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Soy Protein Isolates of Varying Isoflavone Content Exert Minor Effects on Serum Reproductive Hormones in Healthy Young Men[1,2]

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ABSTRACT Inverse associations between soy and prostate cancer and the contribution of hormones to prostate cancer prompted the current study to determine whether soy protein could alter serum hormones in men. Thirty-five men consumed milk protein isolate (MPI), low-isoflavone soy protein isolate (SPI) (low-iso SPI; 1.64 ± 0.19 mg isoflavones/d), and high-iso SPI (61.7 ± 7.35 mg isoflavones/d) for 57 d each in a randomized crossover design. Twenty-four-hour urine samples indicated that urinary isoflavones were significantly increased by the high-iso SPI relative to the low-iso SPI and MPI. Serum collected on d 1, 29, and 57 of each treatment revealed that dihydrotestosterone (DHT) and DHT/testosterone were significantly decreased by the low-iso SPI [9.4% (P = 0.036) and 9.0% (P = 0.004), respectively] and the high-iso SPI [15% (P = 0.047) and 14% (P = 0.013), respectively], compared with the MPI at d 57. Other significant effects included a decrease in testosterone by the low-iso SPI relative to the MPI (P = 0.023) and high-iso SPI (P = 0.020) at d 29; an increase in dehydroepiandrosterone sulfate by the low-iso SPI relative to the MPI at d 29 (P = 0.001) and relative to the MPI (P = 0.0003) and high-iso SPI (P = 0.005) at d 57; and increases in estradiol and estrone by the low-iso SPI relative to the MPI at d 57 (P = 0.010 and P = 0.005, respectively). In conclusion, soy protein, regardless of isoflavone content, decreased DHT and DHT/testosterone with minor effects on other hormones, providing evidence for some effects of soy protein on hormones. The relevance of the magnitude of these effects to future prostate cancer risk requires further investigation. J. Nutr. 135: 584–591, 2005.

KEY WORDS: • *soy protein* • *isoflavones* • *healthy men* • *reproductive hormones*

The frequent occurrence of prostate cancer in North American men (1) has prompted investigation into strategies for its prevention. Ecological data reveal that Asian populations have significantly lower prostate cancer rates (1) and migration studies (2,3) suggest that this difference may be related to Asian lifestyle factors such as their high consumption of soy.

There is a growing body of literature supporting a role for soy and its constituent isoflavones in the reduction of prostate cancer risk. Epidemiological studies conducted within multiple ethnicities have related higher soy food (4–8) and soy isoflavone (8) consumption to reduced prostate cancer risk, including one study that reported a 70% reduction in prostate cancer risk associated with consumption of soy milk among Seventh Day Adventist men (5). Serum concentrations of isoflavones have also been related to prostate cancer risk as demonstrated in a recent Japanese case-control study reporting dose-dependent inverse associations between prostate cancer risk and serum genistein, daidzein, and equol (9). In vitro studies have repeatedly demonstrated the ability of isoflavones to inhibit the growth of prostate cancer cells (10–15) and studies in rats have revealed that consumption of isoflavone-rich soy protein isolate (SPI)[4] (16–19), isoflavone-rich soy flour (20,21), soy phytochemical concentrate (22), and genistin (22) inhibit prostate tumor growth. Also related are reports of increased apoptosis in prostatectomy specimens of prostate cancer patients following consumption of isoflavones extracted from red clover (23,24).

The role of reproductive hormones in the etiology of prostate cancer (25–29), the inverse relation between serum reproductive hormones and prostate cancer risk (26,30), and the structural and functional similarities of soy isoflavones to endogenous estrogen (31–33) have prompted investigation into the relation between soy isoflavones and reproductive hormones. In vitro studies lend support to this relation by demonstrating the ability of soy isoflavones to inhibit steroid

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[4] Abbreviations used: BIA, bioelectrical impedance analysis; DHEA, dehydroepiandrosterone; DHEA-S, dehydroepiandrosterone sulfate; DHT, dihydrotestosterone; DHT/T, dihydrotestosterone to testosterone; FSH, follicle stimulating hormone; high-iso SPI, high-isoflavone soy protein isolate; HNRU, Human Nutraceutical Research Unit; LH, luteinizing hormone; low-iso SPI, low-isoflavone soy protein isolate; MPI, milk protein isolate; ODMA, *O*-desmethylangolensin; SHBG, sex hormone binding globulin; 3α-AG, 3α-androstanediol glucuronide.

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biosynthetic enzymes (34–39); numerous animal studies have reported reduced serum androgens in rats fed isoflavone-rich SPI (18,19), high-isoflavone soy flour (40), high-isoflavone soy flour extract (40), soy meal (41), and genistin (22). Epidemiologic studies relating isoflavone-rich soy consumption to serum hormones in healthy men are less consistent, reporting significant inverse associations with estradiol (42), inverse associations with estrone ($r = -0.24$, $P = 0.05$), testosterone ($r = -0.25$, $P = 0.05$), and free testosterone ($r = -0.25$, $P = 0.06$) (42), and no significant associations with testosterone (43), free testosterone (43), dihydrotestosterone (DHT) (42), 3α-androstanediol glucuronide (3α-AG) (43), sex hormone binding globulin (SHBG) (43), or luteinizing hormone (LH) (43).

The limited intervention studies conducted in healthy men have employed variable study designs, ranged from 3 to 12 wk in duration, and investigated effects of consuming soy foods (44–46), SPI (47–49), or extracted isoflavones (50,51) on circulating reproductive hormones in samples of 6 to 48 men per treatment. Studies investigating soy foods have reported significant decreases in serum estrone (45), testosterone (46), and testosterone/estradiol (44), a significant increase in serum SHBG (44), and no significant changes in serum testosterone (44,45), free testosterone (45), DHT (44,46), 3α-AG (44), estradiol (44–46), estrone (46), or SHBG (45,46) following consumption of soy milk (45), tofu (44), or soy flour scones (46). Studies investigating consumption of 20–40 g of SPI have observed significantly decreased serum SHBG (47), increased serum dehydroepiandrosterone (DHEA) ($P = 0.06$), (47) and no significant effects on circulating testosterone (48,49), free testosterone (47), androstenedione (47), LH (47,48), or follicle stimulating hormone (FSH) (47,48). Finally, within the 2 studies that have investigated extracted isoflavones, one observed a significant increase in plasma DHT (50), but no significant effects were observed on circulating levels of other androgens (50,51), estradiol (51), gonadotropins (51), or SHBG (50).

The purpose of the current randomized crossover study was to advance this research area by investigating the effects of soy protein of varying isoflavone content on a wide profile of serum reproductive hormones in a sample of healthy young men between 20 and 40 y old. The current study included a focus on the isoflavone component of soy by investigating soy protein isolates high and low in isoflavone content in relation to a milk protein isolate.

SUBJECTS AND METHODS

Experimental design. The study protocol was approved by the Human Research Ethics Board of the University of Guelph. The 32-wk study employed a randomized crossover design consisting of three 57-d treatment periods, each separated by 28-d washout periods.

Subjects. Potential subjects were recruited from the local community and screened using a brief phone/e-mail questionnaire followed by a more in-depth questionnaire and meeting with a study coordinator. Inclusion criteria included healthy males, 20 to 40 y old, with a BMI of 19–29 kg/m^2. Exclusion criteria included diagnosis with a disease or serious medical condition, family history of prostate cancer, vasectomy, regular use of medication, smokers, abuse of drugs or alcohol, use of recreational drugs, antibiotic use within the past 3 mo, allergy to milk or soy protein, vegan diet, elite athletes, recent body weight change (>5 kg in the past 6 mo), and intention to gain or lose weight within the following year. All subjects provided written informed consent, attended a study orientation session, and were provided with a study handbook.

Before completing the study, 4 subjects dropped out [job relocation ($n = 3$), disliked the study treatment powder ($n = 1$)] and 3 subjects were excluded [initiated anti-depressants ($n = 1$), initiated antibiotics ($n = 2$)]. In addition, 1 subject who completed the study was excluded from the analyses following examination of urinary isoflavone data that raised concern regarding compliance. A total of 35 subjects were included in the statistical analysis.

Study treatments and diet. During the study, subjects consumed their habitual diets with specific instructions to minimize phytoestrogen consumption through avoidance of soy and soy products, flaxseed, sprouts, beans and legumes, high-fiber foods, and whole grain foods. Subjects were also instructed to avoid milk (due to the calcium content of the study treatment powders), dietary supplements, and green tea and to limit alcohol consumption to 7 drinks per wk with no more than 2 drinks per sitting.

Subjects supplemented their diets with 3 study protein powders including milk protein isolate (MPI), low-isoflavone ethanol-washed soy protein isolate (low-iso SPI), and high-isoflavone soy protein isolate (high-iso SPI) (The Solae Company). Study treatment powders were analyzed in duplicate for isoflavone composition by HPLC in the laboratory of Dr. Patricia Murphy, Iowa State University. The study treatment powders were consumed per kilogram of body weight, matched for protein amount, and provided 0 (MPI), 0.02 ± 0.001 (low-iso SPI), and 0.75 ± 0.01 (high-iso SPI) mg isoflavones/kg body weight/d (mean ± SD), equivalent to total intakes of 0 (MPI), 1.64 ± 0.19 (low-iso SPI), and 61.7 ± 7.35 (high-iso SPI) mg isoflavones/d (mean ± SD), expressed as unconjugated phytoestrogen units. The mean percentage distributions of genistein, daidzein, and glycitein were 78.9, 12.7, and 8.4% within the low-iso SPI and 53.3, 35.6, and 11.1% within the high-iso SPI, respectively. The daily energy, macronutrient, and calcium contribution of the protein powders averaged 236.1 kcal, 31.9 g protein, 23.1 g carbohydrate, 1.48 g fat, and 1269 mg calcium.

Study treatment powders were provided to subjects every 2 wk in daily packets and were available in unsweetened, chocolate, and vanilla flavors. Subjects were provided with multiple suggestions of how to consume the protein powders that were outlined in their study handbook, although the most frequent method was reconstitution with water or fruit juice. Subjects were instructed to record the time and method of how they consumed their study protein powder in their daily study diary.

Data collection. Subjects reported to the Human Nutraceutical Research Unit (HNRU) of the University of Guelph for their study visits. Height without shoes was measured before the study using a metric measuring tape. Fasting body weight was measured before the study and on d 1, 15, 29, 43, and 57 of each treatment period, using a digital scale with subjects wearing a light shirt, pants or shorts, and no shoes. Body composition was measured on d 1 and 57 of each treatment period using bioelectrical impedance analysis (BIA) (BodyStat 1500). BIA measurements were taken with subjects in the prone position with 2 electrodes placed on their right hand (1 over the knuckles and 1 over the wrist bone) and 2 electrodes placed on their right foot (1 over the ankle bone and 1 over the base of the middle toe). To ensure hydration and maximize accuracy of the BIA measurements, subjects were instructed to consume 2 to 4 glasses of water in the 12 h preceding their measurement.

Food records were completed for 3 days before the study and on d 1–3, 26–28, and 54–56 of each treatment period. Subjects received detailed training on the completion of accurate food records and were provided with forms, which included space for time, food or beverage consumed, quantity, description, and preparation method. Subjects were encouraged to provide as much detail as possible, to use household scales and measures, to submit food labels and recipes, and to ask questions at any time.

Fasting blood samples were obtained on d 1, 29, and 57 of each treatment period. Subjects were instructed to avoid food and beverages (except water) for 12 h and avoid alcohol, medications, and ejaculation for 72 h before each blood draw. Timing of blood draws were kept consistent within each subject (20.4 ± 10.0 min; mean ± SD) to minimize confounding due to diurnal variation in reproductive hormones. Blood was drawn into anti-coagulant-free vacutainers, left at room temperature for 30 min, and centrifuged at 1200

× g at 4 C for 15 min. Serum was then divided into aliquots in cryovials and stored at −80 C until analysis.

Complete 24-h urine samples were collected for 3 consecutive days on d 54–56 of each treatment period. Urine was collected into opaque 3-L plastic bottles (VWR International) containing 3 g of ascorbic acid. Subjects were also provided with a 1-L wide-mouth bottle (Nalgene), a lunch-size cooler bag, and an ice pack and were given instructions to transfer their urine to the 3-L container and keep refrigerated at all times. Volume of each 24-h urine collection was recorded and the urine was divided into aliquots in 15-mL polypropylene tubes (Sarstedt) and stored at −20 C until analysis.

Analytical methods. Food records were analyzed using NutriBase IV Clinical Edition 2001 (Cybersoft) and for each 3-d food record mean intakes of energy, macronutrients, calcium, and dietary fiber were calculated.

All serum samples were analyzed in duplicate for androgens (DHT, testosterone, free testosterone, androstenedione, 3α-AG, DHEA, DHEA-S), estrogens (estradiol, estrone), gonadotropins (LH, FSH), and SHBG. Laboratory personnel were unaware of the sample identities during laboratory analysis. To reduce the effects of interassay variability, all samples from the same subject were analyzed in the same batch, along with commercial kit and laboratory-specific control samples. DHT was analyzed using an extraction and oxidation procedure followed by a single antibody-coated tube RIA with ^{125}I-labeled hormone (Diagnostic Systems Laboratories). Testosterone, free testosterone, androstenedione, DHEA-S, and 3α-AG were analyzed using a single antibody-coated tube RIA with ^{125}I-labeled hormone (Diagnostic Systems Laboratories). DHEA, estradiol, and estrone were analyzed using a double-antibody RIA with ^{125}I-labeled hormone (Diagnostic Systems Laboratories). LH and FSH were analyzed using an immunoradiometric assay with ^{125}I-labeled antibody (Diagnostic Systems Laboratories). SHBG was analyzed using an immunoradiometric assay with ^{125}I-labeled antibody (Diagnostic Products). Intra- and interassay variability was 2.64 and 16.2% for DHT, 2.39 and 7.87% for testosterone, 2.47 and 8.14% for free testosterone, 1.53 and 6.64% for 3α-AG, 2.36 and 4.05% for androstenedione, 3.47 and 6.13% for DHEA, 1.74 and 5.73% for DHEA-S, 1.60 and 5.47% for estradiol, 1.54 and 8.55% for estrone, 6.59 and 11.0% for LH, 6.71 and 11.2% for FSH, and 2.80 and 5.38% for SHBG, respectively.

All 24-h urine samples were analyzed for creatinine using an enzymatic ultraviolet method (Randox Laboratories Canada) on a Roche Hitachi 911 with an interassay variability of 1.36%. All 24-h urine samples collected over 3 consecutive days were proportionally pooled and analyzed for urinary isoflavones (genistein and daidzein) and isoflavone metabolites [equol and O-desmethylangolensin (ODMA)] using GC-MS as described previously (52). For isoflavone and isoflavone metabolite results that were below the detection limit in urine, a value halfway between zero and the lowest detection limit was used. Intra- and interassay variability was 4.20 and 11.1% for genistein, 4.27 and 9.74% for daidzein, 5.30 and 16.2% for ODMA, and 4.04 and 16.0% for equol, respectively.

Statistical analysis. Following examination of all data using box plots and residual error plots, all urinary isoflavones and serum concentrations of DHT, 3α-AG, DHEA, DHEA-S, LH, and FSH required log transformation to comply with the normality and equal variance assumptions of the statistical analyses. To ensure the adequacy of the washout period, repeated-measures ANOVA was performed on d-1 values of all serum hormones and SHBG. The effect of treatment on serum hormones at d 29 and 57 was determined using repeated-measures ANOVA, controlling for subject, treatment order, and treatment and including d-1 values as a covariate, followed by the Tukey's test for multiple comparisons. The effect of treatment on anthropometric, food record, and urinary isoflavone data was determined using repeated-measures ANOVA, controlling for subject, treatment order, and treatment, followed by the Tukey's test for multiple comparisons. All data that were log transformed were exponentiated back to the natural scale and presented with their 95% CI. The Statistical Analysis System, version 8.2 (SAS Institute) was used for all statistical analyses with $P < 0.05$ considered significant.

RESULTS

Subject characteristics. Subjects ($n = 35$) had a baseline mean age of 27.9 y, body weight of 82.5 kg, BMI of 25.4 kg/m^2, and body fat of 16.4%. During the study, body weight, BMI, or percentage body fat did not change (**Table 1**).

Energy, macronutrient, dietary fiber, and calcium intakes. The intakes of energy, macronutrients, dietary fiber, or calcium among the 3 treatments did not differ (**Table 2**). However, comparison of food records completed during the study with those completed before the study revealed that subjects consumed more protein ($P = 0.0005$), more calcium ($P < 0.001$), and less fat ($P = 0.0153$) during the study compared with before the study (Table 2).

Urinary isoflavones. Urinary excretion of isoflavones (genistein, daidzein) and isoflavone metabolites (equol, ODMA) was higher following consumption of the high-iso SPI compared with the low-iso SPI and the MPI ($P < 0.0001$ for all comparisons) (**Table 3**). Further analysis of the variability in urinary equol excretion within the high-iso SPI treatment revealed that 12 subjects could be categorized as equol excretors [urinary equol > 1000 nmol/24 h (53)] and 23 subjects could be categorized as equol nonexcretors. Inclusion of equol excretor status as a covariate in the statistical analyses did not change any of the study results nor was there a significant interaction between equol excretor status and treatment for any of the study endpoints. Within the high-iso SPI treatment, equol excretion among the equol excretors ($n = 12$) was 11,731 (7229, 19,039) nmol/24 h [geometric mean (95% CI)].

Serum reproductive hormones and SHBG. Day-1 concentrations of serum androgens did not differ among the 3 treatments, providing evidence that the washout periods between treatments were sufficient (**Table 4**). During the study, serum DHT and the DHT/testosterone ratio were decreased by the low-iso SPI ($P = 0.036$ and $P = 0.004$, respectively) and the high-iso SPI ($P = 0.047$ and $P = 0.013$, respectively) when compared with the MPI at d 57 (Table 4). Other significant effects of treatment on serum androgens occurred within serum testosterone and DHEA-S. Specifically, serum testosterone was decreased by the low-iso SPI relative to the MPI ($P = 0.023$) and the high-iso SPI ($P = 0.020$) at d 29 (Table 4). Serum DHEA-S was increased by the low-iso SPI relative to the MPI at d 29 ($P = 0.001$) and relative to both the MPI ($P = 0.0003$) and the high-iso SPI ($P = 0.005$) at d 57 (Table 4). Effects of treatment on serum concentrations of free testosterone, 3α-AG, androstenedione, or DHEA were not significant (Table 4).

Day-1 concentrations of serum estrogens, SHBG, LH, or FSH did not differ among the 3 treatments, providing evidence that the washout periods between treatments were sufficient (**Table 5**). During the study, serum estradiol and es-

TABLE 1

Subject characteristics at prestudy and during MPI, low-iso SPI, and high-iso SPI treatments[1]

	Prestudy	MPI	Low-iso SPI	High-iso SPI
Age, *y*	27.9 ± 5.7			
Body weight, *kg*	82.5 ± 9.5	82.3 ± 9.9	81.7 ± 9.5	82.2 ± 9.9
BMI, *kg/m2*	25.4 ± 3.0	25.3 ± 3.1	25.1 ± 3.0	25.3 ± 3.2
Body fat, *%*	16.4 ± 4.6	16.7 ± 4.9	16.5 ± 4.3	16.8 ± 5.9

[1] Values are means ± SD, $n = 35$.

TABLE 2

Energy, macronutrient, dietary fiber, and calcium intakes of healthy young men at prestudy and during MPI, low-iso SPI, and high-iso SPI treatments[1]

	Prestudy[2]	MPI[3]	Low-iso SPI[3]	High-iso SPI[3]
Energy, *kJ/d*	11118 ± 409	10770 ± 244	10650 ± 244	10865 ± 244
Protein, *g/d*	105.7 ± 4.91[a]	123.5 ± 2.78[b]	122.8 ± 2.78[b]	125.6 ± 2.78[b]
Carbohydrate, *g/d*	339.2 ± 13.7	334.9 ± 8.31	320.1 ± 8.31	326.9 ± 8.31
Fat, *g/d*	96.4 ± 4.79[b]	81.2 ± 3.00[a]	84.9 ± 3.00[a]	86.3 ± 3.00[a]
Dietary fiber, *g/d*	14.9 ± 0.78	13.7 ± 0.46	13.9 ± 0.46	13.4 ± 0.46
Calcium, *mg/d*	770.6 ± 94.0[a]	1905 ± 54.5[b]	1856 ± 54.5[b]	2004 ± 54.5[b]

[1] Values are least squares means ± SE, $n = 35$. Means in a row without a common letter differ, $P < 0.05$.
[2] Values are based on the results of one 3-d food record completed before the study.
[3] Values are based on the mean results of three 3-d food records completed on d 1–3, 26–28, and 54–56 and include contributions from the study treatment powders.

trone were increased by the low-iso SPI compared with the MPI at d 57 ($P = 0.010$ and $P = 0.005$, respectively) (Table 5). Effects of treatment on serum concentrations of SHBG, LH, or FSH were not significant (Table 5).

DISCUSSION

This human intervention study is the first to assess the effects of soy protein of varying isoflavone content on serum reproductive hormones through comparison of a high-isoflavone SPI with a low-isoflavone SPI and a nonsoy treatment (MPI), the first to include a homogeneous sample of 35 healthy young men, and the first to evaluate a wide profile of serum hormones including androgens, estrogens, gonadotropins, and SHBG.

Urinary isoflavones were significantly higher following consumption of the high-iso SPI compared with the low-iso SPI and the MPI, suggesting that subjects complied with consumption of the high-iso SPI treatment powder. The relatively low isoflavone excretion during the low-iso SPI and MPI treatment periods suggests that subjects also complied with the avoidance of outside dietary isoflavones. Further, during the high-iso SPI treatment period the isoflavone metabolite equol was excreted in amounts >1000 nmol/24 h by 34% of subjects, a proportion consistent with previous reports in men (53–55) and women (53–56).

TABLE 3

Urinary isoflavone excretion of healthy young men following consumption of MPI, low-iso SPI, and high-iso SPI[1,2]

	MPI	Low-iso SPI	High-iso SPI
		nmol/24 h	
Genistein	754[a] (587, 970)	1144[a] (889, 1471)	20,957[b] (16,305, 26,938)
Daidzein	1648[a] (1274, 2131)	2283[a] (1765, 2954)	43,572[b] (33,708, 56,322)
ODMA	161[a] (118, 219)	186[a] (136, 254)	8519[b] (6240, 11,629)
Equol	150[a] (98, 231)	163[a] (106, 251)	626[b] (407, 962)

[1] All data were transformed on the natural log scale prior to statistical analysis. Values are geometric means (95% CI), $n = 35$. Means in a row without a common letter differ, $P < 0.001$.
[2] Similar results were obtained when data were analyzed relative to urinary creatinine (nmol isoflavone/mmol creatinine) or relative to body weight (nmol isoflavone/24 h per kg body wt).

Serum DHT was significantly reduced following consumption of the low-iso SPI and the high-iso SPI compared with the MPI at d 57 in the current study. These results are in contrast to previous studies in healthy men that have utilized different treatments and reported no significant effects in DHT following consumption of tofu (44) or soy flour scones (46) or a significant increase in DHT in a study that evaluated extracted red clover isoflavones (50).

Similar to DHT, the DHT/testosterone ratio was significantly reduced in the current study following consumption of the low-iso SPI and the high-iso SPI compared with the MPI at d 57. No previous human studies have evaluated DHT/testosterone; however, animal studies have demonstrated significant reductions in serum DHT (22,40) and DHT/testosterone (22) in mice fed genistin (22) and rats fed isoflavone-rich soy flour or isoflavone-rich soy flour extract (40). Although the reduced DHT/testosterone in the current study suggests inhibition of 5α-reductase, there was no significant effect in serum 3α-AG, a marker of 5α-reductase activity (26,44,57). Previous studies have been equally unable to demonstrate significant effects of soy on circulating 3α-AG, including a study in men who consumed tofu (44) and a study in rats fed isoflavone-rich soy meal (41).

Collectively, the significant reductions in serum DHT and DHT/testosterone by the low-iso SPI (9.4 and 9.0%, respectively) and high-iso SPI (15 and 14%, respectively) relative to the MPI in the current study may be suggestive of effects related to a reduced prostate cancer risk. Evidence providing support for the magnitude of these differences in reproductive hormones relating to prostate cancer risk stems from a study by Ross et al. (58) that showed a significant 15% higher serum testosterone in 50 young African American men (mean age of 20.6 y) compared with 50 young Caucasian American men (mean age of 19.9 y). In reference to these findings, Ross and Henderson (59) suggest that a difference in serum testosterone of 10% at this young age, if sustained over an extended period of time, is probably sufficient to explain the 60–70% higher prostate cancer rates in older African American men compared with Caucasian men. The relevance of reduced serum DHT/testosterone to prostate cancer requires more investigation but is supported by one epidemiological study reporting lower serum DHT/testosterone in Asian American men compared with Caucasian American and African American men;

TABLE 4

Serum androgen concentrations in healthy young men consuming MPI, low-iso SPI, and high-iso SPI

	MPI	Low-iso SPI	High-iso SPI
DHT,[1] *pmol/L*			
d 1	2196 (2083, 2309)	2192 (2079, 2305)	2190 (2077, 2302)
d 29	2157 (2015, 2300)	1987 (1856, 2119)	2042 (1907, 2177)
d 57	2155 (2035, 2275)[b]	1952 (1843, 2060)[a]	1962 (1852, 2071)[a]
Testosterone,[2] *nmol/L*			
d 1	21.6 (20.6, 22.7)	21.9 (20.8, 23.0)	21.6 (20.5, 22.7)
d 29	22.1 (20.8, 23.3)[b]	19.8 (18.5, 21.0)[a]	22.0 (20.8, 23.3)[b]
d 57	20.3 (18.9, 21.7)	21.9 (20.5, 23.3)	21.7 (20.3, 23.1)
DHT/T[1]			
d 1	0.106 (0.100, 0.112)	0.104 (0.099, 0.110)	0.105 (0.100, 0.111)
d 29	0.101 (0.094, 0.108)	0.104 (0.097, 0.112)	0.096 (0.089, 0.103)
d 57	0.110 (0.102, 0.118)[b]	0.093 (0.086, 0.100)[a]	0.095 (0.088, 0.102)[a]
Free testosterone,[2] *nmol/L*			
d 1	66.1 (62.7, 69.5)	67.3 (63.9, 70.7)	65.2 (61.8, 68.6)
d 29	68.9 (64.3, 73.4)	62.1 (57.5, 66.7)	65.6 (61.0, 70.2)
d 57	63.5 (59.5, 67.5)	65.5 (61.4, 69.5)	65.2 (61.2, 69.2)
3α-AG,[1] *nmol/L*			
d 1	18.4 (17.2, 19.5)	19.0 (17.8, 20.2)	18.7 (17.6, 19.9)
d 29	17.8 (16.6, 19.1)	18.8 (17.5, 20.2)	19.0 (17.6, 20.3)
d 57	18.2 (17.0, 19.4)	19.4 (18.1, 20.6)	18.6 (17.3, 19.8)
Androstenedione,[2] *nmol/L*			
d 1	7.28 (6.97, 7.60)	7.54 (7.22, 7.86)	7.46 (7.15, 7.78)
d 29	7.36 (7.03, 7.68)	7.01 (6.69, 7.34)	7.35 (7.03, 7.67)
d 57	7.35 (7.01, 7.69)	7.64 (7.30, 7.98)	7.56 (7.22, 7.89)
DHEA,[1] *nmol/L*			
d 1	62.1 (57.6, 66.6)	69.4 (64.4, 74.5)	66.7 (61.9, 71.5)
d 29	61.1 (56.5, 65.6)	57.9 (53.6, 62.3)	62.6 (58.0, 67.2)
d 57	61.3 (56.0, 66.6)	63.3 (57.8, 68.7)	64.8 (59.3, 70.3)
DHEA-S,[1] *nmol/L*			
d 1	6761 (6484, 7039)	6535 (6267, 6803)	6614 (6343, 6886)
d 29	6380 (6105, 6655)[a]	7215 (6904, 7526)[b]	6721 (6431, 7011)[ab]
d 57	6522 (6281, 6763)[a]	7266 (6998, 7534)[b]	6674 (6428, 6920)[a]

[1] Data were transformed on the natural log scale prior to statistical analysis. Values are geometric means (95% CI).
[2] Values are least squares means (95% CI), $n = 35$. Means in a row without a common letter differ, $P < 0.05$.

TABLE 5

Serum estrogen, SHBG, and gonadotropin concentrations in healthy young men consuming MPI, low-iso SPI, and high-iso SPI

	MPI	Low-iso SPI	High-iso SPI
Estradiol,[1] *pmol/L*			
d 1	81.5 (78.2, 84.8)	82.3 (79.0, 85.7)	81.1 (77.7, 84.4)
d 29	82.8 (78.5, 87.0)	82.9 (78.6, 87.1)	84.3 (80.1, 88.6)
d 57	77.9 (74.4, 81.4)[a]	85.1 (81.6, 88.6)[b]	82.0 (78.5, 85.5)[ab]
Estrone,[1] *pmol/L*			
d 1	159.4 (151.9, 166.8)	158.3 (150.8, 165.8)	163.8 (156.3, 171.2)
d 29	160.8 (152.9, 168.8)	162.7 (154.7, 170.7)	168.6 (160.6, 176.6)
d 57	155.7 (148.4, 163.0)[a]	172.4 (165.0, 179.7)[b]	164.1 (156.7, 171.4)[ab]
SHBG,[1] *nmol/L*			
d 1	21.9 (21.2, 22.7)	22.3 (21.5, 23.0)	21.7 (20.9, 22.4)
d 29	21.1 (20.4, 21.8)	21.0 (20.3, 21.8)	21.2 (20.5, 21.9)
d 57	21.3 (20.4, 22.1)	21.1 (20.3, 22.0)	21.0 (20.2, 21.9)
LH,[2] *IU/L*			
d 1	2.63 (2.30, 3.01)	2.75 (2.40, 3.14)	2.70 (2.36, 3.08)
d 29	2.95 (2.62, 3.31)	2.76 (2.46, 3.11)	2.93 (2.61, 3.30)
d 57	2.60 (2.30, 2.94)	2.63 (2.32, 2.97)	2.77 (2.44, 3.13)
FSH,[2] *IU/L*			
d 1	1.25 (1.15, 1.36)	1.24 (1.14, 1.35)	1.22 (1.12, 1.33)
d 29	1.35 (1.25, 1.46)	1.29 (1.20, 1.40)	1.27 (1.18, 1.38)
d 57	1.26 (1.16, 1.37)	1.21 (1.11, 1.31)	1.27 (1.17, 1.38)

[1] Values are least squares means (95% CI), $n = 35$. Means in a row without a common letter differ, $P < 0.05$.
[2] Data were transformed on the natural log scale prior to statistical analysis. Values are geometric means (95% CI).

a difference that corresponds to their respective prostate cancer incidence rates (60).

Somewhat similar to serum DHT and DHT/testosterone, serum testosterone was significantly decreased in the current study, but only following consumption of the low-iso SPI relative to the high-iso SPI and the MPI, and only at d 29. Seven of the 8 previous studies evaluating the effects of soy and/or soy isoflavones on hormones in men measured testosterone and other than the Gardner-Thorpe et al. (46) study they reported no significant effects on circulating testosterone (44,45,48–51). Although the Gardner-Thorpe et al. (46) study observed a significant decrease in testosterone, its comparability to the current study is limited because it provided 120 mg isoflavones/d (in the form of soy flour scones) whereas the low-iso SPI in the current study provided only 1.64 mg isoflavones/d.

The less commonly assessed serum free testosterone was measured in the current study but was not significantly affected by treatment. This is consistent with 2 previous studies in men that included free testosterone in their evaluation of the hormonal effects of soy milk (45) and high-iso SPI (47) consumption. Although there was no significant effect of treatment on free testosterone in the current study, there was a trend ($P = 0.095$) toward its decrease by the low-iso SPI relative to the MPI at d 29. This is noteworthy because it corroborates with the potential transient reduction in serum testosterone although it does not further explain why testosterone was only significantly decreased at d 29 and only by the low-iso SPI or why DHT and DHT/testosterone were only significantly decreased at d 57 and not at d 29. In combination, however, because testosterone is a substrate for DHT (29), the significantly reduced serum total testosterone and nonsignificantly reduced free testosterone at d 29 may have occurred as prerequisites for the later-observed significant reductions in serum DHT and DHT/testosterone at d 57.

In contrast to the decreases observed in serum DHT, DHT/testosterone, and testosterone, serum DHEA-S was significantly increased following consumption of the low-iso SPI compared with the MPI and/or the high-iso SPI. A previous study in men reported no significant change in plasma DHEA-S following 3 wk of extracted red clover isoflavones (50), although the study's absence of a control group and its use of red clover isoflavones limit its comparison with the current study. In vitro studies indicate the ability of isoflavones to influence steroidogenic enzymes involved in androgen synthesis (35,37–39) and increase the production of DHEA and DHEA-S (61); however, these observations do not explain the results of the current study due to the relatively low isoflavone content of the low-iso SPI and the fact that the high-iso SPI had no significant effect on DHEA-S.

Estradiol and estrone levels were significantly increased following consumption of the low-iso SPI relative to the MPI at d 57 in the current study. Although comparison is limited by methodological differences and the lack of a low-iso SPI treatment, previous studies have reported no significant effects on estradiol following consumption of soy milk (45), soy flour scones (46), tofu (44), or soy isoflavone extract (51) and no significant effects (46) or a significant reduction (45) in serum estrone following consumption of soy flour scones (46) or soy milk (45), respectively. A possible explanation for the increased estrogens by the low-iso SPI in the current study could be due to increased aromatization of excess androgen in peripheral tissues, supported by the similarly observed increase in the adrenal DHEA-S androgen by the low-iso SPI and the physiological observation that peripheral aromatization of androgens to estrogens is the primary source of estrogen in human males (62). Also of possible relevance is the documented influence of SPI on thyroid hormone receptors in rats, which may interfere with estrogen function (63).

Other results of the current study included no significant effects of SPI treatments on serum concentrations of SHBG, LH, or FSH. Previous studies in men reported inconsistent effects on circulating SHBG including no significant change following consumption of soy milk (45), soy flour scones (46), and extracted red clover isoflavones (50), a significant increase following consumption of tofu (44), and a significant decrease following consumption of a high-iso SPI (47). The lack of significant treatment effects on serum LH and FSH in the current study is consistent with previous studies in men evaluating effects of SPI (47,48) or extracted isoflavone (51) consumption and suggests that the significant effects observed in the current study did not occur as a result of influences on the hypothalamic-pituitary-gonadal axis.

The low-iso SPI resulted in numerous significant effects on serum hormones in the current study and although its use for focus on isoflavones is supported by its similar use in previous studies in men (64,65) and women (64–68), it is relevant to identify its potential limitations. First, the extraction of the isoflavones to produce the low-iso SPI was incomplete, establishing the possibility that the remaining isoflavone quantity was sufficient to exert significant effects on serum hormones. In the current study, however, with the exception of DHT and DHT/testosterone, the effects of the low-iso and high-iso SPIs were not similar. A second limitation of the low-iso SPI is the potential that the ethanol extraction procedure used in its production removed and/or altered nonisoflavone constituents. This is of particular importance due to interest in the ability and potential for soy peptide molecules to exert biologically relevant effects (69).

In conclusion, the current randomized crossover intervention study in healthy young men demonstrated significant effects of SPI on serum reproductive hormones, regardless of high or low isoflavone content, when compared with a non-soy control. The significant reductions in serum DHT and DHT/testosterone following consumption of both low-iso and high-iso SPI are postulated to be the most relevant with respect to male health. These results may indicate soy protein's potential to contribute toward prostate cancer prevention, although this is complicated by the increases in androgens and estrogens following consumption of the low-iso SPI and the dissimilarity between the SPIs and whole soy foods that have been related to reduced prostate cancer risk (4–8). Future research is required to better understand how soy protein–induced changes in reproductive hormones of the magnitudes observed in the current study relate to prostate cancer risk.

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