Neither soy nor isoflavone intake affects male reproductive hormones: An expanded and updated meta-analysis of clinical studies

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ARTICLE INFO

Keywords:
Soy
Isoflavones
Testosterone
Estrogen
Phytoestrogens

ABSTRACT

Concerns that the phytoestrogens (isoflavones) in soy may feminize men continue to be raised. Several studies and case-reports describing feminizing effects including lowering testosterone levels and raising estrogen levels in men have been published. For this reason, the clinical data were meta-analyzed to determine whether soy or isoflavone intake affects total testosterone (TT), free testosterone (FT), estradiol (E2), and sex hormone binding globulin (SHBG). PubMed and CAB Abstracts databases were searched between 2010 and April 2020, with use of controlled vocabulary specific to the databases. Peer-reviewed studies published in English were selected if (1) adult men consumed soyfoods, soy protein, or isoflavone extracts (from soy or red clover) and (2) circulating TT, FT, SHBG, E2 or E1 was assessed. Data were extracted by two independent reviewers. With one exception, studies included in a 2010 meta-analysis were included in the current analysis. A total of 41 studies were included in the analyses. TT and FT levels were measured in 1753 and 752 men, respectively; E2 and E1 levels were measured in 1000 and 239 men, respectively and SHBG was measured in 967 men. Regardless of the statistical model, no significant effects of soy protein or isoflavone intake on any of the outcomes measured were found. Sub-analysis of the data according to isoflavone dose and study duration also showed no effect. This updated and expanded meta-analysis indicates that regardless of dose and study duration, neither soy protein nor isoflavone exposure affects TT, FT, E2 or E1 levels in men.

1. Introduction

For centuries foods made from soybeans, such as tofu and miso, have played an important role in the diets of many Asian countries [1,2]. Much more recently, soyfoods have become popular in many non-Asian countries because of their purported nutritional and health benefits and the increased interest in plant-based diets and plant protein [3–7]. In addition to the traditional Asian soyfoods, soy protein can be incorporated into the diet via supplementation with and/or by consuming foods containing soy protein ingredients, namely soy flour, soy protein concentrate (SPC) and soy protein isolate (SPI). On a moisture free basis these products are approximately 50, 65 and 90 % protein, respectively [8].

Much of the soy-related health research published over the past 3 decades has taken place because among commonly consumed foods, the soybean is a uniquely rich source of isoflavones [9,10]. Mean isoflavone intake in Japan among older adults ranges from approximately 30–50 mg/d [11,12] whereas per capita isoflavone intake in the United States [13] and Europe [14] is <3 mg/d. The three isoflavones, genistein, daidzein and glycitein and their respective glycosides, comprise approximately 50, 40 and 10 % of the total soybean isoflavones content, respectively [15]. Each gram of soy protein in traditional soyfoods is associated with approximately 3.5 mg isoflavones (expressed as the aglycone equivalent weight) [11]. In contrast, much of the isoflavone content is lost in the production of SPI and SPC, although the degree of loss depends upon the method of manufacture [15,16]. Isoflavone values in this manuscript refer to the aglycone equivalent weight.

Isoflavones have a chemical structure similar to the hormone...
estrogen which allows them to bind to both estrogen receptors (ER) – ERα and ERβ [17,18], and to exert estrogen-like effects under certain experimental conditions. For this reason, they are commonly classified as phytoestrogens. Circulating levels of isoflavones in response to the ingestion of approximately two servings of traditional soyfoods are three orders of magnitude higher than estrogen [19]. However, isoflavones differ from estrogen at the molecular level in that they preferentially bind to and activate ERβ in comparison to ERα whereas estrogen has equal affinity for both receptors [20–23]. This difference in binding preference is important because the two ERs have different tissue distributions and, when activated, can exert different and sometimes opposite physiological effects [24,25]. The preference of isoflavones for ERβ is the primary reason that isoflavones are seen as capable of having tissue-selective effects and the reason they are often classified as selective estrogen receptor modulators (SERMs) [26–30].

Isoflavones have been rigorously investigated over the past 30 years for a number of potential health benefits in both men and women [31–37]. However, isoflavones are not without controversy as there is concern that isoflavones feminize men. This concern, which coincided with the rising apprehension that environmental estrogens play a role in the declining sperm count occurring among men worldwide [38–40], has some support from animal studies [41,42].

Some clinical studies have also reported decreases in testosterone levels in response to soy consumption [43,44]. In addition, one case-report described a 60-year-old male who developed gynecomastia allegedly as a result of his soy intake [45] and a small case control study found that soy intake was associated with lower sperm concentration among male partners in subfertile couples who presented for semen analyses to the Massachusetts General Hospital Fertility Center [46].

As a result of feminization concerns, in 2010, three of us co-authored a meta-analysis of clinical studies that examined the effects of isoflavone exposure via supplements and soyfoods on circulating levels of total TT, FT and SHBG [47]. This analysis found no statistically significant effects of soy protein or isoflavone intake on any of the outcomes assessed. That same year also saw the publication of a narrative review which found soy/isoflavones had no effect on estrogen levels in men or other end points related to feminization [48]. Nevertheless, reports of soy exerting estrogenic or feminizing effects subsequent to the 2010 meta-analysis [47] and narrative review [48] have been published. For example, a case-report by Siepman et al. [49] described a 19-year-old vegan who developed hypogonadism and erectile dysfunction allegedly as a result of his soy consumption. It is notable that the man described in this case-report and in the previously cited one [45], consumed an estimated 360 mg/d isoflavones, which is approximately 9 times the typical intake of older native Japanese men [11]. Also, in young resistance-trained men supplementation with soy protein resulted in lower testosterone levels shortly after exercise performance in comparison to whey protein and carbohydrate supplementation [50]. Observational studies have also reported associations between isoflavone exposure and decreased sperm concentration and/or poor semen quality [51–53].

Given the conflicting reports and the number of relevant studies published within the past decade, we have updated and expanded the 2010 meta-analysis [47]. In addition to including levels of SHBG, TT and FT, levels of E2 and E1 were also meta-analyzed since no previous statistical analysis of the effect of soy on these latter two hormones has been published.

2. Materials and methods

2.1. Study identification

Intervention trials were identified on PubMed (National Library of Medicine, Bethesda, MD), with the search dates of 2010 to April 10, 2020. The keywords used were soybeans, soy, soyfoods, soy foods, isoflavones, genistein, daidzein, phytoestrogens, red clover, androgen, estradiol, estrogen, estrone, hormones, testosterone, and sex hormone-binding globulin. Peer-reviewed studies published in English were selected based upon two criteria: 1) if adult men consumed soyfoods, soy protein isolate (SPI), soy protein concentrate (SPC) or isoflavone extracts (from soy or red clover) and 2) if studies assessed circulating TT, FT, E2, E1 or SHBG. Two independent reviewers extracted data. Isoflavone exposure was extracted directly from studies. With one exception, studies published before 2010 included in a previously published meta-analysis were included in the current analysis [47]. Clinical trials (parallel or crossover) and single-group studies were included. Data from single-group studies were analyzed separately from two-group comparisons in the manner described below. A total of 141 articles were examined. Reports that did not match the selection criteria were excluded (n = 101) from the analysis.

2.2. Data analysis

Data were analyzed using Review Manage (RevMan) version 5.3 (Copenhagen: The Nordic Cochrane Centre, Cochrane Collaboration). Data were extracted or calculated in accordance with the Cochrane Collaboration. Missing standard deviations (SD) were generated using available data from the study (standard error (SE) or confidence interval (CI)) or imputed using evidence from similar studies. SD of change (when not given) was calculated using baseline and final SD as suggested by Cochrane. Two analytical comparisons were made: 1) effect sizes (standardized mean difference, SMD) were calculated by comparing the change between baseline and end values in active treatment arms with the change between baseline and end values in the control arm, of all parallel (controlled) and crossover trials (analysis A); and 2) effect size was calculated for the difference between baseline and end values in the treatment arm only of parallel, crossover and single-group studies (analysis B).

The SMD was calculated for both comparisons, for the five outcomes (hormones of interest) measured (TT, TF, SHBG, E2 and E1), 10 such models were calculated in total. A random effects model was used to calculate the SMD difference and the 95% CI, to account for differences in measurement units and techniques.

The data were also analyzed using the statistical models A and B described above to determine whether isoflavone exposure duration (<12 weeks vs >12 weeks) or dose (<75 mg/d vs ≥75 mg/d) affected outcomes. Heterogeneity among studies was assessed using I² and broad cutoff points of <40 %, 40%–60%, 61%–90% and 100 % were used to establish the importance of heterogeneity (non-important to considerable). Finally, funnel plots were used to assess publication bias, and the effect of other influential studies on model change was examined by removing studies one at a time.

3. Results

Based on the established criteria a total of 41 studies were included in the analyses [37,43,44,54–91]. Of the 41 studies, 20 utilized a parallel design (controlled), eight a crossover design and 15 single arm or parallel arms designs. Two studies identified by the literature search were excluded from the analysis because of their short duration as one measured TT and SHBG over a 60-minute period following a bout of resistance exercise [50], and one measured E2, TT and FT after one week exposure [92]. In the latter study, which did not find a significant effect on hormone levels, it was not possible to determine the soy protein or isoflavone dose based on the description of the intervention product (900 g soybeans) [92]. In addition, a study by Lephart et al. [93] was excluded from analysis because the intervention supplement was comprised of equal, which is a bacterially-derived metabolite of daidzein that is not found in soybeans. Also, a study by Grainger et al. [94] was not included in the current analysis despite being included in the 2010 meta-analysis [47] because the original data, which was not published in the paper, is no longer accessible.
Some studies included soy groups that were excluded from the analyses due to the addition of other potentially bioactive ingredients to the test product. This included soy breed with linsen [54], soymilk fortified with stanols [95] and a mixture of soy and whey [60]. Selected details of studies included in the meta-analysis are shown in Table 1. TT and FT levels were measured in 1753 and 752 men, respectively; E2 and E1 levels were measured in 1000 and 239 men, respectively and SHBG was measured in 967 men. The youngest men were aged 18 years [55, 84] and the oldest participants were aged 81 years [77]. Several studies included more than one experimental arm, for example a SPC arm and a SPI arm, thus the total number of groups included in analyses exceeded the total number of studies. Some studies involved multiple quantities of soy, or soy in different forms. For example, both Swart et al. [70] and Hamilton-Reeves et al. [57] included a SPI with added isoflavones group and a SPI alone group. Kalman et al. [66] included a SPI group and a SPC group and the study by Kumar et al. [63] included 3 groups who consumed supplements providing different amounts of isoflavones. Most studies that did not intervene with supplements used SPI or SPC, several studies used other forms including red clover [68, 70], soy milk/yogurt [66, 76], tofu [75] or soybeans [93].

3.1. Effect of soy and isoflavone exposure on circulating reproductive hormone concentrations

As shown in Table 2, there were no significant effects of soy or isoflavone exposure on any of the hormones considered regardless of whether the data were analyzed using statistical approach A) comparison of change in the treatment versus the control arms of parallel/controlled and crossover trials or B) change over time in all active arms.

Subanalysis revealed that neither study duration (<12 weeks vs >12 weeks) (Table 3) nor dose (<75 mg/d vs >75 mg/d) (Table 4) affected the impact of isoflavone exposure on hormone concentrations although in the case of the statistical model A (change in treatment arm compared with change in control arm) in several cases there were insufficient studies (N < 3) to conduct an analysis.

3.2. Publication bias and over-influential studies

No publication bias was noted in the funnel plots (data not shown). There were no over-influential studies in either analysis method for TT, FT or SHBG when studies were removed one at a time and the models then re-estimated. In analysis A (change in treatment versus change in control) for E2, removal of the 2010 study by Kumar et al. [63] had the largest influence, although the model still remained non-significant (p = 0.06). In the study by Haun et al. [59], the control group experienced a drop in E2 that was more than double the drop in the intervention arm, but removal of this study had little to no influence on the effect size. In fact, in the 13 studies that measured E2, there was a drop in the control group in all but two studies [63, 70]. However, as can be seen from analysis B (Table 2), there is little actual change over time in the active arms (p = 0.43).

In the E1 analysis, removal of the study by Nagata et al. [66] from analysis B had the largest influence, resulting in a relatively large change in the SMD as it changed from 0.40 [-0.27, 0.07] to 0.62 [-0.03, 1.27], although the effect remained non-significant (p = 0.06). Given the small number of groups in this analysis, removal of single studies can have a large influence. As was the case for E2, there is little actual change over time in the active arms (model B).

4. Discussion

The results of this meta-analysis confirm the findings of a meta-analysis published in 2010 that found neither soy nor isoflavone intake affects total or bioavailable circulating testosterone concentrations in men [47]. Given that the current analysis includes 41 studies (TT) and 1753 men (versus 31 studies and 939 men in the 2010 analysis [47]), it is unlikely that additional research will alter this conclusion, especially when considering the low heterogeneity (model B, change over time; I², 30 %) among studies. The lack of effect on TT and FT held when the data were sub-analyzed according to study duration (<12 weeks vs >12 weeks) and isoflavone dose (<75 mg/d vs >75 mg/d).

Evidence indicates that testosterone levels can change very quickly so it is unlikely that longer studies would produce different results. For example, in healthy male volunteers, testosterone levels began to decrease from baseline values after 72 h of ethanol ingestion and reached levels similar to those of alcoholic men after 30 days [96]. Importantly, none of the four longer-term studies (>12 months) in this analysis found a statistically significant effect on testosterone levels [64, 65, 67, 72].

Regarding dose, mean isoflavone intake of older native Japanese men ranges from about 30–50 mg/d [11, 12, 97]. Relatively few Asians (<10 %) consume more than 75 mg/d, which is the amount provided by approximately three servings of traditional soyfoods. A serving being one cup (240 mL) of soymilk, ½ cup (~85 g) of tofu or one ounce (28 g) of soy nuts. Thus, by Asian standards, the cutoff of 75 mg/d would cover a high intake of soy foods. Whether greater isoflavone exposure than can reasonably be achieved via the consumption of traditional soyfoods impacts testosterone levels is difficult to assess, but the existing evidence suggesting that it does is unimpressive.

In eight studies included in this analysis men consumed >100 mg/d isoflavones [44, 54, 74, 79, 80, 85, 88]. Of these, Gardner-Thorpe et al. [44] reported an approximate 5% decrease in TT whereas Pendleton et al. [85] reported an approximate 6% decrease in FT, but no effect on TT. In the former study, the decrease in TT was in comparison to baseline values as data for the control group were not reported. van Veldhuizen et al. [88] reported a change in TT from 5.004 ng/mL at baseline to 3.175 ng/mL (no statistics reported) among 11 prostate cancer patients who consumed between 112 and 224 mg/d isoflavones. This study did not include a control group. In contrast to these three studies, no effects on TT and/or FT were noted by several other investigators [54, 80, 89–91]. Finally, among nine men with histologically proven prostate cancer whose prostate specific antigen (PSA) levels decreased in response to 900 mg/d isoflavones, de Vere White et al. [79] reported that one patient had a reduced TT level at three months but five others had increased levels at 6 months.

We did not examine the effects of isoflavone exposure on circulating levels of dihydrotestosterone (DHT), which is the 5α-reduced metabolite of testosterone that is principally converted from its parent hormone in target organs such as prostate, skin, and liver [98]. The reason is that as noted by Swerdlow et al. [98] and as first concluded by Horton [99], blood levels of DHT “provide only a hint of tissue levels as DHT should be regarded as a paracrine hormone formed and acting primarily within target tissues.” The impact of isoflavone exposure on DHT has been studied to a much lesser degree than has testosterone, but the evidence indicates that like testosterone, there is no effect on this testosterone metabolite [44, 54, 57, 68, 74, 75, 89, 90].

Three studies did find changes in DHT in response to isoflavone intake [73, 77, 82], two of which found decreases and one of which, that intervened with isoflavones derived from red clover, found an increase [82]. In addition, a small study by Tanaka et al. [87] found isoflavones decreased DHT levels (also free testosterone levels) in equol-, but not equol-producers. Approximately 25 % of Westerners and 50 % of Asians host the intestinal bacteria that convert the isoflavone daidzein into equol - a conversion that some speculate will benefit individuals consuming isoflavones [100]. The finding by Tanaka et al. [87] is interesting because equal is able to specifically bind to 5α-DHT (to decrease negative androgen impact in the prostate) by sequestering 5α-DHT from the androgen receptor, thus altering growth and physiological hormone responses regulated by 5α-DHT [101, 102]. However, in a small pilot study by LePhart et al. [93], no effect of equol supplementation (12 mg/d) was found on serum DHT levels in 18 men with benign prostatic hyperplasia (BPH) although this study did find some
### Table 1
Description of the studies included in the meta-analysis.

| Location/Year | Mean or median age (y) | Intervention | N | Health outcome | Protein (g/d) | Isoflavones (mg/d) | Duration | Outcome measured |
|---------------|------------------------|--------------|---|----------------|---------------|-------------------|----------|------------------|
| **Parallel Studies** | | | | | | | | |
| Dalai/2004/Australia | 62 ± 5 | Soy grits bread Wheat bread | 8 | Prostate cancer | 18 | 117 | 25 d | T, SHBG |
| Dalai/2004/Australia | 61 ± 5 | Whey protein | 8 | Healthy | 42 | 98 | 4 wk | T |
| Disilvestro/2005/USA | 38-30 | SPI | 10 | Healthy | 42 | | | |
| Deibert/2011/Germany | 56 | SPI | 13 | Healthy | 117 | | | |
| Hamilton-Reeves/2007/USA | 68 ± 8 | Milk protein isolate | 20 | Cancer | 40 | | | |
| Hamilton-Reeves/2013/USA | 62 ± 12 | Capsules | 42 | Prostate cancer | 0 | 51 | 2–6 wk | T, FT, E2 |
| Haan/2018/USA | 21 ± 2 | Carbohydrate | 12 | Prostate cancer | 0 | 36 | 4 wk | FT, SHBG, E2 |
| Kalman/2007/USA | 30 | SPI | 5 | Prostate cancer | 30 | | | |
| Kumar/2004/USA | 71 ± 5 | SPI | 29 | Prostate cancer | 29 | | | |
| Kumar/2007/USA | 72 ± 6 | Capsules | 22 | Prostate cancer | 0 | 48 | 12 wk | T, FT, SHBG, E2 |
| Kumar/2010/USA | 60 ± 7 | Capsules | 12 | Prostate cancer | 0 | 36 | 4 wk | FT, SHBG, E2 |
| Li/2008/USA | 60 ± 1 | SPI | 5 | Prostate cancer | 30 | | | |
| Miyama/2012/Japan | 63 ± 2 | SPI | 5 | Healthy | 30 | | | |
| Nagata/2001/Japan | 32 ± 8 | SPI | 26 | Prostate cancer | 40 | | | |
| Yamashita/2005/USA | 65 ± 7 | SPI | 17 | Healthy | 13.40 | | | |
| Whittey/2005/USA | 67 ± 8 | SPI | 17 | Healthy | 48 | | | |
| Rammikko/2006/Finland | 64 ± 3 | SPI | 35 | Prostate cancer | 0 | 0 | 3–6 wk | TT, FT, E2, SHBG |
| Reidy/2016/USA | 24 ± 1 | SPI | 22 | Prostate cancer | 60 | | | |
| Swart/2019/England | 52 | SPI | 85 | Prostate cancer | 0 | 10 | 12 mo | T |
| Teede/2001/Australia | 50–75 | SPI | 48 | Rising prostate specific antigen | 0 | 0 | 12 mo | T, FT, SHBG, E2 |
| Wong/2012/Hong Kong | 65 ± 9 | SPI | 22 | Prostate cancer | 0 | 0 | 12 mo | T |
| **Cross-over Studies** | | | | | | | | |
| Dillingham/2005/USA | 28 ± 6 | SPI | 35 | Healthy | 32 | 2 | 57 d | T, FT, SHBG, E1, E2 |
| Gardner-Thorpe/2003/UK | 35 ± 11 | SPI | 35 | Healthy | 32 | | | |
| Goldin/2005/USA | 61 | SPI | 18 | Moderately hypercholesterolemic | 0 | | | |
| Hibble/2000/Japan | 46 ± 8 | SPI | 18 | Healthy | 71 | 139 | 6 wk | T, E1, E2 |
| Hiigashi/2001/Japan | 31 ± 4 | SPI | 18 | Healthy | 710 | 9 | 6 wk | T, E1, E2 |
| Krane/2005/Netherlands | 54–81 | SPI | 18 | Healthy | 0 | | | |
| Mavrikaki/2006/USA | 58.7 ± 7.2 | SPI | 18 | Healthy | 0 | | | |
| Schroder/2005/Netherlands | 70 ± 7 | SPI | 18 | Healthy | 0 | | | |
| **Single or dual arm studies (no control)** | | | | | | | | |
| 73.6 | | Capsules | 52 | Prostate cancer | <1 | 900 | 6 mo | T |

(continued on next page)
evidence that BPH symptoms were alleviated. Also, in contrast to the finding by Tanaka et al. [87], in a case-control study involving Japanese men with rising PSA levels, Miyanaga et al. [65] found that DHT levels did not differ between non-equol producers and equol producers. They would almost certainly be underpowered to detect a difference given the low prevalence of producers among non-Asian men. In addition to equol, there were insufficient data to evaluate the effects of isoflavone exposure on androgen receptor (AR) expression. Of note in addition to equol, there were insufficient data to evaluate the effects of isoflavone exposure on androgen receptor (AR) expression. Of note in this regard, Hamilton-Reeves et al. [57] found that AR expression in the

| Author/year/Location/Ref | Mean or median age (y) ± SD or range | Intervention | N | Health status | Protein (g/d) | Isoflavone (mg/d) (aglycone weight) | Duration | Outcome measured |
|--------------------------|-------------------------------------|--------------|---|---------------|--------------|-----------------------------------|---------|-----------------|
| DeVere White/2004/USA/(79) | 32.25 | SPI | 12 | Healthy | 56 | NI | 12 wk | T |
| Goodin/2007/USA/(43) | 68.9 ± 7.3 | Tablets | 20 | Prostate cancer | 0 | 300–600 | 84 d | T, FT |
| Fischer/2004/USA/(90) | 73 (55–82) | Tablets | 39 | Prostate cancer | 0 | 200 | 5.5 mo | T |
| Hussain/2003/USA/(91) | 60 ± 7 | Red clover | 20 | Prostate cancer | 0 | 160 | 20 d | T |
| Jarred/2002/Australia/(90) | 78 | Soymilk | 29 | Prostate cancer | 12 | 65–90 | 6 mo | T |
| Kwan/2010/Canada/(81) | 40–53 | Tablets, red clover | 6 | Healthy | 0 | 40 | 3 wk | T |
| Mackey/2000/Australia/(83) | 51.8 | SPI | 27 | Healthy | 28 | 65 | 12 wk | T, SHBG |
| Mitchell/2001/Scotland/(84) | 18–35 | Tablets, red clover | 11 | Healthy | 0 | 40 | 2 mo | T, E2 |
| Pendleton/2008/USA/(85) | 73 | Soymilk | 12 | Prostate cancer | NI | 141 | 12 mo | T |
| Spentzos/2003/USA/(86) | 71 | SPI | 18 | Prostate cancer | 34 | 68 | 2 mo | T, FT, E2 |
| Tanaka/2009/Japan/(87) | 30–59 | Tablets | 28 | Healthy | <2 | 36 | 3 mo | T, FT, SHBG, E2 |
| Van Veldhuizen/2006/USA/(88) | 63 | Tablets | 11 | Prostate cancer | 0 | 112–224 | 4 wk | T |

* Converted to aglycone value.

### Table 2

| Outcome/statistical model | No of groups (subjects) | Effect size SMD (95 % CI) | P value for overall effect | P² % |
|---------------------------|-------------------------|---------------------------|---------------------------|------|
| **Total testosterone**    |                         |                           |                           |      |
| Treatment vs Control      | 20 (1241)               | −0.06 [-0.29, 0.17]       | 0.59                      | 72   |
| Change over time (active) | 42 (1101)               | 0.09 [-0.02, 0.20]        | 0.12                      | 29   |
| Free (unbound) testosterone | 15 (724)               | 0.01 [-0.33, 0.32]        | 0.98                      | 76   |
| Change over time (active) | 18 (474)                | −0.06 [-0.24, 0.13]       | 0.54                      | 0    |
| SHBG                     | 18 (856)                | −0.03 [-0.45, 0.38]       | 0.88                      | 87   |
| Change over time (active) | 25 (662)                | −0.02 [-0.17, 0.14]       | 0.84                      | 0    |
| Estradiol                | 16 (835)                | 0.18 [-0.04, 0.41]        | 0.12                      | 56   |
| Change over time (active) | 25 (622)                | −0.06 [-0.09, 0.22]       | 0.43                      | 0    |
| Estrone                  | 6 (220)                 | 0.40 [-0.27, 1.07]        | 0.24                      | 83   |
| Change over time (active) | 8 (184)                 | 0.18 [-0.12, 0.47]        | 0.24                      | 0    |

* Model A: Treatment vs control is an analysis of the change (in the treatment arms vs the change in the control arms); model B: the change over time in the all active arms. SMD is standardized mean difference.

### Table 3

| Outcome¹ | Statistical model | N | Protein (g/d) | Isoflavone (mg/d) (aglycone weight) | Duration | Outcome measured |
|----------|-------------------|---|---------------|-----------------------------------|---------|-----------------|
| Testosterone | A | N = 15 (372; 372) | SMD - 0.11 | [0.43; 0.20] | Z = 0.69 | 12 weeks Studies (n, active arm; n, control arm) | >12 weeks |
| |   | N = 5 (255; 242) | SMD - 0.01 | [0.28; 0.30] | Z = 0.96 | 12 weeks Studies (n, active arm; n, control arm) | >12 weeks |
| SHBG | A | N = 6 (32; 546) | SMD 0.00 | [0.51; 0.51] | Z = 0.62 | 12 weeks Studies (n, active arm; n, control arm) | >12 weeks |
| |   | N = 3 (124; 103) | SMD 0.21 | [0.97; 0.55] | Z = 1.00 | 12 weeks Studies (n, active arm; n, control arm) | >12 weeks |
| | B | N = 14 (304; 342) | SMD 0.45 | [0.74; 0.74] | Z = 0.74 | 12 weeks Studies (n, active arm; n, control arm) | >12 weeks |
| Estradiol | A | N = 1 (530; 530) | SMD 0.21 | [0.40; 0.48] | Z = 0.13 | 12 weeks Studies (n, active arm; n, control arm) | >12 weeks |
| |   | N = 3 (142; 142) | SMD 0.01 | [0.29; 0.29] | Z = 0.54 | 12 weeks Studies (n, active arm; n, control arm) | >12 weeks |
| Estrone | B | N = 6 (142; 142) | SMD 0.15 | [0.18; 0.18] | Z = 0.89 | 12 weeks Studies (n, active arm; n, control arm) | >12 weeks |

* Model A: Treatment vs control is an analysis of the change (in the treatment arms vs the change in the control arms); model B: the change over time in the all active arms. SMD is standardized mean difference.
Reproductive Toxicology 100 (2021) 60–67

Finally, while the results of this meta-analysis are based on a large dataset it is important to acknowledge, as noted in the methods section, that it was necessary to make a number of assumptions when full data for the individual studies were not available. In addition, many of the trials did not indicate whether the isoflavone intervention dose was expressed in aglycone equivalent or glycoside weight. We attempted to ascertain the aglycone equivalent dose based on general knowledge of the intervention product, but uncertainty still existed in many cases.

In conclusion, extensive clinical data published over the past two decades shows that in men neither soy nor isoflavone intake, even when exposure occurs for an extended period of time and exceeds typical Japanese intake, affects levels of total testosterone, free testosterone, estradiol or estrone.

Funding
This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of Competing Interest
The authors declare no conflict of interest.

Acknowledgements
There is no funding source. However, Mark Messina has a conflict of interest. Dr. Messina regularly consults for companies that manufacture and/or sell soy products. Mindy Kurzer is on the scientific advisory board of the Soy Nutrition Institute.

References
[1] F.-J. He, J.-Q. Chen, Consumption of soybean, soy foods, soy isoflavones and breast cancer incidence: differences between Chinese women and women in Western countries and possible mechanisms, Food Sci. Hum. Wellness 2 (2013) 146–161.
[2] T. Hymowitz, W. Shurtleff, Debunking soybean myths and legends in the historical and popular literature, Crop Sci. 45 (2005) 473–476.
[3] M. Fabich, M. Materka, Biological effect of soy isoflavones in the prevention of civilization diseases, Nutrients 11 (2019).
[4] M. Messina, Soy and health update: evaluation of the clinical and epidemiologic literature, Nutrients 8 (2016).
[5] M.J. Messina, Legumes and soybeans: overview of their nutritional profiles and health effects, Am. J. Clin. Nutr. 70 (1999) 439S–450S.
[6] S.A. Bawa, Functional and edible uses of soy protein products, Compr. Rev. Food Sci. Food Saf. 7 (2008) 14–28.
[7] U. Thompson, B.A. Boucher, Z. Liu, M. Cotterchio, N. Kreiger, Phytoestrogen content of foods consumed in Canada, including isoflavones, lignans, and coumestans, Nutr. Cancer 54 (2006) 184–201.
[8] A.A. Franke, L.J. Custer, W. Wang, C.Y. Shi, HPLC analysis of isoflavonoids and other phenolic agents from foods and human fluids, Proc. Soc. Exp. Biol. Med. 217 (1998) 263–273.
[9] M. Messina, C. Nagata, A.H. Wu, Estimated Asian adult soy protein and isoflavone intakes, Nutr. Cancer 55 (2006) 1–12.
[10] K. Konishi, K. Wada, M. Yamakawa, Y. Goto, F. Mizuta, S. Koda, et al., Dietary soy intake is inversely associated with risk of type 2 diabetes in Japanese women but not in men, J. Nutr. 149 (2019) 1208–1214.
[11] W. Bai, C. Wang, C. Ren, Intakes of total and individual flavonoids by US adults, Int. J. Food Sci. Nutr. 65 (2014) 9–20.
[12] R. Zamora-Ros, P. Ferrari, A.C. Gonzalez, A. Tjonneland, A. Olsen, L. Bredsdorff, et al., Dietary flavonoids and lignan intake and breast cancer risk according to menopausal status and menopausal hormone replacement status in the European Prospective Investigation into Cancer and Nutrition (EPIC) Study, Breast Cancer Res. Treat. 139 (2013) 163–176.
[13] P.A. Murphy, K. Barua, C.C. Hauck, Solvent extraction selection in the determination of isoflavones in soy foods, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 777 (2002) 129–138.
[14] P.A. Murphy, T. Song, G. Buseman, K. Barua, G.R. Beecher, D. Trainer, et al., Isoflavones in retail and institutional soy foods, J. Agric. Food Chem. 47 (1999) 2697–2704.

Table 4
Effect of dose on the impact of isoflavone exposure on reproductive hormone concentrations.

| Outcome | Statistical model | Intervention isoflavone dose (mg/d) |
|---------|------------------|-----------------------------------|
|         |                  | <75 mg Studies (n, active arm; n, control arm) | >75 mg Studies (n, active arm; n, control arm) |
|         |                  | N = 14 (536; 540) SMD -0.13 [-0.39, 0.14] Z = 0.41 | N = 6 (91; 74) SMD 0.14 [-0.33, 0.61] Z = 0.57 |
| Testosterone | A | p = 0.35 | Z = 0.57 p = 0.57 |
|         | B | p = 0.35 | Z = 0.57 |
|         | N = 27 (859) SMD | N = 15 (322) SMD |
|         | Z = 0.82, p = 0.41 | Z = 1.6 p = 0.10 |
|         | N = 13 (337, 365) SMD 0.05 [-0.31, 0.41] Z = 0.25 | Insufficient groups to analyze |
| SHBG | A | p = 0.80 | N = 15 (404) SMD |
|         | B | p = 0.80 | N = 3 (69) SMD |
|         | p = 0.46 | Z = 0.85 |
|         | N = 13 (359; 366) SMD 0.01 [-0.50, 0.49] Z = 0.05 | Z = 0.54 p = 0.59 |
| Estradiol | A | p = 0.96 | N = 19 (542) SMD |
|         | B | p = 0.96 | N = 6 (120) SMD |
|         | p = 0.40 | Z = 0.85 |
|         | N = 14 (388; 425) SMD 0.18 [-0.17, 0.43] Z = 1.39 | Insufficient groups to analyze |
| Estrogen | A | p = 0.17 | N = 21 (560) SMD |
|         | B | p = 0.17 | N = 4 (62) SMD |
|         | p = 0.95 | Z = 0.07 |
|         | N = 5 (91; 91) SMD 0.07 [-0.49, 0.52] Z = 0.07 | Z = 0.95 |
|         | A | p = 0.20 | N = 5 (91; 91) SMD 0.45 [-0.36, 1.28] Z = 1.10 p = 0.27 |
|         | Insufficient groups to analyze |
|         | B | p = 0.20 | N = 5 (127) SMD 0.24 [-0.11, 0.60] |
|         | Z = 1.37 p = 0.17 | Z = 0.10 p = 0.92 |

a Model A: Treatment vs control is an analysis of the change (in the treatment arm vs the change in the control arm); model B: the change over time in the all active arms. SMD is standardized mean difference.

It should be emphasized that the lack of effect of soy intake and isoflavone exposure on these reproductive hormones in men does not necessarily mean that soy or isoflavone intake does not exert any hormonal effects. Isoflavones could exert biological effects independent of effects on hormone levels, such as by directly interacting with ERs and/or the AR. However, clinically relevant endpoints can therefore also inform about the possible impact of soy related to the effects of reproductive hormones. In this regard, it is notable that clinical studies show no effect of soy on sperm and semen parameters [84,104] and soy protein supplementation leads to similar gains in muscle mass and strength among men engaged in resistance exercise training as animal protein, including whey protein supplementation [105].
