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

Katharine E. Reed, Juliana Camargo, Jill Hamilton-Reeves, Mindy Kurzer, Mark Messina

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Running title: Soy does not lower testosterone levels

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

Authors:

Katharine E. Reed,[a] Juliana Camargo,[b] Jill Hamilton-Reeves,[c] Mindy Kurzer,[d] Mark Messina[e]*

[a]School of Sport, Rehabilitation and Exercise Sciences, University of Essex, ESA.3.11, Colchester Campus, Wivenhoe Park, Colchester CO4 3SQ, United Kingdom email: reedk@essex.ac.uk [b]University of Kansas Medical Center, Department of Dietetics & Nutrition, 3901 Rainbow Blvd, MS 4013, Kansas City, KS 66160, United States email: jcamargo@kumc.edu [c]Kansas University Department of Urology and Department of Dietetics & Nutrition, University of Kansas Medical Center, Mail Stop 3016, 3901 Rainbow Blvd., Kansas City, KS 66160 email: jhamilton-reeves@kumc.edu; [d]Mindy S. Kurzer, Dept. of Food Science and Nutrition, University of Minnesota, 1334 Eckles Ave, St. Paul, MN 55108 (USA), email: mkurzer@umn.edu [e*]Nutrition Matters, Inc. 26 Spadina Parkway, Pittsfield, MA, 01201 United States email: markjohnmessina@gmail.com (corresponding author) Phone 1-413-464-0565

Highlights

- Soy is a uniquely rich source of isoflavones, compounds classified as phytoestrogens
- Concerns have been raised that because it contains isoflavones, soy feminizes men
- Clinical data were analyzed to examine effects of soy/isoflavones on hormone levels
- 38 clinical studies were deemed suitable for inclusion in the meta-analysis
- No effects of soy/isoflavones on testosterone or estrogen levels in men were noted

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 (E_2), estrone (E_1), 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; E_2 and E_1 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, E_2 or E_1 levels in men.

Key words: Soy, isoflavones, testosterone, estrogen, phytoestrogens

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 to 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) [26-29]. [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 endpoints 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 E_2 and E_1 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, E_2, E_1 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, E_2 and E_1), thus 10 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 I^2 and broad cutoff points of <40%, 40% to 60%, 61% to 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 over 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 E_2, 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 [93] was excluded from analysis because the intervention supplement was comprised of equol, 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 bread with linseed [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.

Table 1. Description of the studies included in the meta-analysis

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
Parallel Studies								
Dalais/2004/ Australia/(54)	62 ± 5 61 ± 5	Soy grits bread Wheat bread	8 8	Prostate cancer	18 11	117	25 d	T, SHBG
DiSilvestro/2006/ USA/(55)	18-30	SPI Whey protein	10 10	Healthy	42 42	98	4 wk	T
Deibert/2011/ Germany/(56)	56 55	SPI Usual diet	13 13	Healthy	27 0	NI	12 wk	FT
Hamilton- Reeves /2007/USA/(57)	68 ± 8 68 ± 5 68 ± 7	SPI SPI Milk protein isolate	20 20 18	High risk for prostate cancer	40 40 40	107 <6 0	6 mo	T, FT, SHBG, E1, E2
Hamilton-Reeves /2013/USA/(58)	62 ± 12 62 ± 7	Capsules Capsules	42 44	Prostate cancer	0 0	51 0	2-6 wk	T, FT, E2
Haun/2018/ USA/(59)	21± 2 21± 2	SPC Whey	11 9	Healthy	39 26	32 1	12 wk	T, E2

	21± 1	Carbohydrate	12		<1	1		
Kalman/2007/	30	SPI	5	Healthy	30	29	12 wk	T, FT, E2
USA/(60)	31	SPC	5		30	77		
	29	Whey protein	5		30	1		
Kumar/2004/	71 ± 5	SPI	29	Prostate cancer	29	60	12 wk	T, FT,
USA/(61)	72 ± 5	SPI	30		30	0		SHBG, E2
Kumar/2007/	72 ± 6	Capsules	22	Prostate cancer	0	48[a]	12 wk	T, FT,
USA/(62)	72 ± 6	Capsules	27		0	0		SHBG, E2
Kumar/2010/	60 ±7	Capsules	12	Prostate cancer	0	24[a]	4 wk	FT, SHBG,
USA/(63)	59 ±6	Capsules	11		0	36[a]		E2
	59 ±7	Capsules	10		0	48[a]		
Kumar/2020/(37)	58.8 ± 7.5	Capsules	36	Prostate cancer	0	40	3-6 wk	TT, FT, E2,
	59.1 ± 7.4	Capsules	35		0	0		SHBG
Li/2008/USA/(64)	60 ± 1	SPI	26	Prostate cancer	40	80	12 mo	T
	63 ± 2	Usual diet	14		0	0		
Miyanaga/2012/	66	Capsules	78	Rising prostate	0	36	12 mo	T, SHBG,
Japan/(65)	65	Capsules	80	specific antigen	0	0		E2
Nagata/2001/	32 ± 8	Soymilk	17	Healthy	13.40	55[a]	8 wk	T, FT,
Japan/(66)	33 ± 8	Usual diet	17					SHBG, E1, E2

Ornish/2005/ USA/(67)	65± 7 67± 8	Tofu/SPI Usual diet	43 41	Prostate cancer	~60 0	Not indicated 0	12 mo	T
Rannikko/(2006/ Finland/(68)	64 ± 3 64 ± 3	Red clover tablets Tablets	20 20	Prostate cancer	0 0	NI	2 wk	T, FT, SHBG, E1, E2
Reidy/(2016/ USA/(69)	24 ±1 25 ±1	ISP+Whey+Casein Whey	22 18	Healthy	25.2 (6.3 soy) 26.2	NI 0	12 wk	T
Swart/2019/ England/(70)	52 52	SPI SPI	85 86	Diabetes	15 15	66 0	3 mo	T, SHBG, E2
Teede/2001/ Australia/(70)	50-75	SPI Casein	48 48	Healthy	40 40	71[a]0	12 wk	T
Wong/2012/ Hong Kong/(72)	65 ± 9 65 ± 9	Capsule Capsule	72 74	Benign prostatic hyperplasia	0 0	40 0	12 mo	T
Cross-over studies								
Dillingham/2005/ USA/(73)	28 ± 6	SPI SPI Milk protein isolate	35 35 35	Healthy	32 32 32	2 62 0	57 d	T, FT, SHBG, E1, E2
Gardner-Thorpe/ 2003/UK/(44)	35 ±11	Soy flour Wheat flour	19 19	Healthy	NI	120 0	6 wk	T, SHBG, E1, E2

Goldin/2005/ USA/(74)	61	SPI SPI Capsules Capsules	18 18 18 18	Moderately hypercholesterolemic	71 710 0	139[c] 9[c] ≥50[c] 0	6 wk	T, E1, E2
Habito/2000/ Japan/(75)	46 ± 8	Tofu Lean meat	42 42	Healthy	35 0	Not indicated	4 wk	T, SHBG, E2
Higashi/2001/ Japan/(76)	31 ±. 4	SPI Usual diet	14 14	Healthy	20 0	Not indicated	4 wk	T, E2
Kranse/2005/ Netherlands/(77)	54-81	Tablets Tablets		Prostate cancer	0 0	100 0	6 wk	T
Maskarinec/2006/ USA/(78)	58.7 ± 7.2	Soyfoods Usual diet	23 23	Healthy	NI	~69 <5	12 wk	T
Schroder/2005/ Netherlands/(89)	70 ± 7	Tablets Tablets	49 49	Prostate cancer or rising PSA	0 0	0 62.5	10 wk	T, SHBG
Single or dual arm studies (no control)								
DeVere White/ 2004/USA/(79)	73.6	Capsules	52	Prostate cancer	<1	900	6 mo	T
Goodin/2007/	32.25	SPI	12	Healthy	56	NI	12 wk	T

USA/(43)								
Fischer/2004/ USA/(90)	68.9 ± 7.3	Tablets	20	Prostate cancer	0	300-600	84 d	T, FT
Hussain/2003/ USA/(91)	73 (55-82)	Tablets	39	Prostate cancer	0	200	5.5 mo	T
Jarred/2002/ Australia/(80)	60 ± 7	Red clover	20	Prostate cancer	0	160	20 d	T
Kwan/(2010/ Canada/(81)	78	Soymilk	29	Prostate cancer	12	65-90	6 mo	T
Lewis/2002/New Zealand/(82)	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[a]	2 mo	T, FT, E2

Tanaka/2009/ Japan/(87)	30-59	Tablets	28	Healthy	<2	36 [a]	3 mo	T, FT, SHBG, E2
Van Veldhuizen/ 2006/USA/(88)	63	Tablets	11	Prostate cancer	0	112-224	4 wk	T

[a]Converted to aglycone value

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. [60] 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, 80], soymilk/yogurt [66, 76], tofu [75] or soybeans [92].

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.

Table 2. Effects of isoflavone exposure on reproductive hormone levels in men

Outcome/statistical model[1]	No of groups (subjects)	Effect size SMD (95% CI)	P value for overall effect	I^2 %
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				
Treatment vs Control	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				
Treatment vs Control	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				
Treatment vs Control	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				
Treatment vs Control	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

[1]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

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 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.

Table 3. Effect of duration on the impact of isoflavone exposure on reproductive hormone concentrations

Outcome[1]	Statistical model	≤12 weeks Studies (n, active arm; n, control arm)	>12 weeks
Testosterone	A	N=15 (372; 372) SMD - 0.11 [-0.43,0.20] Z=0.69 p=0.49	N=5 (255; 242) SMD 0.01 [-0.28,0.30] Z=0.05 p=0.96
	B	N=32 (821) SMD 0.05 [-0.08, 0.19] Z=0.76 p=0.45	N= 10 (360) SMD 0.18 [-0.13,0.39] Z= 1.67 p=0.10
Free (unbound) testosterone	A	N=14 (340; 342) SMD 0.03 [-0.31, 0.36] z=0.16 p=0.87	Insufficient groups
	B	N=16 (410) SMD -0.32 [-0.23,0.16] Z = 0.33 p=0.74	Insufficient groups
SHBG	A	N=15 (312; 317) SMD 0.00 [-0.51,0.51] Z= 0.0 p=0.99	N=3 (124; 103) SMD -0.21 (-0.97, 0.55) Z =0.54 p=0.59
	B	N=22 (546) SMD -0.03 [-0.20,0.14) Z=0.38 p=0.70	N= 3 (124) SMD 0.06 (-0.29, 0.41) Z=0.33 p=0.74
Estradiol	A	N=14 (315; 322) SMD 0.21 (-0.04,0.48) Z=1.5 p=0.13	Insufficient groups
	B	N=23 (530) SMD 0.10 [-0.07, 0.26] Z=0.16 p = 0.25	Insufficient groups
Estrone	A	N=4 (7; 73) SMD 0.24 [-0.64,1.13] Z=0.54 p=0.59	Insufficient groups
	B	N= 6 (142) SMD 0.15(-0.18,0.48) Z= 0.89, p=0.37	Insufficient groups

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

Outcome[1]	Statistical model	Intervention isoflavone dose (mg/d)	
		≤75 mg Studies (n, active arm; n, control arm)	>75 Studies (n, active arm; n, control arm)
Testosterone	A	N=14 (536; 540) SMD -0.13 [-0.39, 0.14] Z=0.93 p=0.35	N=6 (91; 74) SMD 0.14 [-0.33, 0.61] Z=0.57 p=0.57
	B	N=27 (859) SMD 0.06 [-0.08, 0.19] Z=0.82, p=0.41	N=15 (322) SMD 0.08 [-0.04, 0.40] Z= 1.6 p=0.10
Free (unbound) testosterone	A	N=13 (337; 365) SMD 0.05 [-0.31, 0.41] Z=0.25 p=0.80	Insufficient groups to analyze
	B	N=15 (404) SMD -0.03 [-0.23, 0.17] Z=0.31 p-0.76	N=3 (69) SMD -0.20 [-0.68, 0.27] Z=0.85 p=0.40
SHBG	A	N=13 (359; 366) SMD -0.01 [-0.50, 0.49] Z=0.05 p=0.96	N=5 (77+54) SMD -0.16 [-0.75, 0.42] Z=0.54 p=0.59
	B	N= 19 (542) SMD -0.00 [-0.17, 0.17] Z=0.04 p=0.97	N=6 (120) SMD -0.07 [-0.43, 0.29] Z=0.39 p=0.70
Estradiol	A	N=14 (388; 425) SMD 0.18 [-0.17, 0.43} Z=1.39 p=0.17	Insufficient groups to analyze
	B	N=21 (560) SMD 0.07 [-0.10, 0.24] Z=0.82 p=0.41	N=4 (62) SMD 0.02 [-0.49, 0.52} Z=0.07 p=0.95
Estrone	A	N=5 (91; 91) SMD 0.45 [-0.36, 1.28] Z=1.10 p=0.27	Insufficient groups to analyze
	B	N=5 (127) SMD 0.24 [-0.11, 0.60] Z=1.37 p=0.17	N=3 (57) SMD 0.03 [-0.49, 0.55] Z= 0.10 p=0.92

[1]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

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 E_2, 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 E_2 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, 01.07] 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; I^2 , 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 hours of ethanol ingestion and reached levels similar than 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 to 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 soynuts. Thus, by Asian standards, the cutoff of 75 mg/d would cover a high intake of soyfoods. 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 histological proven prostate cancer whose prostate specific antigen (PSA) levels decreased in response to 900 mg/d isoflavones, deVere 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 Swerdloff 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 equol 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 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.

There were insufficient data upon which to determine whether equol per se, alters reproductive hormone levels. Most studies in this analysis did not determine equol producer status. Furthermore, even if more had, 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 this regard, Hamilton-Reeves et al. [57] found that AR expression in the prostate was suppressed (~8%) in response to isoflavone intake. In future research it may be worth comparing isoflavones with other agents that inhibit AR expression to determine their potential in prostate cancer treatment.

To our knowledge, the current meta-analysis is the first to examine the effects of soy intake and isoflavone exposure on estrogen levels in men. A meta-analysis published in 2009 found no

effect on estrogen levels in pre- or postmenopausal women [103]. One year later, a narrative review based on nine studies concluded there was no effect of isoflavone exposure on estrogen levels in men [48]. These publications concur with the findings of the current analysis in that statistically significant changes were not found for E_2 or E_1 [48]. There was only a moderate amount of heterogeneity (model A, I^2 =56%) among the 16 studies. Only eight studies evaluated E_1.

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].

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.

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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 Human 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. Pabich, M. Materska, 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. G. J. Hughes, D. J. Ryan, R. Mukherjea, C. S. Schasteen, Protein digestibility-corrected amino acid scores (PDCAAS) for soy protein isolates and concentrate: Criteria for evaluation, J Agric Food Chemistry 59 (2011) 12707-12712.
7. Z. Shan, C. D. Rehm, G. Rogers, M. Ruan, D. D. Wang, F. B. Hu, et al, Trends in dietary carbohydrate, protein, and fat intake and diet quality among US adults, 1999-2016, JAMA 322 (2019) 1178-1187.
8. P. Singh, R. Kumar, S. N. Sabapathy, A. S. Bawa, Functional and edible uses of soy protein products, Comprehensive Rev Food Sci Food Safety 7 (2008) 14-28.
9. L. U. Thompson, B. A. Boucher, Z. Liu, M. Cotterchio, N. Kreiger, Phytoestrogen content of foods consumed in Canada, including isoflavones, lignans, and coumestan, Nutr Cancer 54 (2006) 184-201.
10. A. A. Franke, L. J. Custer, W. Wang, C. Y. Shi, HPLC analysis of isoflavonoids and other phenolic agents from foods and from human fluids, Proc Soc Exp Biol Med 217 (1998) 263-273.
11. M. Messina, C. Nagata, A. H. Wu, Estimated Asian adult soy protein and isoflavone intakes, Nutr Cancer 55 (2006) 1-12.
12. 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.
13. W. Bai, C. Wang, C. Ren, Intakes of total and individual flavonoids by US adults, Int J Food Sci Nutr 65 (2014) 9-20.
14. R. Zamora-Ros, P. Ferrari, C. A. Gonzalez, A. Tjonneland, A. Olsen, L. Bredsdorff, et al, Dietary flavonoid and lignan intake and breast cancer risk according to menopause and hormone receptor status in the European Prospective Investigation into Cancer and Nutrition (EPIC) Study, Breast Cancer Res Treat 139 (2013) 163-176.
15. 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.
16. 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.

17. G. G. Kuiper, B. Carlsson, K. Grandien, E. Enmark, J. Haggblad, S. Nilsson, et al, Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta, Endocrinology 138 (1997) 863-870.
18. G. G. Kuiper, J. G. Lemmen, B. Carlsson, J. C. Corton, S. H. Safe, P. T. van der Saag, et al, Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta, Endocrinology 139 (1998) 4252-4263.
19. V. van der Velpen, P. C. Hollman, M. van Nielen, E. G. Schouten, M. Mensink, P. Van't Veer, et al, Large inter-individual variation in isoflavone plasma concentration limits use of isoflavone intake data for risk assessment, Eur J Clin Nutr 68 (2014) 1141-1147.
20. J. An, C. Tzagarakis-Foster, T. C. Scharschmidt, N. Lomri, D. C. Leitman, Estrogen Receptor beta -Selective Transcriptional Activity and Recruitment of Coregulators by Phytoestrogens, J Biol Chem 276 (2001) 17808-17814.
21. E. Margeat, A. Bourdoncle, R. Margueron, N. Poujol, V. Cavailles, C. Royer, Ligands Differentially Modulate the Protein Interactions of the Human Estrogen Receptors alpha and beta, J Mol Biol 326 (2003) 77-92.
22. D. Kostelac, G. Rechkemmer, K. Briviba, Phytoestrogens modulate binding response of estrogen receptors alpha and beta to the estrogen response element, J Agric Food Chem 51 (2003) 7632-7635.
23. A. C. Pike, A. M. Brzozowski, R. E. Hubbard, T. Bonn, A. G. Thorsell, O. Engstrom, et al, Structure of the ligand-binding domain of oestrogen receptor beta in the presence of a partial agonist and a full antagonist, EMBO J 18 (1999) 4608-4618.
24. V. Speirs, P. J. Carder, S. Lane, D. Dodwell, M. R. Lansdown, A. M. Hanby, Oestrogen receptor beta: what it means for patients with breast cancer, Lancet Oncol 5 (2004) 174-181.
25. D. G. Pons, M. Nadal-Serrano, M. Torrens-Mas, J. Oliver, P. Roca, The phytoestrogen genistein affects breast cancer cells treatment depending on the ERalpha/ERbeta ratio, J Cell Biochem 117 (2016) 218-229.
26. A. Brzezinski, H. Adlercreutz, R. Shaoul, R. Rösler, A. Shmueli, V. Tanos, et al, Short-term effect of phytoestrogen-rich diet on postmenopausal women, Menopause 4 (1997) 89-94.
27. P. Diel, R. B. Geis, A. Caldarelli, S. Schmidt, U. L. Leschowsky, A. Voss, et al, The differential ability of the phytoestrogen genistein and of estradiol to induce uterine weight and proliferation in the rat is associated with a substance specific modulation of uterine gene expression, Mol Cell Endocrinol 221 (2004) 21-32.
28. M. F. Yildiz, S. Kumru, A. Godekmerdan, S. Kutlu, Effects of raloxifene, hormone therapy, and soy isoflavone on serum high-sensitive C-reactive protein in postmenopausal women, Int J Gynaecol Obstet 90 (2005) 128-133.
29. T. Oseni, R. Patel, J. Pyle, V. C. Jordan, Selective estrogen receptor modulators and phytoestrogens, Planta Med 74 (2008) 1656-1665.
30. M. Russo, G. L. Russo, M. Daglia, P. D. Kasi, S. Ravi, S. F. Nabavi, et al, Understanding genistein in cancer: The "good" and the "bad" effects: A review, Food Chem 196 (2016) 589-600.
31. M. Akhlaghi, M. Ghasemi Nasab, M. Riasatian, F. Sadeghi, Soy isoflavones prevent bone resorption and loss, a systematic review and meta-analysis of randomized controlled trials, Crit Rev Food Sci Nutr (2019) 1-15.

32. K. Taku, M. K. Melby, F. Kronenberg, M. S. Kurzer, M. Messina, Extracted or synthesized soybean isoflavones reduce menopausal hot flash frequency and severity: systematic review and meta-analysis of randomized controlled trials, Menopause 19 (2012) 776-790.
33. M. D. van Die, K. M. Bone, S. G. Williams, M. V. Pirotta, Soy and soy isoflavones in prostate cancer: a systematic review and meta-analysis of randomized controlled trials, BJU Int 113 (2014) E119-130.
34. C. C. Applegate, J. L. Rowles, K. M. Ranard, S. Jeon, J. W. Erdman, Soy consumption and the risk of prostate cancer: An updated systematic review and meta-analysis, Nutrients 10 (2018).
35. Q. Tang, J. Ma, J. Sun, L. Yang, F. Yang, W. Zhang, et al, Genistein and AG1024 synergistically increase the radiosensitivity of prostate cancer cells, Oncol Rep 40 (2018) 579-588.
36. A. M. Mahmoud, W. Yang, M. C. Bosland, Soy isoflavones and prostate cancer: a review of molecular mechanisms, J Steroid Biochem Mol Biol 140 (2014) 116-132.
37. N. B. Kumar, J. Pow-Sang, P. Spiess, S. Dickinson, M. J. Schell, A phase II randomized clinical trial using aglycone isoflavones to treat patients with localized prostate cancer in the pre-surgical period prior to radical prostatectomy, Oncotarget 11 (2020) 1218-1234.
38. R. M. Sharpe, N. E. Skakkebaek, Are oestrogens involved in falling sperm counts and disorders of the male reproductive tract?, Lancet 341 (1993) 1392-1395.
39. J. Toppari, J. C. Larsen, P. Christiansen, A. Giwercman, P. Grandjean, L. J. Guillette, Jr., et al, Male reproductive health and environmental xenoestrogens, Environ Health Perspect 104 Suppl 4 (1996) 741-803.
40. N. E. Skakkebaek, E. Rajpert-De Meyts, K. M. Main, Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects, Hum Reprod 16 (2001) 972-978.
41. K. S. Weber, K. D. Setchell, D. M. Stocco, E. D. Lephart, Dietary soy-phytoestrogens decrease testosterone levels and prostate weight without altering LH, prostate 5alpha-reductase or testicular steroidogenic acute regulatory peptide levels in adult male Sprague-Dawley rats, J Endocrinol 170 (2001) 591-599.
42. L. Strauss, S. Makela, S. Joshi, I. Huhtaniemi, R. Santti, Genistein exerts estrogen-like effects in male mouse reproductive tract, Mol Cell Endocrinol 144 (1998) 83-93.
43. S. Goodin, F. Shen, W. J. Shih, N. Dave, M. P. Kane, P. Medina, et al, Clinical and biological activity of soy protein powder supplementation in healthy male volunteers, Cancer Epidemiol Biomarkers Prev 16 (2007) 829-833.
44. D. Gardner-Thorpe, C. O'Hagen, I. Young, S. J. Lewis, Dietary supplements of soya flour lower serum testosterone concentrations and improve markers of oxidative stress in men, Eur J Clin Nutr 57 (2003) 100-106.
45. J. Martinez, J. E. Lewi, An unusual case of gynecomastia associated with soy product consumption, Endocr Pract 14 (2008) 415-418.
46. J. E. Chavarro, T. L. Toth, S. M. Sadio, R. Hauser, Soy food and isoflavone intake in relation to semen quality parameters among men from an infertility clinic, Hum Reprod 23 (2008) 2584-2590.
47. J. M. Hamilton-Reeves, G. Vazquez, S. J. Duval, W. R. Phipps, M. S. Kurzer, M. J. Messina, Clinical studies show no effects of soy protein or isoflavones on reproductive hormones in men: results of a meta-analysis, Fertil Steril 94 (2010) 997-1007.

48. M. Messina, Soybean isoflavone exposure does not have feminizing effects on men: a critical examination of the clinical evidence, Fertil Steril 93 (2010) 2095-2104.
49. T. Siepmann, J. Roofeh, F. W. Kiefer, D. G. Edelson, Hypogonadism and erectile dysfunction associated with soy product consumption, Nutrition 27 (2011) 859-862.
50. W. J. Kraemer, G. Solomon-Hill, B. M. Volk, B. R. Kupchak, D. P. Looney, C. Dunn-Lewis, et al, The effects of soy and whey protein supplementation on acute hormonal reponses to resistance exercise in men, J Am Coll Nutr 32 (2013) 66-74.
51. H. Toshima, Y. Suzuki, K. Imai, J. Yoshinaga, H. Shiraishi, Y. Mizumoto, et al, Endocrine disrupting chemicals in urine of Japanese male partners of subfertile couples: a pilot study on exposure and semen quality, Int J Hyg Environ Health 215 (2012) 502-506.
52. Y. Xia, M. Chen, P. Zhu, C. Lu, G. Fu, X. Zhou, et al, Urinary phytoestrogen levels related to idiopathic male infertility in Chinese men, Environ Int 59 (2013) 161-167.
53. G. Yuan, Y. Liu, G. Liu, L. Wei, Y. Wen, S. Huang, et al, Associations between semen phytoestrogens concentrations and semen quality in Chinese men, Environ Int 129 (2019) 136-144.
54. F. S. Dalais, A. Meliala, N. Wattanapenpaiboon, M. Frydenberg, D. A. Suter, W. K. Thomson, et al, Effects of a diet rich in phytoestrogens on prostate-specific antigen and sex hormones in men diagnosed with prostate cancer, Urology 64 (2004) 510-515.
55. R. A. DiSilvestro, C. Mattern, N. Wood, S. T. Devor, Soy protein intake by active young adult men raises plasma antioxidant capacity without altering plasma testosterone, Nutr Res 26 (2006) 92-95.
56. P. Deibert, F. Solleder, D. Konig, M. Z. Vitolins, H. H. Dickhuth, A. Gollhofer, et al, Soy protein based supplementation supports metabolic effects of resistance training in previously untrained middle aged males, Aging Male 14 (2011) 273-279.
57. J. M. Hamilton-Reeves, S. A. Rebello, W. Thomas, J. W. Slaton, M. S. Kurzer, Isoflavone-rich soy protein isolate suppresses androgen receptor expression without altering estrogen receptor-{beta} expression or serum hormonal profiles in men at high risk of prostate cancer, J Nutr 137 (2007) 1769-1775.
58. J. M. Hamilton-Reeves, S. Banerjee, S. K. Banerjee, J. M. Holzbeierlein, J. B. Thrasher, S. Kambhampati, et al, Short-term soy isoflavone intervention in patients with localized prostate cancer: a randomized, double-blind, placebo-controlled trial, PLoS One 8 (2013) e68331.
59. C. T. Haun, C. B. Mobley, C. G. Vann, M. A. Romero, P. A. Roberson, P. W. Mumford, et al, Soy protein supplementation is not androgenic or estrogenic in college-aged men when combined with resistance exercise training, Sci Rep 8 (2018) 11151.
60. D. Kalman, S. Feldman, M. Martinez, D. R. Krieger, M. J. Tallon, Effect of protein source and resistance training on body composition and sex hormones, J Int Soc Sports Nutr 4 (2007) 4.
61. N. B. Kumar, A. Cantor, K. Allen, D. Riccardi, K. Besterman-Dahan, J. Seigne, et al, The specific role of isoflavones in reducing prostate cancer risk, Prostate 59 (2004) 141-147.
62. N. B. Kumar, J. P. Krischer, K. Allen, D. Riccardi, K. Besterman-Dahan, R. Salup, et al, A Phase II randomized, placebo-controlled clinical trial of purified isoflavones in modulating steroid hormones in men diagnosed with localized prostate cancer, Nutr Cancer 59 (2007) 163-168.
63. N. B. Kumar, L. Kang, J. Pow-Sang, P. Xu, K. Allen, D. Riccardi, et al, Results of a randomized phase I dose-finding trial of several doses of isoflavones in men with

localized prostate cancer: administration prior to radical prostatectomy, J Soc Integr Oncol 8 (2010) 3-13.
64. Z. Li, W. J. Aronson, J. R. Arteaga, K. Hong, G. Thames, S. M. Henning, et al, Feasibility of a low-fat/high-fiber diet intervention with soy supplementation in prostate cancer patients after prostatectomy, Eur J Clin Nutr 62 (2008) 526-536.
65. N. Miyanaga, H. Akaza, S. Hinotsu, T. Fujioka, S. Naito, M. Namiki, et al, A prostate cancer chemoprevention study: an investigative randomized control study using purified isoflavones in men with rising prostate-specific antigen, Cancer Sci 103 (2012) 125-130.
66. C. Nagata, N. Takatsuka, H. Shimizu, H. Hayashi, T. Akamatsu, K. Murase, Effect of soymilk consumption on serum estrogen and androgen concentrations in Japanese men, Cancer Epidemiol Biomarkers Prev 10 (2001) 179-184.
67. D. Ornish, G. Weidner, W. R. Fair, R. Marlin, E. B. Pettengill, C. J. Raisin, et al, Intensive lifestyle changes may affect the progression of prostate cancer, J Urol 174 (2005) 1065-1069; discussion 1069-1070.
68. A. Rannikko, A. Petas, T. Raivio, O. A. Janne, S. Rannikko, H. Adlercreutz, The effects of short-term oral phytoestrogen supplementation on the hypothalamic-pituitary-testicular axis in prostate cancer patients, Prostate 66 (2006) 1086-1091.
69. P. T. Reidy, M. S. Borack, M. M. Markofski, J. M. Dickinson, R. R. Deer, S. H. Husaini, et al, Protein supplementation has minimal effects on muscle adaptations during resistance exercise training in young men: A double-blind randomized clinical trial, J Nutr 146 (2016) 1660-1669.
70. A. C. Swart, I. D. Johannes, T. Sathyapalan, S. L. Atkin, The effect of soy isoflavones on steroid metabolism, Front Endocrinol (Lausanne) 10 (2019) 229.
71. H. J. Teede, F. S. Dalais, D. Kotsopoulos, Y. L. Liang, S. Davis, B. P. McGrath, Dietary soy has both beneficial and potentially adverse cardiovascular effects: a placebo-controlled study in men and postmenopausal women, J Clin Endocrinol Metab 86 (2001) 3053-3060.
72. W. C. Wong, E. L. Wong, H. Li, J. H. You, S. Ho, J. Woo, et al, Isoflavones in treating watchful waiting benign prostate hyperplasia: a double-blinded, randomized controlled trial, J Altern Complement Med 18 (2012) 54-60.
73. B. L. Dillingham, B. L. McVeigh, J. W. Lampe, A. M. Duncan, Soy protein isolates of varying isoflavone content exert minor effects on serum reproductive hormones in healthy young men, J Nutr 135 (2005) 584-591.
74. B. R. Goldin, E. Brauner, H. Adlercreutz, L. M. Ausman, A. H. Lichtenstein, Hormonal response to diets high in soy or animal protein without and with isoflavones in moderately hypercholesterolemic subjects, Nutr Cancer 51 (2005) 1-6.
75. R. C. Habito, J. Montalto, E. Leslie, M. J. Ball, Effects of replacing meat with soyabean in the diet on sex hormone concentrations in healthy adult males, Br J Nutr 84 (2000) 557-563.
76. K. Higashi, S. Abata, N. Iwamoto, M. Ogura, T. Yamashita, O. Ishikawa, et al, Effects of soy protein on levels of remnant-like particles cholesterol and vitamin E in healthy men, J Nutr Sci Vitaminol (Tokyo) 47 (2001) 283-288.
77. R. Kranse, P. C. Dagnelie, M. C. van Kemenade, F. H. de Jong, J. H. Blom, L. B. Tijburg, et al, Dietary intervention in prostate cancer patients: PSA response in a randomized double-blind placebo-controlled study, Int J Cancer 113 (2005) 835-840.

78. G. Maskarinec, Y. Morimoto, S. Hebshi, S. Sharma, A. A. Franke, F. Z. Stanczyk, Serum prostate-specific antigen but not testosterone levels decrease in a randomized soy intervention among men, Eur J Clin Nutr 60 (2006) 1423-1429.
79. R. W. deVere White, R. M. Hackman, S. E. Soares, L. A. Beckett, Y. Li, B. Sun, Effects of a genistein-rich extract on PSA levels in men with a history of prostate cancer, Urology 63 (2004) 259-263.
80. R. A. Jarred, M. Keikha, C. Dowling, S. J. McPherson, A. M. Clare, A. J. Husband, et al, Induction of apoptosis in low to moderate-grade human prostate carcinoma by red clover-derived dietary isoflavones, Cancer Epidemiol Biomarkers Prev 11 (2002) 1689-1696.
81. W. Kwan, G. Duncan, C. Van Patten, M. Liu, J. Lim, A phase II trial of a soy beverage for subjects without clinical disease with rising prostate-specific antigen after radical radiation for prostate cancer, Nutr Cancer 62 (2010) 198-207.
82. J. G. Lewis, J. C. Morris, B. M. Clark, P. A. Elder, The effect of isoflavone extract ingestion, as Trinovin, on plasma steroids in normal men, Steroids 67 (2002) 25-29.
83. R. Mackey, A. Ekangaki, J. A. Eden, The effects of soy protein in women and men with elevated plasma lipids, Biofactors 12 (2000) 251-257.
84. J. H. Mitchell, E. Cawood, D. Kinniburgh, A. Provan, A. R. Collins, D. S. Irvine, Effect of a phytoestrogen food supplement on reproductive health in normal males, Clin Sci (Lond) 100 (2001) 613-618.
85. J. M. Pendleton, W. W. Tan, S. Anai, M. Chang, W. Hou, K. T. Shiverick, et al, Phase II Trial of Isoflavone in prostate specific antigen recurrent prostate cancer after previous local therapy, BMC Cancer 8 (2008) 132.
86. D. Spentzos, C. Mantzoros, M. M. Regan, M. E. Morrissey, S. Duggan, S. Flickner-Garvey, et al, Minimal effect of a low-fat/high soy diet for asymptomatic, hormonally naive prostate cancer patients, Clin Cancer Res 9 (2003) 3282-3287.
87. M. Tanaka, K. Fujimoto, Y. Chihara, K. Torimoto, T. Yoneda, N. Tanaka, et al, Isoflavone supplements stimulated the production of serum equol and decreased the serum dihydrotestosterone levels in healthy male volunteers, Prostate Cancer Prostatic Dis 12 (2009) 247-252.
88. P. J. van Veldhuizen, J. B. Thrasher, G. Ray, R. Cherian, J. Ward, J. Holzbeierlein, et al, Dose effect of soy supplementation in prostate cancer: A pilot study, Oncol Rep 16 (2006) 1221-1224.
89. F. H. Schroder, M. J. Roobol, E. R. Boeve, R. de Mutsert, S. D. Zuijdgeest-van Leeuwen, I. Kersten, et al, Randomized, double-blind, placebo-controlled crossover study in men with prostate cancer and rising PSA: effectiveness of a dietary supplement, Eur Urol 48 (2005) 922-930; discussion 930-921.
90. L. Fischer, C. Mahoney, A. R. Jeffcoat, M. A. Koch, B. E. Thomas, J. L. Valentine, et al, Clinical characteristics and pharmacokinetics of purified soy isoflavones: multiple-dose administration to men with prostate neoplasia, Nutr Cancer 48 (2004) 160-170.
91. M. Hussain, M. Banerjee, F. H. Sarkar, Z. Djuric, M. N. Pollak, D. Doerge, et al, Soy isoflavones in the treatment of prostate cancer, Nutr Cancer 47 (2003) 111-117.
92. P. Celec, D. Ostatnikova, J. Hodosy, Z. Putz, M. Kudela, Increased one week soybean consumption affects spatial abilities but not sex hormone status in men, Int J Food Sci Nutr 58 (2007) 424-428.
93. E. D. Lephart, Severe and moderate BPH symptoms in mid-aged men improve with isoflavonoid-equol treatment: Pilot intervention study, Open J Urology 3 (2013) 21-27.

94. E. M. Grainger, S. J. Schwartz, S. Wang, N. Z. Unlu, T. W. Boileau, A. K. Ferketich, et al, A combination of tomato and soy products for men with recurring prostate cancer and rising prostate specific antigen, Nutr Cancer 60 (2008) 145-154.
95. W. Kriengsinyos, K. Sumriddetchkajorn, U. Yamborisut, Reduction of LDL-cholesterol in mildly hypercholesterolemic Thais with plant stanol ester-fortified soy milk, J Med Assoc Thai 94 (2011) 1327-1336.
96. D. H. Van Thiel, J. S. Gavaler, C. F. Cobb, L. Santucci, T. O. Graham, Ethanol, a Leydig cell toxin: evidence obtained in vivo and in vitro, Pharmacol Biochem Behav 18 Suppl 1 (1983) 317-323.
97. A. Hara, S. Sasazuki, M. Inoue, M. Iwasaki, T. Shimazu, N. Sawada, et al, Isoflavone intake and risk of gastric cancer: a population-based prospective cohort study in Japan, Am J Clin Nutr 95 (2012) 147-154.
98. R. S. Swerdloff, R. E. Dudley, S. T. Page, C. Wang, W. A. Salameh, Dihydrotestosterone: Biochemistry, physiology, and clinical implications of elevated blood levels, Endocr Rev 38 (2017) 220-254.
99. R. Horton, Dihydrotestosterone is a peripheral paracrine hormone, J Androl 13 (1992) 23-27.
100. K. D. Setchell, N. M. Brown, E. Lydeking-Olsen, The clinical importance of the metabolite equol-a clue to the effectiveness of soy and its isoflavones, J Nutr 132 (2002) 3577-3584.
101. T. D. Lund, D. J. Munson, M. E. Haldy, K. D. Setchell, E. D. Lephart, R. J. Handa, Equol is a novel anti-androgen that inhibits prostate growth and hormone feedback, Biol Reprod 70 (2004) 1188-1195.
102. T. D. Lund, C. Blake, L. Bu, A. N. Hamaker, E. D. Lephart, Equol an isoflavonoid: potential for improved prostate health, in vitro and in vivo evidence, Reprod Biol Endocrinol 9 (2011) 4.
103. L. Hooper, J. J. Ryder, M. S. Kurzer, J. W. Lampe, M. J. Messina, W. R. Phipps, et al, Effects of soy protein and isoflavones on circulating hormone concentrations in pre- and post-menopausal women: a systematic review and meta-analysis, Hum Reprod Update 15 (2009) 423-440.
104. L. K. Beaton, B. L. McVeigh, B. L. Dillingham, J. W. Lampe, A. M. Duncan, Soy protein isolates of varying isoflavone content do not adversely affect semen quality in healthy young men, Fertil Steril 94 (2010) 1717-1722.
105. M. Messina, H. Lynch, J. M. Dickinson, K. E. Reed, No difference between the effects of supplementing with soy protein versus animal protein on gains in muscle mass and strength in response to resistance exercise, Int J Sport Nutr Exerc Metab 28 (2018) 674-685.