Edward E. Wallach, M.D.
Associate Editor

Soybean isoflavone exposure does not have feminizing effects on men: a critical examination of the clinical evidence

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Objective: To critically evaluate the clinical evidence, and when not available, the animal data, most relevant to concerns that isoflavone exposure in the form of supplements or soy foods has feminizing effects on men.
Design: Medline literature review and cross-reference of published data.
Result(s): In contrast to the results of some rodent studies, findings from a recently published metaanalysis and subsequently published studies show that neither isoflavone supplements nor isoflavone-rich soy affect total or free testosterone (T) levels. Similarly, there is essentially no evidence from the nine identified clinical studies that isoflavone exposure affects circulating estrogen levels in men. Clinical evidence also indicates that isoflavones have no effect on sperm or semen parameters, although only three intervention studies were identified and none were longer than 3 months in duration. Finally, findings from animal studies suggesting that isoflavones increase the risk of erectile dysfunction are not applicable to men, because of differences in isoflavone metabolism between rodents and humans and the excessively high amount of isoflavones to which the animals were exposed.
Conclusion(s): The intervention data indicate that isoflavones do not exert feminizing effects on men at intake levels equal to and even considerably higher than are typical for Asian males. (Fertil Steril® 2010;93:2095–104. ©2010 by American Society for Reproductive Medicine.)

Key Words: Isoflavones, testosterone, estrogen, men, feminization, gynecomastia, erectile dysfunction, clinical

For several decades, soy-based foods have been recognized as plant sources of high-quality protein (1), but in recent years, these foods have received considerable attention for their possible role in providing health benefits independent of their nutritional content (2, 3). In large part, this is because they are essentially unique dietary sources of isoflavones, a group of diphenolic chemicals classified as phytoestrogens (4). However, isoflavones are also the reason that soy foods have become controversial, because there are concerns that the estrogen-like properties of these soybean constituents might lead to adverse effects in some individuals. Most notable in this regard is the concern that isoflavones pose a risk to patients with breast cancer and women at high risk of developing it, although the relevant clinical and newly published epidemiologic data are reassuring (5, 6), in contrast to the animal data upon which concerns are based (7).

More recently, questions have been raised regarding possible adverse effects of soy consumption in men, including feminization (8) and infertility (9–11). Sensationalized media stories on these topics (12, 13) may have led some men to avoid soy foods, which may be particularly unfortunate because speculative but intriguing evidence suggests that soy foods reduce risk of developing prostate cancer (14) and perhaps even inhibit prostate cancer metastasis (15, 16). Peripherally related to feminization concerns is the hypothesis that xenoestrogen exposure is responsible for an assortment of male ailments, including testicular germ cell cancer, cryptorchidism, and some cases of hypospadias and low sperm counts—collectively referred to as testicular dysgenesis syndrome (TDS)—although TDS is thought to result primarily from exposure to xenoestrogens in utero or during the neonatal period rather than adulthood (9, 17, 18).

Despite the large populations in soy food–consuming countries (19), suggesting that soy foods might impair fertility in Western men is not as nonsensical as might first appear. Estrogen is involved in sperm production (20, 21), and isoflavones (especially genistein) affect sperm in vitro (22–26) and reproductive health in animals (27–31). In addition, it is yet to be definitively established whether Asians and non-Asians respond similarly to isoflavones. In theory, the long history of exposure, traditional lifestyle factors (including but not limited to dietary habits), and possible genetic differences could limit the ability to extrapolate findings from Asians to non-Asians. In fact, preliminary evidence indicates there may be some differences in isoflavone metabolism between these two groups (32, 33). Another obvious difference is that Asian men are exposed to isoflavones from an early age via the consumption of

Received March 3, 2010; accepted March 3, 2010; published online April 8, 2010.
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traditional soy foods, whereas exposure in non-Asian men usually begins later in life, although there is no evidence indicating that this differing exposure pattern would affect the possible effects of isoflavones on fertility-related parameters.

Of potential relevance to the fertility issue is the recently expressed concern that environmental hormones, including dietary phytoestrogens (9), but especially pesticides (34), act through multiple mechanisms to adversely affect reproduction. To this point, in a small Indian study, infertile men ($n = 21$) with low sperm count (<20 million/ml) and no obvious etiology for their condition were found to have higher seminal plasma levels of polychlorinated biphenyls and phthalate esters than fertile controls ($n = 32$). Furthermore, xenoestrogen concentrations were inversely related to total motile sperm counts in the former group of men (35).

Interestingly, the possibility that isoflavones impair fertility has been a subject of discussion for more than 60 years (36). The potential biologic effects of isoflavones first came to the attention of the scientific community in the 1940s, because of breeding problems in female sheep in Western Australia that grazed on a type of clover rich in isoflavones (36–38). Three decades later, Setchell et al. (39) established that the isoflavone-rich soy, which was part of the standard diet of captive cheetahs in North American zoos, was a factor in the decline of their fertility.

However, the fertility problems in these species occurred in females and not males, and problems likely occurred in the cheetah because felines are barely able to glucuronidate phenolic compounds (40-43). Because glucuronidation is a primary step in the bodily elimination of isoflavones, circulating levels in the cheetah are much higher than they would be in species that readily possess this ability, such as humans (44, 45). It is widely recognized that there is much species variation in the metabolism of and biological response to isoflavones (38, 43). In the case of sheep, serum levels of the isoflavone equol (a bacterially-derived metabolite of the soybean isoflavone daidzein) far exceeded anything approaching human levels simply because daily isoflavone intake was estimated to be as much as several grams, which dwarfs the average Japanese intake of approximately 40 mg/d (46).

The aim of this review is to evaluate evidence most relevant to the possible feminizing effects of soy food and isoflavone exposure in men with a specific focus on the clinical data. Before doing so, brief background information on isoflavones is provided.

BACKGROUND ON ISOFLAVONES

Isoflavones have been the subject of intense investigation, as evidenced by the more than 10,000 peer-reviewed journal articles published during the past 20 years since the U.S. National Cancer Institute first announced a research program aimed at establishing the possible chemopreventive properties of these diphenolic molecules (47). Isoflavones have a limited distribution in nature, and among commonly consumed foods they are found in physiologically relevant amounts only in soybeans and foods derived from this legume (4), although a variety of plants such as red clover (48) are also rich sources. One serving of a traditional soy food contains approximately 25 mg of isoflavones (approximately 3.5 mg per gram of protein; isoflavone amounts in this text refer to the aglycone weight) (46).

In total, there are 12 different soybean isoflavone isomers. These are the three aglycones genistein (4',5,7-trihydroxyisoflavone), daidzein (4',7-dihydroxyisoflavone), and glycitein (7,4'-dihydroxy-6-methoxyisoflavone); their respective β-glycosides genistin, daidzin, and glycitin; and the three β-glucosides esterified with either malonic or acetic acid. In nonfermented soyfoods, nearly all of the isoflavones are present as glycosides; however, more of the isoflavones are present as aglycones in fermented soy foods because of microbial hydrolysis (49). Typically, there is somewhat more genistein/genistin than daidzein/daidzin in soybeans and soy foods, whereas glycitein/glycitin comprises only 5%–10% of the total isoflavone content.

To absorb isoflavones present in the intestine as glycosides, the sugar molecule must first be hydrolyzed, which occurs in a relatively efficient manner (45, 50). Once absorbed, isoflavones circulate primarily as the glucuronide and to a lesser extent sulfate conjugate. Generally, only 1%–2% is in the unconjugated and biologically active form (44, 51). In response to an isoflavone intake of between 50 and 100 mg/d, peak serum levels can reach the low micromolar range, although there are huge interindividual differences in isoflavone metabolism such that levels of the parent isoflavones and their metabolites vary markedly among subjects in clinical studies (52).

Isoflavones have a chemical structure similar to the hormone estrogen, bind to and transactivate estrogen receptors (ER) (53–55), and exert estrogen-like effects under certain experimental conditions in vitro and in vivo (56). Isoflavones have been classified by some as selective estrogen receptor modulators (mixed estrogen agonists/antagonists) (57–59), in part because of their preferential binding to and transactivation of ER-β compared with ER-α (54, 60). Therefore, isoflavones have been discussed as possible natural alternatives to conventional hormone therapy, and most of the clinical research involving isoflavones has focused on understanding their effects in postmenopausal women (61, 62).

Aside from ER binding, isoflavones also exert nongenomic actions that modulate a diverse array of intracellular signaling cascades (63, 64), including affecting the activity of enzymes involved in hormone synthesis and metabolism (65–67), although the concentrations required to exert these effects often exceed circulating levels of isoflavones attained in vivo. There is a need to better establish tissue isoflavone concentration, because relatively limited work in this area has been conducted, although there is some suggestion that isoflavones may be concentrated in tissues relative to serum and plasma (68–70). Given the potential hormone-dependent and independent effects of isoflavones, the interest in establishing their effects on hormone status and balance in both men and women is understandable.

GYNECOMASTIA, ESTROGEN, AND TESTOSTERONE

Findings from case reports are not a basis for forming conclusions, but they can be grounds for hypothesis generation. In 2008, Martinez and Lewis (8) published a case report of a 60-year old man with gynecomastia and dramatically elevated estrogen levels thought to have resulted from the consumption of isoflavone-containing soymilk. Gynecomastia is not merely excessive breast adipose tissue, rather it is a benign enlargement of the male breast attributable to proliferation of the ductular elements (71). This condition is actually common, occurring in 50%–70% of boys during puberty (72) and 30%–70% of men (73-76).

The subject described in the case report (8) was said to have consumed 3 quarts of soymilk daily, an amount (assuming it is made using the whole soybean) that would be expected to provide approximately 300 mg of isoflavones (the authors suggested 361 mg) (46). In comparison, typical intake among older men in Japan (46) and Shanghai (77) is approximately 40 mg/d. Clearly, excessive intake of even very nutritious foods can produce untoward effects. In fact,

aside from the extreme isoflavone intake, it is worth noting that if the soymilk in question was calcium-fortified, or the subject had instead consumed a similar amount of cow's milk, calcium intake would have exceeded the upper safe limit by approximately 50%, which could have led to serious adverse effects such as hypercalemia (78).

The biologic basis for the gynecomastia described in the case report appears to be the rise (9- to 25-fold compared with levels following discontinuation of soymilk intake) in estrogen levels, which were 5- to 10-fold above average reference values. Therefore, regarding gynecomastia and isoflavones, a critical issue is to establish the effect of the latter on circulating estrogen levels. Because an imbalance between estrogens, which stimulate, and androgens, which inhibit breast tissue proliferation, is often the cause of gynecomastia (71), it is also necessary to consider the effects of isoflavones on androgen levels.

Although the principle sex hormone in men is T, estradiol (E_2), and estrone are also endogenously synthesized. Approximately 20% of estrogen is produced via testicular secretion (79), whereas the remainder is derived from peripheral aromatization of androgens mainly in the adipose tissue, skin, and muscle (80). As is the case for T concentrations, estrogen levels in men decline with age (81); nevertheless, blood E_2 levels are actually higher in older men than in postmenopausal women (80–82). Knowledge is increasing about the important role estrogen plays in the health of men. For example, epidemiologic research suggests that circulating estrogen levels are better than T levels as predictors of bone mineral density in older men (83).

Soy, Isoflavones, and Estrogen

Nine studies were identified that examined the effects of isoflavone supplements or soy foods on 17β-E_2 and/or estrone levels in men (84–92). As shown in Table 1, three studies (85, 86, 92) included older (>40 years) and six (84, 87–91) included younger men. One study involved men at elevated risk for prostate cancer or who had low-grade prostate cancer (85). Study duration ranged from 3 weeks to 6 months, and in five studies daily isoflavone dose exceeded 80 mg. The highest exposure level in any study was 139 mg/d. Because changes in hormone levels respond quickly to a variety of influences including dietary changes, the relatively short duration of several studies would appear not to limit their ability to observe effects (93).

Of the nine studies, two reported that following soy/isoflavone exposure, statistically significant changes in estrogen levels occurred in comparison with baseline values and/or control group (85, 87). One of these was a 6-month study involving older men that found that estrogen levels increased by approximately 20% in response to the consumption of 40 g/d isolated soy protein (ISP; ISP by definition is at least 90% protein) that provided 6 mg of isoflavones (85). In the other study, which involved young men, much smaller increases were noted in response to the consumption of 32 g/d ISP that was almost devoid of isoflavones (87). In both studies, estrogen levels remained well within the normal range. In contrast to these two studies, Nagata et al. (90) noted that estrone concentrations tended to decrease in the soymilk-supplemented group and increase in the control group.

No significant changes were noted in the other six studies. Furthermore, in the two studies in which estrogen levels increased, there were no changes in the men consuming ISP that provided much larger amounts of isoflavones—107 mg in one case (85) and 62 mg in the other (87). Thus, it is likely the small increase in estrogen levels in response to ISP low in or nearly devoid of isoflavones in these two studies occurred by chance. Finally, aside from the clinical data, one relevant epidemiologic investigation was identified. In this small study ($n = 69$) of older Japanese men (mean age $\pm$ SD, 60.5 $\pm$ 10.7 years) an inverse association between soy product and isoflavone intake (mean intake, 21.9 mg/d $\pm$ 8.7) and serum E_2 levels was noted (94).

Overall, the findings indicate that, even in response to isoflavone intakes that greatly exceed typical Japanese values and amounts obtainable through reasonable dietary behavior, isoflavones do not affect estrogen levels. Therefore, if isoflavones were responsible for the rise in estrogen levels and the gynecomastia noted in the case report (8), it was almost certainly caused by excessive isoflavone intake—and possibly also because the subject in question was particularly sensitive to isoflavones—as the highest isoflavone exposure (86) in the studies in Table 1 was less than half the estimated exposure that reportedly led to the gynecomastia.

Soy, Isoflavones, and Testosterone

As noted previously, androgens inhibit breast tissue proliferation; this accounts for why prostate cancer patients treated by androgen deprivation or blockade frequently experience new-onset breast pain, tenderness, and enlargement (95). Several animal studies (96–99), but not all (100, 101), found that isoflavone exposure decreases circulating T levels, but increases have also been noted (102). Furthermore, in some cases in which decreases occurred, isoflavones were presented in isolated form, not isoflavone-rich soy, and exposure included the in utero and/or neonatal or infancy periods (98, 99). The focus of this article is on isoflavone exposure during adulthood. Nevertheless, because one animal study that reported decreases has received much attention, it is discussed here.

In this study, the feeding of soy formula (estimated isoflavone exposure, 1.6–3.5 mg/kg/d) to male infant marmoset monkeys decreased serum T concentrations, increased Leydig cell numbers at the end of formula feeding, and led to larger testes and lower serum T concentrations during adulthood (103). However, these animals went through normal puberty and were fertile as adults (104). Furthermore, infant monkeys convert most of their ingested daidzein into the highly estrogenic isoflavone metabolite equol (44), which has been shown to drive conversion of T to dihydrotestosterone (105). Human infants do not produce equol, and there are no reports of enlarged testes in infants fed soy formula (44). As discussed by Badger et al. (44), when piglets were fed milk infant formula, soy infant formula or were fed by a sow (breast fed) from 48 h through 21 d, testicular weights did not differ among diet groups (106). The neonatal pig models human growth, development, metabolism, and endocrine systems and like human infants, piglets do not produce equol.

Considerably more studies have examined the effects of isoflavone-containing products on circulating T levels in men than have evaluated estrogen. Although some studies reported decreases (88, 107), a recently published metaanalysis by Hamilton-Reeves et al. (108) concluded that neither soy protein nor isoflavone intake affects circulating levels of total T, sex hormone binding globulin, free T, or the free androgen index. The metaanalysis included 15 placebo-controlled treatment groups, with baseline and ending measures, and 32 reports involving 36 treatment groups in which hormones were assessed in simpler models. In general, isoflavone intake in these studies greatly exceeded typical dietary Japanese intake, although the duration of most trials was <6 mo. However, a recently presented 2-year study that is available only as an abstract found no effects of 20 g/d ISP that provided 43 mg isoflavones (24–26 mg genistein) on T levels in men with high-grade prostatic intraepithelial neoplasia (109).

Finally, there is one additional report of relevance to the subject of isoflavone exposure and breast enlargement (110). In this study, 20

men aged ≥40 years (mean age ± SD, 68.9 ± 7.3 years) with stage B, C, or D adenocarcinoma of the prostate, were treated with 450 or 900 mg isoflavones per day for 84 days. Three men, all of whom were consuming the higher dose of isoflavones, developed gynecomastia. However, one subject in which gynecomastia occurred was given a stable regimen of the anti-androgen bicalutamide and exhibited breast tenderness at baseline. Moreover, the gynecomastia continued after discontinuing isoflavones. In another subject with grade 2 gynecomastia, the condition was present at baseline and was attributed to the use of PC-SPES, a proprietary combination of eight herbs known to have associated estrogenic side effects, including gynecomastia (111, 112). It was unclear whether the third case of gynecomastia was caused by isoflavones, but it was shown to be a transient effect. Considering the extreme dose to which the men were exposed, the general lack of gynecomastia in this study attributable to isoflavones is notable.

ERECTILE DYSFUNCTION

Erectile dysfunction (ED) affects more than 18 million men in the United States or 18.4% of the male population aged 20 and over when defined as "sometimes able" or "never able" to get and keep an erection (113). Furthermore, it is estimated that in 1995 there were >152 million men worldwide who experienced ED, and by 2025 the prevalence will increase to approximately 322 million (114). Risk factors include diabetes, hypertension and cardiovascular disease (113). ED can have a neurogenic, psychogenic, or endocrinologic basis. For example, it was reported recently that hypothyroidism is associated with ED and that treatment of thyroid dysfunction restores function (115). For this reason, it is important to note that there has been considerable investigation of the effects of soy on thyroid function, but no further comment on this issue will be made because there is essentially no evidence that soy adversely affects the thyroid in healthy individuals (116).

The most common cause of ED is thought to be related to vascular abnormalities of the penile blood supply and erectile tissue, which are often associated with cardiovascular disease and its risk factors (117). ED is also thought to result from a functional excess of E_2 or a decrease in T that offsets the normal E_2-T balance (118). It has been suggested that inadvertent exposure to environmental estrogens could disturb this balance and result in ED, especially if exposure comes during older age, when T levels naturally decline and obesity (possibly leading to hyperestrogenism) and the metabolic syndrome are more likely to be present (119, 120).

Claims that isoflavones can lead to ED are based primarily on research in male Sprague-Dawley rats conducted by Chinese investigators (97, 121, 122). In their most relevant study, when 9-10-week-old rats were orally gavaged daily with daidzein at a dose of 20 or 100 mg/kg body weight (bw) for 90 d, the relative content of the collagen fibers in the corpora cavernosa (corpus cavernosum penis is one of a pair of sponge-like regions of erectile tissue that contain most of the blood in the male penis during erection) was significantly increased, and the smooth muscle cell and elastic fiber content significantly reduced compared with that of the controls (122). In contrast, no effects were noted in response to a daidzein dose of 2 mg/kg bw. The authors of this study commented that the higher-dose findings might be suggestive of ED. However, assuming that the model is potentially predictive of effects in humans, the dose of daidzein required to exert effects on the corpora cavernosa raises questions about the relevance of the findings.

Humans consuming soy foods are exposed to all three isoflavones, so it is difficult to speculate about the extent to which exposure to isolated daidzein mimics the effects of daidzein when combined with genistein and glycitein as they occur in soy foods, because there is evidence that interactions occur among the isoflavones (123). In any event, the lowest daidzein dose (20 mg/kg bw) at which effects occurred is approximately 100-fold higher than typical human exposure. Of the 40 mg/d isoflavones typically consumed by older Japanese and Chinese men from Shanghai, approximately 15 mg of that is daidzein. Therefore, assuming a body weight of 55 kg (124), daidzein exposure on a body weight basis in Asian men is approximately 0.27 mg/kg. When correcting the dose for differences in body surface area (metabolic rate) between rats and humans, the effective human dose is still 13-fold higher (125). Although, if one assumes that genistein has a similar effect as daidzein, the effective dose is reduced by approximately half.

The major issue, however, is not necessarily daidzein exposure per se, rather that rats convert essentially all of the daidzein they absorb into the isoflavone equol (33). For this reason, in response to the consumption of soy foods and mixed isoflavones as found in soybeans or daidzein, circulating equol levels in rats and monkeys (126) far exceed those of daidzein and genistein (44, 127). Chemically, equol and daidzein are different molecules, and they often exert different physiologic effects (33). Although relative potency varies according to the outcome measure in question, at least in regard to ERα relative binding affinity, equol can be 100-fold more potent than daidzein (128, 129). Furthermore, in the circulation, the percentage of free and biologically active versus bound equol is much higher than it is for daidzein, further increasing the difference in potency between the two molecules (130).

In contrast to rats, only approximately 25% of Westerners (approximately 50% of Asians) possess the intestinal bacteria that convert daidzein into equol, and in those who do, less than half is converted (33). Consequently, in response to soy food intake, even in equol producers, circulating levels of genistein (and to a lesser extent daidzein) far exceed equol levels (131). Thus, there is little justification for extrapolating results from rodents given daidzein to humans given soy foods or mixed isoflavones. Furthermore, and of critical importance, is the finding that in Sprague-Dawley rats the two doses (20 and 100 mg/kg bw) of daidzein that affected the corpora cavernosa also decreased circulating T levels by more than 50% (97). It is this decrease that is thought to account for effects on the corpora cavernosa (122). As already discussed, no such decrease in T occurs in men (108). Thus, it would appear that the rodent findings do not provide a reasonable basis for raising a concern that soy food consumption increases the risk of developing ED (97, 121, 122). Of potential relevance, and in support of this conclusion, is the recent finding in prostate cancer patients undergoing androgen deprivation therapy that exposure to high dose isoflavones (160 mg/d) was neither beneficial nor harmful in terms of libido or erectile function (132). However, the extent to which these data apply to healthy eugonadal men is uncertain.

Finally, it should be noted that in New Zealand rabbits, a 1000-fold lower daidzein dose (100 μg/d or approximately 30 μg/kg bw) than was used in the previously discussed rat studies (97, 121, 122) significantly potentiated norepinephrine-induced antierectile contraction of the corpora cavernosa. In addition, in organ bath experiments, relaxant responses to acetylcholine, nitroglycerin, and nitrergic transmission were significantly attenuated compared with the control response (133). However, these changes occurred in animals whose circulating T levels were also dramatically lowered by more than 50% in response to the intervention.

TABLE 1

Studies evaluating the effects of isoflavone exposure on circulating estrogen levels in men.

Reference	Study length	Study type	N	Age (y)	BMI	Experimental group	17β-Estradiol		Estrone	
Tanaka, 2009 (84)	3 mo	SG	10	36.7 ± 5.2	64.8 ± 4.1	60 mg IF	Pre[a]	Post[a]		
			18	43.2 ± 7.8	67.8 ± 3.9	(Equol non-producers)	25.5 ± 7.2	27.0 ± 7.6		
						60 mg IF (Equol producers)	24.7 ± 4.7	24.6 ± 5.4		
Hamilton-Reeves, 2007 (85)	6 mo	P	18	68 ± 6.7	32 ± 6	Milk protein isolate (40 g)	Pre[b]	Post[b]	Pre[b]	Post[b]
			20	68 ± 6.5	29 ± 4	ISP-Low (40 g, 6 IF mg)	69 ± 3	66 ± 3	158±8	150±10
			20	68 ± 6.8	30 ± 5	ISP-High (40 g, 107 IF mg)	66 ± 4	79 ± 3	141±10	171±10
							67 ± 4	69 ± 3	157±15	152±10
							$P < 0.05$		$P < 0.05$	
Goldin, 2005 (86)	6 wk	XO	10	61	26	Animal protein	Final values[b]		Final values[b]	
						Animal protein + 139 ± 35 mg IF	52.5 ± 60.2 (56.3)		67.9 ± 95.1 (80.3)	
						ISP –low (71 ± 18 g; minimal IF	52.4 ± 59.6 (55.9)		60.7 ± 84.6 (71.7)	
							44.4 ± 66.5 (54.3)		60.6 ± 141.1 (92.5)	
						ISP –high (71 ± 18 g; 139±35 mg IF)	52.9 ± 59.5 (56.1)		79.0 ± 116.6 (96.0)	
Dillingham, 2005 (87)	57 d	P	35	27.9 ± 5.7	25.3 ± 3.1	Milk protein isolate	Pre[c]	Post[c]	Pre[c]	Post[c]
					25.1 ± 3.0	(31.9 g)	81.5 (78.2, 84.8)	77.9 (74.4, 81.4)	159.4 (151.9, 166.8)	155.7 (148.4, 163.0)
					25.3 ± 3.2	ISP-Low IF (31.9 g, 1.6 ± 0.2 mg IF)	82.3 (79.0, 85.7)	85.1 (81.6, 88.6)	158.3 (150.8, 165.8)	172.4 (165.0, 179.7)
							81.1 (77.7, 84.4)	82.0 (78.5, 85.5)	163.8 (156.3, 171.2)	164.1 (156.7, 171.4)
						ISP-High IF (31.9 g, 61.7 ± 7.4 mg IF)	$P < 0.05$ from milk group		$P < 0.05$ from milk group	
Gardner-Thorp, 2003 (88)	6 wk	SG	19	35.6 ± 11.2	25.6 ± 3	Soy flour (120 mg IF)	Pre[d] 102.80 (93.07, 113.55) Post[d] 98.97 (87.82, 111.53)		Pre[d] 120.23 (107.85, 136.21) Post[d] 115.88 (103.09, 130.26)	
Mitchell, 2001 (89)	2 mo	SG	14	18–35	NR	40 mg IF	Monthly values[e] 38.2, 35.2, 36.2, 36.3, 33.4, 35.6, 34.1			
Nagata, 2001 (90)	8 wk	P	17	32.8 ± 8.3	23.8 ± 2.9	Control (usual diet)	Pre[f]	Post	Pre[f]	Post
			17	32.0 ± 8.4	23.3 ± 3.2	Soymilk (360.0 ± 41.0 ml) (80.8 ± 17.4 mg IF)	29.6 ± 5.3	NR	27.8 ± 9.7	NR
							33.6 ± 15.2	NR	29.5 ± 11.4	NR
							1.46[g]	1.44[g]	1.42[g]	1.44[g]
							1.49[g]	1.50[g]	1.43[g]	1.42[g]
Higashi, 2001 (91)	3 wk	SG	12	30 ± 2	22.4 ± 2.4	ISP (no vitamin E) 20 g[h]	Pre[i]	Post[i]		
						ISP (+ vitamin E) 20 g[h]	35.2 ± 6	36.9 ± 11.5		
							38.5 ± 13.6	35.6 ± 15.1		

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TABLE 1

Continued.

Reference	Study length	Study type	N	Age (y)	BMI	Experimental group	17β-Estradiol	Estrone
Habito, 2000 (92)	4 wk	XO	42	45.7 ± 7.6	26.2 ± 3.3	Red meat (raw weight, 150 g) Tofu (raw wt, 290 g; 35 g protein)[n]	Post 69.6 (60.6, 78.6)[j] Post 74.2 (65.6, 82.9)	

Note: BMI = body mass index; SG = single group; IF = isoflavones; P = parallel; XO = cross-over; NR = not reported.

[a] Value (pg/ml) is mean ± standard deviation.

[b] Value (pmol/l) is mean ± standard error mean (geometric means).

[c] Value (pmol/l) is least squares mean (95% confidence interval).

[d] Value (pmol/l) is geometric mean (95% confidence interval).

[e] Value (pg/ml) is mean for two months prior to, two months during, and three months following, isoflavone supplementation.

[f] Value (pg/ml) is mean ± standard deviation.

[g] Value (pg/ml, $\log_{10}$ transformed) was estimated from the graphs in the published paper.

[h] Isoflavone content not reported.

[i] Value (ng/dl) is mean ± standard deviation.

[j] Value (pmol/l) is mean plus (95% confidence interval).

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SPERM AND SEMEN EFFECTS

Infertility affects approximately 10%–15% of reproductive-age couples, and infertility is attributable to poor semen quality in 25% of cases (134). Because the etiology and pathogenesis are still not fully understood, a significant proportion of male infertility is considered to be idiopathic (135). In 1992, on the basis of a comprehensive review of the literature, Carlsen et al. (136) concluded that there had been a decline in semen quality over the past 50 years and that "as male fertility is to some extent correlated with sperm count the results may reflect an overall reduction in male fertility." However, Fisch (137) concluded that the bulk of the evidence refutes claims for a widespread decline in semen parameters. Interestingly, recent evidence suggests that good semen quality might be a fundamental biomarker of overall male health (138).

One theory proposed to explain the possible decrease in semen quality is exposure to xenoestrogens and antiandrogens, some of which are present in the diet (139). As noted, soy phytoestrogen exposure has been suggested to adversely affect male reproduction, although the data in support of this concern come largely from in vitro and animal studies. Furthermore, proponents of this hypothesis primarily cite in utero and infancy as the key exposure periods for effects to occur (9, 22, 140). Hypothyroidism can adversely affect spermatogenesis (141), but because only morphology is affected and, as noted previously, there is essentially no evidence that soy adversely affects thyroid function in healthy individuals, no further comment about this relationship will be made (116).

The effects of isoflavone exposure on sperm and semen parameters have been examined in vitro (22, 24), in animals (142, 143), and in clinical trials, but a pilot cross-sectional epidemiologic study by Chavarro et al. (10) brought widespread attention to this area of research. This study involved 99 male partners of subfertile couples who received semen analyses at the Massachusetts General Hospital Fertility Center. The intake of 15 soy-based foods in the previous 3 months was assessed. In multivariate analysis, men in the highest intake category of soy foods had an average of 41 million sperm/ml less than men who did not eat soy foods (P=0.02).

The inverse association between soy food intake and sperm concentration remained significant after accounting for age, abstinence time, body mass index, caffeine and alcohol intake, and smoking. There was a monotonic response between soy intake and sperm concentration, and there were no associations between soyfoods or isoflavones and total sperm count, ejaculate volume, sperm motility, or morphology in univariate or multivariate analyses. Furthermore, soy food intake had little effect on sperm concentration at the lower end of the distribution, and there was a nonsignificant trend toward the association between soy food intake and sperm concentration being more pronounced among overweight and obese men. If there is an interaction between obesity and isoflavone exposure, the general thinness of Asian compared to non-Asian men could explain why isoflavones have not led to any obvious fertility problems in soy food–consuming countries (124).

Despite the preliminary nature of the findings by Chavarro et al (10), they have raised concern because this epidemiologic study is the first of its kind, aside from a small pilot epidemiologic study that found isoflavone intake among fertile controls (n = 10) was higher (genistein, 527 ± 183 μg/d versus 1,722 ± 714 μg/d) than among infertile men (n = 48) (144). Higher isoflavone intake was also associated with levels of good sperm DNA integrity, sperm count, and sperm motility. However, this study is published only as an abstract, and the low isoflavone intake among the Caucasian

U.S. men in this study raises questions about the biologic relevance of the findings (145).

For at least two reasons, the findings by Chavarro et al. (10) would appear to have little if any implication for fertility. First, as noted, soy intake had relatively little effect on men with the lowest sperm concentration. Thus, men who were most affected by soy still had a sperm concentration above the level (>20 million/ml) classified by the World Health Organization as oligospermia (146). Second, soy had no effect on sperm morphology or motility, two measures of semen quality that are thought to impact fertility (146).

Furthermore, there were several weaknesses to the study design by Chavarro et al. (10) that highlight the pilot nature of this research. For example, other than soy intake, no dietary information was collected, and the instrument used to estimate soy food intake was not validated. The lack of dietary data is potentially important for two reasons. First, foods and specific nutrients are thought to affect sperm quality and concentration. For example, Wong et al. (147) found in a 26-wk intervention study that supplements of folic acid (5 mg/d) and zinc sulfate (66 mg/d) increased sperm count in both fertile and infertile men. In addition, omega-3 fatty acid intake can favorably affect sperm and semen parameters, whereas diets high in omega-6 fatty acids can have the opposite effect (148). In addiiton, dietary protein type can have an influence. Vervet monkeys (*Cercopithecus aethiops*) that consumed diets containing milk solids had significantly lower sperm counts and reduced sperm motility compared with monkeys that consumed plant protein from maize plus legumes, although differences were noted only when the total protein content of the diet was relatively low (9%) (149). Even intelligence, which was not measured, is correlated with semen quality (150). That multiple dietary factors can affect sperm and semen is particularly relevant because in Western countries, where soy foods are not a traditional part of the diet, the dietary habits of soy food consumers are likely to be rather different from their non–soy-consuming counterparts.

In addition to the points raised above, the findings by Chavarro et al. (10) are somewhat puzzling in that much of the decreased sperm concentration appeared to be the result of an increase in ejaculate volume, although this effect was not statistically significant. For example, for soy foods, genistein, and daidzein intakes, ejaculate volume increased 17%, from 3.5 to 4.1 ml, when comparing the fourth with the first intake quartiles, which explains why total sperm count was unaffected. An increased ejaculate volume in response to soy seems biologically implausible. Related to this point is that median soyfood intake was only 0.54 servings/d, an amount providing approximately 13 mg total isoflavones. For such a relatively small amount of isoflavones to have such a pronounced effect on sperm concentration would be unexpected, although this is obviously conjecture.

Three clinical studies (89, 151), one of which is available only as abstract (152) and one as case report (153), have examined the effects of isoflavone exposure on sperm and semen parameters. In contrast to the epidemiologic research by Chavarro et al. (10), the intervention data show that isoflavone exposure does not lower sperm concentration. In the first study to examine this issue, Mitchell et al. (89) took semen and monthly blood samples from 14 healthy young men between the ages of 18 and 35 years, beginning 2 months prior to the administration of a supplement that provided 40 mg/d isoflavones and for 2 months during and for four months after supplementation. Blood genistein levels reached approximately 1 μM indicating compliance was good. There were no changes in ejaculate volume, sperm concentration, count, or motility. In addition, computer-assisted sperm assessment indicated that there was no effect on sperm movement.

In a second study conducted by researchers from the University of Guelph and the Fred Hutchinson Cancer Research Center in Seattle, 32 healthy young men consumed diets in random order that were supplemented with 32 g protein provided in the form of milk protein isolate, low-isoflavone ISP (approximately 1.6 mg/d isoflavones) or high-isoflavone ISP (approximately 62 mg/d isoflavones) for 57 d each, separated by 28-d washout periods (151). Analysis of semen samples collected on d 1 and 57 of each treatment period revealed no significant effects of diet on semen parameters including semen volume, sperm concentration, sperm count, total motile sperm count, sperm motility, and sperm morphology. In a third study by Unfer et al. (152) from the AGUNCO Obstetric and Gynecology Centre, Rome, Italy, 20 volunteers were randomized into three groups and received 160, 320, or 480 mg/d isoflavones, respectively, for 3 months. When compared with baseline, there were no significant differences in ejaculate volume, sperm concentration, sperm count, and motility of spermatozoa in men given isoflavones.

One obvious limitation to the intervention data is that two of three studies lacked a control group. In addition, the ideal length of a trial examining effects on spermatogenesis is likely somewhat longer than the 2-month duration of two of the trials, because spermatogenesis in healthy men takes approximately 64 days (154). It is likely however, that if isoflavones affected spermatogenesis, 2 months would be sufficiently long to observe at least some effects. Furthermore, the 3-month trial exposed men to extremely high amounts of isoflavones.

Aside from design type, one notable difference between the epidemiologic study by Chavarro et al. (10) and the intervention research described above is that the subjects were infertile men in the former. Whether fertility per se affects the response to isoflavones is unknown. For this reason, the case report by Casini et al. (153) involving a 30-year old male with severe oligospermia (10 million/ml) and abnormal sperm motility and morphology, and who was given soybean isoflavones (80 mg/d) for 6 mo, is particular intriguing. The couple had been trying to conceive for 3 years, and the woman was healthy at the clinical and endocrinologic examination. No other parameters except sperm count, motility, and morphology were altered in the man. During the third month of supplementation, semen parameters improved dramatically (sperm count, 45 million/ml; >50% motility; >30% normal sperm morphology); therefore, intrauterine insemination was performed. This treatment resulted in pregnancy, and a healthy baby weighing 3,300 g was born. After 6 mo of treatment, sperm parameters maintained their improvement (sperm count, 50 million/ml; >50% motility; >35% normal sperm morphology). However, 6 mo after termination of isoflavone supplementation, sperm parameters had deteriorated (sperm count, 18 million/ml; <20% motility; <10% normal sperm morphology). Casini et al. (153) commented that their results suggest "a possible therapeutic role for phytoestrogens in the treatment of oligospermia," but also noted that a randomized control trial was needed to confirm their findings.

SUMMARY AND CONCLUSIONS

Isoflavones are diphenolic molecules that have a chemical structure similar to estrogen and exert estrogen-like effects in some tissues under certain conditions. Consequently, concern has been raised that isoflavones have feminizing effects in men and adversely affect male reproductive health. Findings that support this concern include those from a pilot epidemiologic study that linked soy intake with

lower sperm concentration among infertile men (10), a case report linking excessive isoflavone intake with raised estrogen levels and gynecomastia (8), limited clinical research showing soy intake lowers blood T levels (88, 107), and rodent research suggesting isoflavone (daidzein) exposure can lead to ED (97, 121, 123, 133). However, the clinical evidence overwhelmingly indicates that there is essentially no basis for concern. Isoflavone exposure at levels even greatly exceeding reasonable dietary intakes does not affect blood T or estrogen levels in men or sperm and semen parameters. The ED-related findings in rats can be attributed to excessive isoflavone exposure and to differences in isoflavone metabolism between rodents and humans. Thus, men can feel confident that making soy a part of their diet will not compromise their virility or reproductive health.

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