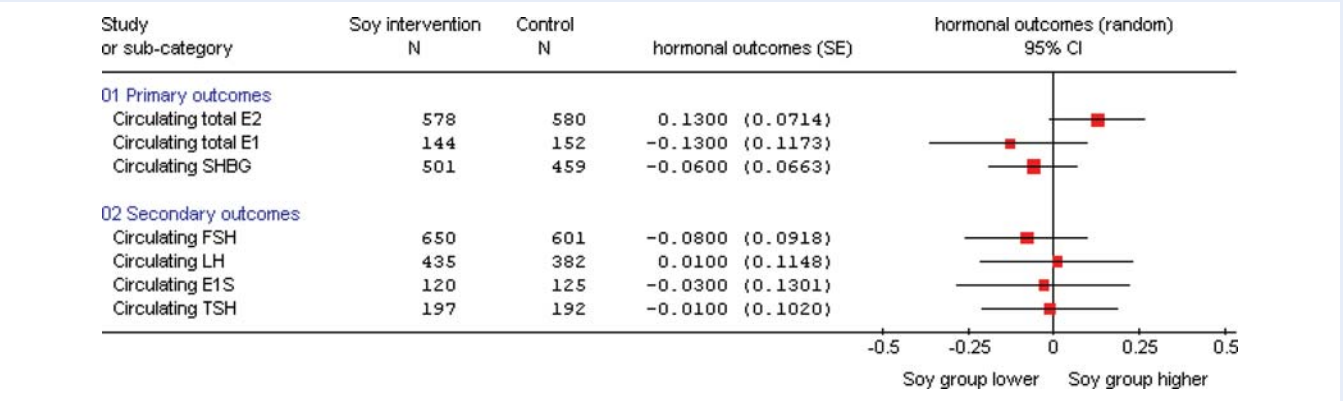

Figure 3 Effects of soy and isoflavones on circulating hormones in post-menopausal women (presenting all outcomes where at least four studies contribute to the data). SMD analysis, all in units of standard deviation.

(including 267 control participants) had analyzed the effect of soy isoflavones on total circulating estradiol in post-menopausal women, but not presented the data in a useable way—addition of the results of these studies could alter both the effect size and the statistical significance of the results of this meta-analysis.

Soy isoflavones had no effect on circulating total estrone (7 studies, 152 in control) or SHBG concentrations (17 studies, 459 women in control groups), combining by either SMD or MD, with no suggestion of important heterogeneity. There were one and three studies, respectively, that clearly assessed estrone and SHBG in post-menopausal women, but did not present data in a useable format—addition of these studies would be unlikely to appreciably alter the results.

Subgrouping

Subgrouping by isoflavone source indicated that consumption of isoflavone extracts (rather than soy foods or ISP) was associated with a significant increase in circulating estradiol among post-menopausal women (Supplementary Fig. 1). The SMD of isoflavone extracts was 0.21 (95% CI 0.03 to 0.40, 14 studies, 398 in control groups, I^2 40.1%). However, given the total number of subgroups analyzed, and the proximity of the confidence interval for this subgroup to zero, this finding may be due to chance alone.

Subgrouping by total isoflavone dose or soy protein intake resulted in no suggestion of an effect moderated by either. It was not possible to subgroup by equol-producer status (as not enough studies reported data on equol concentrations) or by intensity of intervention (as all studies provided a supplement or food and none provided advice alone).

Secondary outcomes

Premenopausal women

Soy isoflavones reduced circulating FSH and LH, and increased menstrual cycle length in premenopausal women, as shown in Supplementary Figs 2–4. Effects on FSH and LH were observed in seven studies (73 participants in control groups) and effects were statistically significant using SMD, but not MD. I^2 was 0%, suggesting no important heterogeneity between the studies. Removing studies not at low risk of bias resulted in no remaining studies (trials were considered to be at low risk of bias if participant and outcome assessor blinding were all coded 'yes', industry funding was not reported, duration was

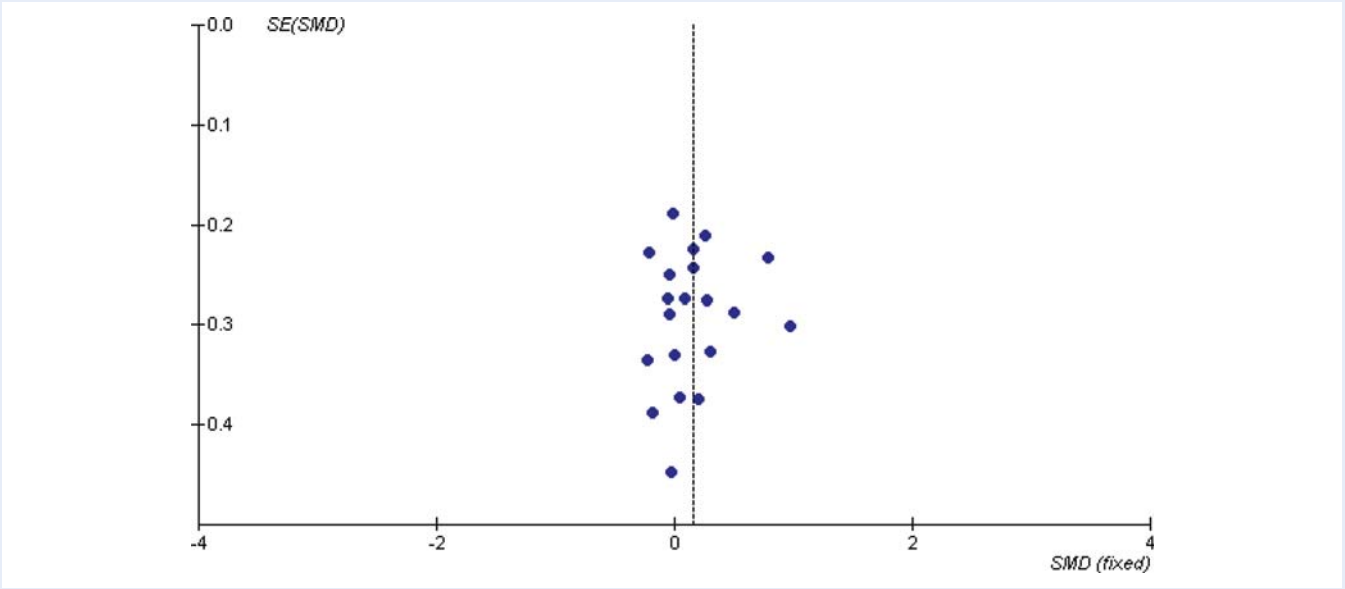

Figure 4 Funnel plot of results from included published studies on the effects of soy protein and isoflavones on circulating total estradiol (E2, pmol/l) in post-menopausal women.

done and dropouts 'done', all other trials were considered at moderate or high risk of bias—for further details, see definition below Supplementary Table S1) whereas removing crossover studies without exact *t*-test or *P*-values resulted in wider confidence intervals, with the effect on FSH remaining statistically significant and loss of such significance for LH and menstrual cycle length.

Interpretation of SMD is problematic as it uses units of standard deviation, but we can use the largest single study (Maskarinec 2002, with 16 participants in the control group) to provide insight into the magnitude of the effect using conventional units. In that study, the control group FSH was 5.4 IU/l at the study end, so that the effect of soy isoflavones on FSH corresponded to −1.2 IU/l (95% CI −0.3 to −2.2), a decrease of ∼22%. Control group LH concentrations at the study end were 4.5 IU/l, giving an effect size of −1.1 IU/l (95% CI −0.03 to −2.2), a decrease of ∼24%. All studies that determined the effects of soy isoflavone intervention on FSH or LH in premenopausal women were useable in meta-analysis.

Combined analysis of 10 studies (148 women in control groups) suggested an increase in menstrual cycle length of 1.1 days with soy isoflavones compared with control. Sensitivity analysis, removing studies not at low risk of bias, removed statistical significance (WMD 1.21 days, 95% CI −0.98 to 3.41, two studies, 62 women in control groups, I^2 28.3%).

Urinary estradiol concentrations were reduced in women taking soy isoflavones, based on only one study of 14 women. Soy isoflavones had no statistically significant effects on progesterone, circulating free estradiol or IGF-1 concentrations (where there were at least three studies and at least 50 women in combined control groups). There were insufficient data to comment on the effects on circulating estrone sulfate, T_4, T_3, TSH, urinary estrone or urinary estradiol.

Using data only from the luteal phase (rather than the point with the highest baseline measure) for circulating FSH and LH, and only from the follicular phase for progesterone, resulted in no suggestions of an effect (circulating FSH SMD −0.07, 95% CI −0.42 to 0.27, I^2 0%; circulating LH SMD 0.05, 95% CI −0.27 to 0.36, I^2 0%; circulating progesterone SMD −0.02, 95% CI −0.30 to 0.26, I^2 17%).

Post-menopausal women

Soy isoflavone intake had no effect on FSH, LH, circulating estrone sulfate, circulating free estradiol, TSH, T_4 or IGF-1 concentrations (where there were at least three studies and at least 50 women in combined control groups). There were insufficient data to comment on the effects on progesterone, T_3, urinary estrone or urinary estradiol.

Perimenopausal women

Two studies included some perimenopausal women, but only Alekel 2000 provided useable data for meta-analysis. Effects on circulating estrone, circulating estradiol, FSH and IGF-1 were all non-significant (24 women in the control group).

Side effects

Gastrointestinal side effects, but not dropouts due to adverse effects or any recorded side effect, were statistically significantly more likely to occur in participants taking any source of soy and/or isoflavones than controls (pre- and post-menopausal women combined, gastrointestinal side effects RR 1.8, 95% CI 1.3 to 2.6, eight studies, 507 in control groups, I^2 0%; any side effect RR 1.9, 95% CI 0.5 to 8.0, two studies, 131 in control groups, I^2 79%; dropouts due to adverse events RR 1.6, 95% CI 0.7 to 3.7, four studies, 357 in control groups, I^2 25%).

Discussion

This systematic review of 47 studies assessed the effects of soy isoflavones on hormone concentrations in pre- and post-menopausal women. In premenopausal women, consumption of soy isoflavones had no effect on circulating total estradiol, estrone or SHBG. There were significant reductions in FSH (by ∼22%, $P = 0.01$) and LH concentrations (by ∼24%, $P = 0.05$), and an increase in menstrual cycle length

of 1.05 days (95% CI 0.13 to 1.97, I^2 0%, 10 studies with 148 women in control groups) as shown in Table I and Fig. 3. However, these effects should be considered tentative because in sensitivity analysis, when only studies at low risk of bias were retained, the results were no longer statistically significant. No statistically significant effects were observed on free estradiol, progesterone or IGF-1 concentrations.

In post-menopausal women, there were no statistically significant effects of soy or isoflavones on circulating total estradiol, estrone or SHBG, although there was a small non-significant increase in total circulating estradiol following soy (~14%, $P = 0.07$, 21 studies, 580 women analyzed in control groups). Soy had no effect on FSH, LH, free estradiol or estrone sulfate, T_4, IGF-1 or TSH.

These data are consistent with at most weak effects of soy isoflavones on the hypothalamic–pituitary–gonadal axis in women. They could occur *via* the effects of isoflavones on endogenous estrogen synthesis, through alterations of enzymes involved in steroid metabolism, including aromatase (Rice *et al*., 2006), 17β-hydroxysteroid dehydrogenases, steroid sulfatases and sulfotransferases (Lacey *et al*., 2005). Alternatively, isoflavones may exert estrogenic or anti-estrogenic effects by binding to estrogen receptors, directly affecting transcription of estrogen-regulated gene products (Kuiper *et al*., 1997; Kuiper *et al*., 1998; Pike *et al*., 1999; Rosselli *et al*., 2000; An *et al*., 2001). Although isoflavones have a weaker binding affinity for estrogen receptors than endogenous estrogens, circulating levels of isoflavones following consumption of soy will exceed endogenous estrogen levels by several orders of magnitude (Axelson *et al*., 1984; Cassidy *et al*., 1994; Adlercreutz and Mazur, 1997). Our data, however, suggest no changes in estrogen status in premenopausal women and borderline effects in post-menopausal women. This lack of effect contrasts with notable and often-cited animal data; for example, infertility in Western Australian sheep grazing on isoflavone-rich clover (Bennetts *et al*., 1946; Adams, 1995) and captive North American cheetahs consuming diets containing soy protein (Setchell *et al*., 1987). However, in both cases, circulating levels of isoflavones were much higher than could realistically be achieved in humans. In Australian sheep, this was because of the high isoflavone exposure and in the cheetah, due to their inability to glucuronidate isoflavones *in vivo*.

Whether the observed but tentative premenopausal changes in FSH and LH reflect an estrogenic or anti-estrogenic effect is not clear. These hormones were assessed in different studies at different points in the menstrual cycle, including, for example, during the mid-cycle gonadotrophin surge, when a decrease in LH is best construed as an anti-estrogenic effect, while during the luteal phase a decrease in LH may be an estrogenic effect. On the other hand, the increase in menstrual cycle length suggests an anti-estrogenic effect, with longer cycles linked to reduced breast cancer risk (Setchell *et al*., 1984; Kelsey *et al*., 1993; Cassidy *et al*., 1994; Duncan *et al*., 1999a; Messina *et al*., 2006a), and a growing body of evidence that increased lifetime soy exposure lowers breast cancer risk (Wu *et al*., 2008).

In post-menopausal women, the small statistically non-significant increase in circulating estradiol concentrations is potentially of concern, as a recent meta-analysis of nine prospective studies showed that increased levels of circulating estradiol were associated with an increased risk of breast cancer in post-menopausal women (Endogenous Hormones and Breast Cancer Collaborative Group, 2002). Being in the top quintile of total estradiol, compared with the bottom quintile, doubled breast cancer risk (RR 2.00, 95% CI 1.47 to 2.71). However it is difficult to assess the absolute effect of the small statistically non-significant increase in circulating estradiol associated with increased intake of soyfoods and supplements observed in our systematic review. In this regard, the fact that neither SHBG nor LH and FSH concentrations were affected argues against a physiologically important estrogenic effect. Furthermore, the available clinical and epidemiological data do not support the idea that soy isoflavone exposure increases breast cancer risk (Messina *et al*., 2006a; Wu *et al*., 2008) or menopausal symptoms (Nelson *et al*., 2006; Lethaby *et al*., 2007), although there is conflicting and limited evidence that isoflavones from red clover reduce hot flush frequency in menopausal women (Nelson *et al*., 2006; Coon *et al*., 2007).

There are many limitations of the data set used in this systematic review and meta-analysis. The diversity of baseline and control group values between studies for most hormones [also seen in prospective studies (Endogenous Hormones and Breast Cancer Collaborative Group, 2002)] was managed by assuming that different studies were effectively using different scales to measure these hormones (e.g. variation in the methodology employed to assay a particular hormone may have generated consistent among-study variation in hormone concentrations), and by carrying out meta-analysis using SMD. Many data appeared to be skewed, so group means may be dependent on a few extreme values and thus unreliable. Some authors appropriately dealt with this by presenting their data as medians or geometric means (only the latter being amenable to meta-analysis). Other studies presented least squares means following model adjustment, but these were not combinable in meta-analysis due to associated smaller standard errors (so would have been weighted incorrectly). Many parallel studies were small, with baseline hormone levels that differed a great deal between the intervention and the control groups (and where changes over time were smaller than the initial difference between the groups, so that outcome data represented baseline levels more strongly than changes engendered by time or the intervention). On the other hand, the crossover data were underweighted in the analyses due to lack of information on the within-participant differences in the studies. We checked that these studies were not providing misleading data by running a sensitivity analysis removing crossover studies without exact *t*-test or *P*-values, and found that the effects were not greatly altered, but that confidence intervals were widened due to reduced power in the remaining studies. Another issue was how to combine data measured at different points during the menstrual cycle. Our decision to take the point at which control data were greatest was not ideal, but appeared the only realistic approach. Finally, a large number of studies assessed the effects of soy isoflavones on at least one of the selected outcomes, but did not report the data in a way that was useable in meta-analysis. As far as possible, we collected details of these missing data to document the extent of the problem.

To our knowledge, this is the first systematic review and meta-analysis to compare the endocrine effects of different soy products on hormonal status in women at different lifecycle stages. It provides weak evidence that soy and isoflavones decrease FSH and LH in premenopausal women, and a suggestion that they may increase estradiol in post-menopausal women. The clinical implications of these relatively modest hormonal changes are unclear and the clinical relevance of these findings for women at different stages of the lifecycle require confirmation in further robust studies.

Author's role

L.H. and A.C. were the principal investigators and the study was initiated by discussion between A.C. and M.J.M. L.H. led the review and is the guarantor of the study. The protocol was drafted by L.H., A.C. and M.J.M., and all authors contributed to, and agreed, the final protocol. J.J.R. and L.H. ran the searches, assessed titles and abstracts for collection, collected relevant full text papers, assessed these for inclusion and merged papers into studies. J.J.R. organized distribution of full text papers to authors for data extraction. All authors contributed to development of the final data extraction form, agreed review methodology and data extracted a share of the included studies. J.J.R., A.C. and L.H. collated the duplicated data extraction forms and adjudicated where differences emerged. J.J.R. and L.H. collated data into tables and ran the analyses in RevMan. L.H. and A.C. prepared the first draft of the paper, and all authors contributed significantly to data interpretation, and preparation of the second draft of the manuscript. All authors agreed the final draft.

Supplementary data

Supplementary data are available at http://humupd.oxfordjournals.org/.

Acknowledgements

Many thanks to the trialists who took the time to reply, provide additional information, expertise and data for the review: D Lee Alekel (Iowa State University, Alekel 2000); Bahram Arjmandi (Florida State University, Arjmandi 2003, 2005); Francesco Branca (World Health Organization, Copenhagen, Brink 2008); Elisabeth Brink (TNO Quality of Life, Zeist, The Netherlands, Brink 2008); Blakely D. Brown (University of Montana, Brown 2002); Michael Daskalakis (Southern Cross Pathology, Australia, Kotsopoulos 2006); Alison Duncan (University of Guelph, Duncan 1999 pre and post); J.A. Eden (University of New South Wales, Australia, Knight 2001); Christopher Gardner (Stanford University School of Medicine, Gardner 2001); Barry Goldin (Tufts University School of Medicine, Lichtenstein 2002); Jinzhu Liu (Barbara Gross Research Centre, Randwick, Australia, Knight 2001); Gertraud Maskarinec (Cancer Research Center of Hawaii, Maskarinec 2004); Jennifer A. Nettleton (University of Minnesota, Nettleton 2004); Eini Nikander (Helsinki University Central Hospital, Nikander 2004); Giovanni Scambia (Catholic University of the Sacred Heart, Rome, Scambia 2000); Helena Teede (Department of Medicine, Dandenong, Australia, Kotsopoulos 2006); Lillian U. Thompson (University of Toronto, Brooks 2004); Margo Woods (Tufts University School of Medicine, Woods 2000); Michelle Warren (Center for Menopause and Hormone Disorders, New York, Upmalis 2000); Anna Wu (University of Southern California, Wu 2005).

Funding

This work was partially funded by the Soy Nutrition Institute, Inc., St Louis, MO, USA. M.J.M. consults for companies that manufacture and/or sell soyfoods, soy protein and isoflavone supplements and is a Scientific Advisory Board Member of the Soy Nutrition Institute.

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Submitted on July 28, 2008; resubmitted on December 23, 2008; accepted on February 18, 2009

Appendix: Characteristics of included studies

Study	Participants	Interventions	Outcomes
Alekel 2000	**Participants:** peri-men (com)	**Design:** parallel	**DO:** 11, unclear from which arms
USA (Alekel *et al.*, 2000; Dent *et al.*, 2001; St Germain *et al.*, 2001; Swain *et al.*, 2002; Moeller *et al.*, 2003)	**Analyzed:** int 24, cont A 21, cont B 24	**Intervention: A:** ISP versus milk protein	**Duration:** 24 weeks
	Med age: int 50, cont A 49, cont B 51	**B:** ISP versus alcohol-washed ISP	
		Isoflav, mg/day: int 80, cont A unclear, cont B 4.4 (**AU**)	
		Soy protein difference: 0 g/day	
Arjmandi 2003	**Participants:** post-men (com)	**Design:** parallel	**DO:** int 16, cont 13
USA (Arjmandi *et al.*, 2003, 2004)	**Analyzed:** int 20, cont 22	**Intervention:** ISP versus milk protein	**Duration:** 12 weeks
	Mean age: int 62, cont 62	**Isoflav, mg/day:** int 88.4, cont 0 (**AU**)	
		Soy protein difference: 37 g/day	
Arjmandi 2005	**Participants:** post-men (com)	**Design:** parallel	**DO:** int 13, cont 12
USA (Arjmandi *et al.*, 2005)	**Analyzed:** int 35, cont 27	**Intervention:** ISP versus no soy	**Duration:** 52 weeks
	Mean age: int 53, cont 56	**Isoflav, mg/day:** int 60, cont unclear (AU?)	
		Soy protein difference: 25 g/day	
Aubertin-Leheudre 2007	**Participants:** obese post-men (com)	**Design:** parallel	**DO:** 14 in each arm
Canada (Aubertin-Leheudre *et al.*, 2007)	**Analyzed:** int 10, cont 10	**Intervention:** isoflav ext versus placebo	**Duration:** 52 weeks
	Mean age, sd: 58 overall	**Isoflav, mg/day:** int 70, cont unclear (AU?)	
		Soy protein difference: 0 g/day	
Baird 1995	**Participants:** post-men (com)	**Design:** parallel	**DO:** 6 (unclear from which arms)
USA (Baird *et al.*, 1995)	**Analyzed:** int 66, cont 25	**Intervention:** whole soy versus usual diet	**Duration:** 4 weeks
	Mean age: unclear	**Isoflav, mg/day:** int 165, cont unclear (AU?)	
		Soy protein difference: unclear	
Baum 1998	**Participants:** HC post-men (com)	**Design:** parallel	**DO:** 15 (unclear from which arms)
USA (Baum *et al.*, 1998; Persky *et al.*, 2002; Potter *et al.*, 1998)	**Analyzed:** ISP90 21, ISP56 23, cont 22	**Intervention:** ISP versus milk protein	**Duration:** 24 weeks
	Mean age: ISP90 61, ISP56 60, cont 61	**Isoflav, mg/day:** ISP90 90, ISP56 56, cont nil (**AU**)	
		Soy protein difference: 37 g/day	

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Study	Participants	Interventions	Outcomes
Brink 2008 (NL, Italy, France)	**Participants:** post-men (com)	**Design:** parallel	**DO:** int NL 5, It 13, Fr 14, cont NL 5, It 11, Fr 15
Netherlands, Italy, France (Brink *et al*., 2008)	**Analyzed: NL** int 45, cont 46; **It** int 39, cont 39; **Fr** int 34, cont 34	**Intervention:** isoflav ext versus nil	**Duration:** 52 weeks
	Mean age: NL int 53, cont 53; **It** int 53, cont 53; **Fr** int 54, cont 54	**Isoflav, mg/day:** int 110, cont unclear (**AU**)	
		Soy protein difference: 0 g/day	
Brooks 2004	**Participants:** post-men (com)	**Design:** parallel	**DO:** int 2, cont 0
Canada (Lewis *et al*., 2006; Brooks *et al*., 2004)	**Analyzed:** int 13, cont 15	**Intervention:** soy foods versus wheat flour	**Duration:** 16 weeks
	Mean age: int 54, cont 53	**Isoflav, mg/day:** int 41.4, cont unclear (AU?)	
		Soy protein difference: 8 g/day	
Brown 2002	**Participants:** pre-men (com)	**Design:** crossover	**DO:** 12
USA (Brown *et al*., 2002)	**Analyzed:** 14	**Intervention** ISP versus alternate foods	**Duration:** 2 cycles
	Mean age: 28	**Isoflav, mg/day:** int 40, cont none (AU?)	
		Soy protein difference: 31 g/day	
Cassidy 1995	**Participants:** pre-men (metabolic unit)	**Design:** crossover	**DO:** 3 drop outs from study 2
UK (Cassidy *et al*., 1994, 1995)	**Analyzed:** Study 1: 6, Study 2: 6, Study 3: 5, Study 4: 6	**Intervention:** soy foods versus nil	**Duration:** 1 cycle
	Mean age: Study 1: 24, Studies 2–4: unclear	**Isoflav, mg/day:** Study 1: int 45, cont 1; Study 2: int 25, cont unclear; Study 3: unclear; Study 4: int 23, cont unclear (AU—No)	
		Soy protein difference: Study 1 30 g/day; Study 2 7 g/day; Study 3 unclear; Study 4 14 g/day	
Cheng 2007	**Participants:** post-men (com)	**Design:** parallel	**DO:** 9 (unclear from which arms)
Sweden (Cheng *et al*., 2007)	**Analyzed:** int 26, cont 24	**Intervention:** isoflav ext versus oatmeal	**Duration:** 12 weeks
	Mean age: int 58, cont 56	**Isoflav, mg/day:** int 60, cont unclear (AU?)	
		Soy protein difference: 0 g/day	
Cuevas 2003	**Participants:** raised LDL post-men (com)	**Design:** crossover	**DO:** unclear
Chile (Cuevas *et al*., 2003)	**Analyzed:** 18	**Intervention:** ISP versus milk protein	**Duration:** 4 weeks
	Mean age: 59	**Isoflav, mg/day:** int 80, cont unclear (AU?)	
		Soy protein difference: 37 g/day	
D'Anna 2007	**Participants:** post-men (com)	**Design:** parallel	**DO:** 48 int, 37 cont at 2 years
Italy (D'Anna *et al*., 2005; Atteritano *et al*., 2007; Marini *et al*., 2007)	**Analyzed:** int 150, cont 154	**Intervention:** isoflav ext versus placebo	**Duration:** 104 weeks
	Mean age, sd: int 55, cont 54	**Isoflav, mg/day:** int 54, cont nil (**AU**)	
		Soy protein difference: 0 g/day	
Dewell 2002	**Participants:** HC post-men (com)	**Design:** parallel	**DO:** 4 (unclear from which arms)
USA (Dewell *et al*., 2002; Bruce *et al*., 2003)	**Analyzed:** int 22, cont 16	**Intervention:** isoflav ext versus placebo	**Duration:** 26 weeks
	Mean age: int 69, cont 70	**Isoflav, mg/day:** int 90 AU, cont unclear (**AU**)	

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Study	Participants	Interventions	Outcomes
		Soy protein difference: 0 g/day	
Duncan 1999 Pre	**Participants:** pre-men (com)	**Design:** crossover	**DO:** 6
USA (Xu *et al*., 1998; Duncan *et al*., 1999a; Merz-Demlow *et al*., 2000; Wangen *et al*., 2000; Phipps *et al*., 2001; Fritz *et al*., 2003)	**Analyzed:** 14	**Intervention:** ISP versus low isoflavone ISP	**Duration:** 13 weeks
	Mean age: 27	**Isoflav, mg/day:** highISO 128.7, medISO 64.7, Cont 10.0 (**AU**)	
		Soy protein difference: 0 g/day	
Duncan 1999 Post	**Participants:** post-men (com)	**Design:** crossover	**DO:** 4
USA (Duncan *et al*., 1999b; Xu *et al*., 2000; Phipps *et al*., 2001; Wangen *et al*., 2001)	**Analyzed:** 18	**Intervention:** ISP versus low isoflavone ISP	**Duration:** 13 weeks
	Mean age: 57	**Isoflav, mg/day:** int 132, cont 7.1 (**AU**)	
		Soy protein difference: 0 g/day	
Gann A 2005	**Participants:** pre-men (com)	**Design:** parallel	**DO:** unclear
USA (Gann *et al*., 2005)	**Analyzed:** int 43, cont 43	**Intervention:** ISP versus low isoflavone ISP	**Duration:** 3 cycles
	Mean age: int 34, cont 33	**Isoflav, mg/day:** int 84.4, cont unclear (**AU**)	
		Soy protein difference: 0 g/day	
Gann B 2005	**Participants:** pre-men (com)	**Design:** parallel	**DO:** unclear
USA (Gann *et al*., 2005)	**Analyzed:** int 38, cont 30	**Intervention:** ISP versus low isoflavone ISP	**Duration:** 3 cycles
	Mean age: int 34, cont 33	**Isoflav, mg/day:** int 84.4, cont unclear (**AU**)	
		Soy protein difference: 0 g/day	
Gardner 2001	**Participants:** HC post-men (com)	**Design:** parallel	**DO:** int 3, cont A 2, cont B 1
USA (Gardner *et al*., 2001)	**Analyzed:** int 31, cont A 30, cont B 33	**Intervention:** ISP versus A milk protein, B low isoflavone ISP	**Duration:** 12 weeks
	Mean age: int 63, cont A 58, cont B 58	**Isoflav, mg/day:** int 80, contA 2, contB 3 (**AU**)	
		Soy protein difference: 0 or 39 g/day	
Garrido 2006	**Participants:** post-men (com)	**Design:** parallel	**DO:** none
Chile (Garrido *et al*., 2006)	**Analyzed:** int 15, cont 14	**Intervention:** isoflav ext versus placebo	**Duration:** 12 weeks
	Mean age: int 54, cont 53	**Isoflav, mg/day:** int 100, cont unclear (**AU**)	
		Soy protein difference: 0 g/day	
Han 2002	**Participants:** post-men (com)	**Design:** parallel	**DO:** int 1, cont 1
Brazil (Han *et al*., 2002)	**Analyzed:** int 40, cont 40	**Intervention:** isoflav ext versus glucose	**Duration:** 16 weeks
	Mean age: int 48, cont 49	**Isoflav, mg/day:** int 100, cont nil (**AU**)	
		Soy protein difference: 0 g/day	
Harkness 2004	**Participants:** post-men (com)	**Design:** crossover	**DO:**1
USA (Harkness *et al*., 2004)	**Analyzed:** 19	**Intervention:** isoflav ext versus placebo	**Duration:** 26 weeks
	Mean age: 71	**Isoflav, mg/day:** int 110, cont unclear (AU - No)	
		Soy protein difference: 0 g/day	
Huang 2006	**Participants:** post-men (com)	**Design:** parallel	**DO:** 1 overall (unclear in which arm)
Taiwan (Huang *et al*., 2006)	**Analyzed:** int IF200 15, int IF100 15, cont 12	**Intervention:** isoflav ext versus nil	**Duration:** 52 weeks

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Study	Participants	Interventions	Outcomes
	Mean age: IF200 int 52, IF100 int 54, cont 51	**Isoflav, mg/day:** IF200 200, IF100 100, cont nil (AU?)	
		Soy protein difference: 0 g/day	
Jayagopal 2002	**Participants:** diabetic post-men (com)	**Design:** crossover	**DO:** 1
UK (Jayagopal *et al*., 2002)	**Analyzed:** 32	**Intervention:** ISP versus microcrystalline cellulose	**Duration:** 12 weeks
	Mean age: 63	**Isoflav, mg/day:** int 132, contl unclear (AU?)	
		Soy protein difference: 30 g/day	
Knight 2001	**Participants:** post-men (com)	**Design:** parallel	**DO:** int 3, cont 1
Australia (Knight *et al*., 2001)	**Analyzed:** int 9, cont 11	**Intervention:** ISP versus milk protein	**Duration:** 12 weeks
	Mean age: int 52, cont 54	**Isoflav, mg/day:** int 77.4, cont unclear (**AU**)	
		Soy protein difference: 55 g/day	
Kotsopoulos 2000 (PEARL)	**Participants:** post-men (com)	**Design:** parallel	**DO:** int 20?, cont 35?
Australia (Kotsopoulos *et al*., 2000; Teede *et al*., 2001, 2004, 2005; Dalais *et al*., 2003)	**Analyzed:** int 30, cont 20	**Intervention:** ISP versus milk protein	**Duration:** 12 weeks
	Mean age: int 60, cont 60	**Isoflav, mg/day:** int 118, cont unclear (AU?)	
		Soy protein difference: 40 g/day	
Kumar 2002	**Participants:** pre-men (com)	**Design:** parallel	**DO:** int 16, cont 15
USA (Kumar *et al*., 2002)	**Analyzed:** int 33, cont 33	**Intervention:** ISP versus milk protein	**Duration:** 3 cycles
	Mean age: int 41, cont 43	**Isoflav, mg/day:** int 40 genistein, cont 0 (AU?)	
		Soy protein difference: unclear	
Lichtenstein 2002	**Participants:** HC post-men (com, AFP)	**Design:** crossover	**DO:** 4 dropped out but were replaced
USA (Lichtenstein *et al*., 2002; Desroches *et al*., 2004; Wang *et al*., 2004; Goldin *et al*., 2005; Vega-Lopez *et al*., 2005)	**Analyzed:** 10 or 11	**Intervention:** isoflav ext versus nil, also ISP versus low isoflavone ISP	**Duration:** 6 weeks
	Mean age: 64	**Isoflav, mg/day:** isoflav ext int 114, cont nil, ISP int 102, cont 3 (**AU**)	
		Soy protein difference: g/day	
Mackey 2000	**Participants:** HC post-men (com?)	**Design:** parallel	**DO:** 5 in total (unclear in which arms)
Australia (Eden *et al*., 2000; Mackey *et al*., 2000)	**Analyzed:** int 25, cont 24	**Intervention:** ISP versus low isoflavone ISP	**Duration:** 12 weeks
	Mean age: int 56, cont 57	**Isoflav, mg/day:** int 65, cont <4 (AU?)	
		Soy protein difference: 0 g/day	
Martini OC 1999	**Participants:** pre-men (com)	**Design:** crossover	**DO:** 4
USA (Rutman *et al*., 1997; Martini *et al*., 1999)	**Analyzed:** 16	**Intervention:** ISP versus milk protein	**Duration:** 2 cycles
	Mean age: 30	**Isoflav, mg/day:** int 38, cont unclear (AU?)	
		Soy protein difference: 20 g/day	
Maskarinec 2002	**Participants:** pre-men (com)	**Design:** parallel	**DO:** 5 (unclear in which arms)
USA (Maskarinec *et al*., 2002a, b, 2003)	**Analyzed:** int 13, cont 16	**Intervention:** isoflav ext versus maltodextrin	**Duration:** 52 weeks
	Mean age: int 42, cont 43	**Isoflav, mg/day:** int 76, cont 0 (**AU**)	
		Soy protein difference: 0 g/day	

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Study	Participants	Interventions	Outcomes
Maskarinec 2004	**Participants:** pre-men (com)	**Design:** parallel	**DO:** 17 int, 14 cont
USA (Maskarinec *et al*., 2004, 2005)	**Analyzed:** int 92, cont 97	**Intervention:** soy foods versus usual diet	**Duration:** 104 weeks
	Mean age: int 43, cont 43	**Isoflav, mg/day:** int 57.1, cont 7.2 (**AU**)	
		Soy protein difference: 10–44 g/day	
Murkies 1995	**Participants:** post-men (com)	**Design:** parallel	**DO:** int 5, cont 6
Australia (Murkies *et al*., 1995)	**Analyzed:** int 24, cont 23	**Intervention:** soy foods versus non-protein cont	**Duration:** 12 weeks
	Mean age: int 54, cont 56	**Isoflav, mg/day:** unclear (AU?)	
		Soy protein difference: 18 g/day	
Nagata 1998	**Participants:** pre-men, (com)	**Design:** parallel	**DO:** 0
Japan (Nagata *et al*., 1998; Takatsuka *et al*., 2000)	**Analyzed:** int 31, cont 29	**Intervention:** soy foods versus usual diet	**Duration:** 8 weeks
	Mean age: int 26, cont 27	**Isoflav, mg/day:** int 71, cont unclear (AU—No)	
		Soy protein difference: 12 g/day	
Nahas 2004	**Participants:** post-men (com)	**Design:** parallel	**DO:** none
Brazil (Nahas *et al*., 2004)	**Analyzed:** int 25, cont 25	**Intervention:** soy foods versus placebo	**Duration:** 26 weeks
	Mean age: int 54, cont 53	**Isoflav, mg/day:** int 60, cont unclear (AU?)	
		Soy protein difference: 1 g/day	
Nettleton 2004	**Participants:** post-men (com)	**Design:** crossover	**DO:** 13
USA (Greany *et al*., 2004, 2008; Nettleton *et al*., 2004, 2005a, b)	**Analyzed:** 20 HO br cancer, 20 without	**Intervention:** ISP versus milk protein	**Duration:** 6 weeks
	Mean age: hO br cancer 60, without 56	**Isoflav, mg/day:** int 44.4, cont unclear (**AU**)	
		Soy protein difference: 27 g/day	
Nikander 2003	**Participants:** post-men HO br cancer (com)	**Design:** crossover	**DO:** 6
Finland (Nikander *et al*., 2003a, b, 2004a, b, 2005; Tormala *et al*., 2006)	**Analyzed:** 56	**Intervention:** isoflav ext versus placebo	**Duration:** 12 weeks
	Mean age: 54	**Isoflav, mg/day:** int 114, cont unclear (**AU**)	
		Soy protein difference: 0 g/day	
Scambia 2000	**Participants:** post-men (com)	**Design:** parallel	**DO:** unclear
Italy (Scambia *et al*., 2000)	**Analyzed:** int 20, cont 19	**Intervention:** isoflav versus placebo	**Duration:** 6 weeks
	Mean age: int 54, cont 53	**Isoflav, mg/day:** int 50, cont unclear (AU?)	
		Soy protein difference: 0 g/day	
Spence 2005	**Participants:** post-men (com, AFP)	**Design:** crossover	**DO:** 0
USA (Spence *et al*., 2005)	**Analyzed:** 15	**Intervention:** ISP versus low isoflavone ISP	**Duration:** 4 wks
	Mean age: 58	**Isoflav, mg/day:** int 65, cont 3.1 (**AU**)	
		Soy protein difference: 0 g/day versus ISP- or 40 g/day versus milk protein	
Squadrito 2002	**Participants:** post-men (com)	**Design:** parallel	**DO:** 3 int, 4 cont
Italy (Squadrito *et al*., 2002, 2003; Crisafulli *et al*., 2005; D'Anna *et al*., 2007)	**Analyzed:** int 27, cont 26	**Intervention:** isoflav ext (genistein) versus placebo	**Duration:** 52 weeks
	M**ean age:** int 56, cont 57	**Isoflav, mg/day:** int 54, cont unclear (AU?)	
		Soy protein difference: 0 g/day	
Uesugi 2003	**Participants:** post-men (com)	**Design:** parallel	**DO:** int 0, cont 1
Japan (Uesugi *et al*., 2003)	**Analyzed:** int 11, cont 10	**Intervention:** isoflav ext versus dextrin	**Duration:** 12 weeks

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Study	Participants	Interventions	Outcomes
	Mean age: int 55, cont 53	**Isoflav, mg/day:** int 61.8, cont unclear (AU—No)	
		Soy protein difference: 0 g/day	
Uesugi 2004	**Participants:** peri- and post-men (com)	**Design:** crossover	**DO:** unclear
Japan (Uesugi *et al*., 2004)	**Analyzed:** 58	**Intervention:** isoflav ext versus placebo	**Duration:** 4 wks
	Mean age: 58	**Isoflav, mg/day:** int 42, cont unclear (**AU**)	
		Soy protein difference: 0 g/day	
Upmalis 2000	**Participants:** post-men, (com)	**Design:** parallel	**DO:** 31 int, 24 cont
USA (Upmalis *et al*., 1999, 2000)	**Analyzed:** int 59, cont 63	**Intervention:** isoflav versus placebo	**Duration:** 12 weeks
	Mean age: int 55, cont 54	**Isoflav, mg/day:** int 50, cont unclear (AU?)	
		Soy protein difference: 0 g/day	
Woods 2000	**Participants:** post-men (com)	**Design:** crossover	**DO:** unclear
USA (Woods *et al*., 2000)	**Analyzed:** 85	**Intervention:** isoflav ext versus placebo	**Duration:** 12 weeks
	Mean age: unclear	**Isoflav, mg/day:** int 45, cont unclear (AU?)	
		Soy protein difference: unclear	
Wu 2005	**Participants:** post-men, (com, AFP)	**Design:** parallel	**DO:** 6 (unclear from which arms)
USA (Wu *et al*., 2005)	**Analyzed:** int 17, cont 20	**Intervention:** soy food versus nil	**Duration:** 8 weeks
	Mean age: int 57, cont 60	**Isoflav, mg/day:** int 51, cont unclear (**AU**)	
		Soy protein difference: 15 g/day	
Wu 2006 A and B	**Participants:** post-men (com)	**Design:** parallel	**DO:** A int 8, cont 4, B int 1, cont 7
Japan (Wu *et al*., 2006a, b)	**Analyzed:** A: int 33, cont 33; B: int 31, cont 31	**Intervention:** isoflav ext versus dextrin	**Duration:** 52 weeks
	Mean age: A: int 54, cont 55, B: int 54, cont 55	**Isoflav, mg/day:** int 75, cont unclear (**AU**)	
		Soy protein difference: 0 g/day	
Zitterman 2004	**Participants:** pre-men (com)	**Design:** crossover	**DO:** 3
Germany (Zittermann *et al*., 2004)	**Analyzed:** 14	**Intervention:** soy foods versus nil	**Duration:** 4 weeks
	Mean age: 24	**Isoflav, mg/day:** int 52, cont <0.1 (**AU**)	
		Soy protein difference: 22 g/day	

ISP, isolated soy protein; AU, aglycone units; Cont, control group; Int, intervention group; Com, community; Isoflav, isoflavone/s; FP, food provided; DO, dropouts; HC, hypercholesterolaemic; Br cancer, breast cancer; HO, history of; AFP, all food provided.