

Clinical and Biological Activity of Soy Protein Powder Supplementation in Healthy Male Volunteers

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Abstract

Purpose: To determine if a commonly used soy protein supplement exhibits biological activity *in vivo* and *in vitro*, we evaluated an over-the-counter soy protein powder supplement using blood from healthy male volunteers and in an estrogen receptor *in vitro* assay.

Subjects and Methods: We recruited healthy male volunteers 18 years of age or older that were in good health. Treatment consisted of consuming two scoops (56 g) of pure soy protein powder (Puritan's Pride, Oakdale, NY) daily for 28 days. Serum testosterone and luteinizing hormone (LH) levels were collected on days -7, 0, 14, and 28 of therapy, and day 42. A reporter estrogen receptor (ER) assay was used to determine the effect on ER- β and ER- α *in vitro*.

Results: Twelve subjects were enrolled with a mean age of

32.25 years (range 25 to 47). Serum testosterone decreased 19% ($\pm 22\%$) during the 4-week use of soy protein powder ($P = 0.021$) and increased within 2 weeks after we discontinued soy protein powder. Serum LH concentrations decreased during the 4-week use of soy protein powder then increased within 2 weeks after we stopped the soy protein powder, but the changes did not reach statistical significance ($P = 0.20$). Soy protein powder was found to induce agonist activity to ER- β using a reporter estrogen receptor assay in yeast.

Conclusion: Soy protein powder decreases serum testosterone levels in healthy men and acts as an ER- β agonist; the significance of this biological effect with respect to cancer prevention needs further study. (Cancer Epidemiol Biomarkers Prev 2007;16(4):829-33)

Introduction

Prior studies of PC-SPES, a herbal combination used by men with prostate cancer, revealed potent estrogenic activity due to phytoestrogens or pharmaceutical estrogen contaminants (1, 2). Soy phytoestrogen supplements may also exhibit potent estrogenic effects. Protein supplements made from soy, a known source of phytoestrogens, are commonly used in the community without a clear understanding of their biological effects in men or women. Prior studies clearly show that pharmaceutical estrogens have profound biological and clinical effects. In contrast, claims about the therapeutic effects of herbal estrogens remain mostly unproven.

Pharmaceutical estrogens are used to induce androgen ablation in men with prostate cancer and normal testosterone levels through the inhibition of luteinizing hormone (LH; ref. 2). Estrogens can also induce clinical tumor response in some men with progression of prostate cancer after initial medical castration, suggesting an additional direct cytotoxic effect (3). Given the biological and clinical activity of pharmaceutical estrogens, the study of herbal products with estrogenic activity is critical. Our prior studies of PC-SPES showed that it induced androgen ablation with resultant testosterone levels at the castrate level in men with initially

normal testosterone levels and also exhibited antitumor effects in men with progression of prostate cancer after medical castration. Additional studies in animals also show a rationale for testing natural products containing phytoestrogens, such as those in soy, in the prevention of prostate cancer (4, 5). Although one might not expect the use of less potent estrogens, such as those found in soy, to exhibit effects as profound as those with PC-SPES, demonstration of a significant reduction of testosterone would further support the rationale for studying the long-term toxicity and benefits of soy supplementation. Furthermore, because phytoestrogens may show differential effects on estrogen receptor (ER)- α and ER- β , characterizing these estrogenic products could help to define their potential use (6-8).

To determine if a commonly used soy protein supplement has biological activity in men, we studied supplementation with soy protein powder in healthy male volunteers. We chose a soy product that would be used for protein supplementation because it is more commonly used than products with concentrated phytoestrogen content. Clinically, we assessed serum testosterone and LH levels in the blood from healthy male volunteers. Additionally, given the known differential estrogen receptor activity of estrogens, we also assessed the effect of this product on ER- α and ER- β in an *in vitro* system.

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Materials and Methods

Materials. Soy protein powder was purchased from Puritan's Pride (Oakdale, NY) in the form of 100% pure soy protein isolate with <2% lecithin in a 908-g canister of powder for laboratory and clinical studies. Stock solutions of soy protein powder and herbal extracts were prepared by exposing them to ethanol (dilution, 1:10 wt/vol) for 24 h. 17 β -estradiol was purchased from Sigma Chemical (St. Louis, MO).

Clinical Study. We recruited healthy male volunteers to this study who were 18 years of age or older, were in good health, and had no known allergy to soy or phenylalanine. Exclusions to the study were strict vegetarians and those taking any nutritional supplements including over-the-counter vitamins within the last 2 months. All subjects provided written informed consent, and the protocol was approved by the Cancer Institute of New Jersey's Scientific Review Board as well as the Institutional Review Board of the University of Medicine and Dentistry of New Jersey/Robert Wood Johnson Medical School.

History and physical examinations were done on every subject upon enrollment (baseline), days 14 and 28 during therapy, and at day 42. Serum testosterone and LH levels were collected before starting supplementation with soy protein powder on days -7, 0, 14, and 28 and day 42 (14 days after the completion of therapy). Because testosterone levels peak in the early morning and decrease by 25% to the evening minimum, all subject samples were drawn at approximately the same time to minimize this daily variation. Additionally, levels increase after exercise and decrease after immobilization and after glucose load. Therefore, subjects were asked to refrain from exercise or eating in the 6 h before returning to the clinic for evaluations and blood samples. Treatment consisted of consuming two scoops (56 g) of pure soy protein powder (Puritan's Pride, Oakdale, NY) daily for 28 days. Suggestions for consuming the soy protein powder were provided to subjects and included mixing in milk or water. Subjects were asked to maintain a food diary throughout the 28-day period. Toxicity assessments occurred by a telephone interview of the participants on days 7 and 21 and when the subject returned to the clinic on days 14, 28, and 42.

In vitro ER- α and ER- β Activity. To determine the estrogenic activity of the soy supplement, we used the *Saccharomyces cerevisiae* yeast, strain PL3, as previously described (2). PL3 carries a URA3 gene that is under the control of the estrogen-response element. Transcription of URA3, which is required for the growth of these cells in a medium lacking uracil, is dependent on the activation of the human ER by the respective ligand. Yeast was seeded in 96-well plates in 190 μ L of medium lacking uracil. In addition, the culture also contained either ER- α or ER- β receptor ligands with or without their respective vector controls. Serial dilutions of 10 μ L of 17 β -estradiol, ethanol, or one of two possible lots of soy protein powder extract were added to the cultures, and growth was observed for 4 days.

Statistical Analysis. Data are presented as means \pm SD. The subjects' testosterone and LH values were compared with the use of the Wilcoxon signed-rank test. All *P* values are two sided.

Results

Clinical Activity. Twelve males were enrolled with a mean age of 32.25 years (range 25 to 47). There was a statistically significant decrease (*P* = 0.021) in the percent change of testosterone from baseline to week 4. The mean \pm SD of testosterone was 411 \pm 175.82 ng/dL (range 198 to 872 ng/dL) at the start of therapy, 393 \pm 194.24 ng/dL (range 182 to 759 ng/dL) at day 28, and 438 \pm 95.46 ng/dL (range 263 to 587 ng/dL) at day 42, 2 weeks after treatment was discontinued (Fig. 1). One subject was started on study with a serum testosterone below the normal reference range (241-827 ng/dL), and it returned to the reference range at day 42; otherwise, the

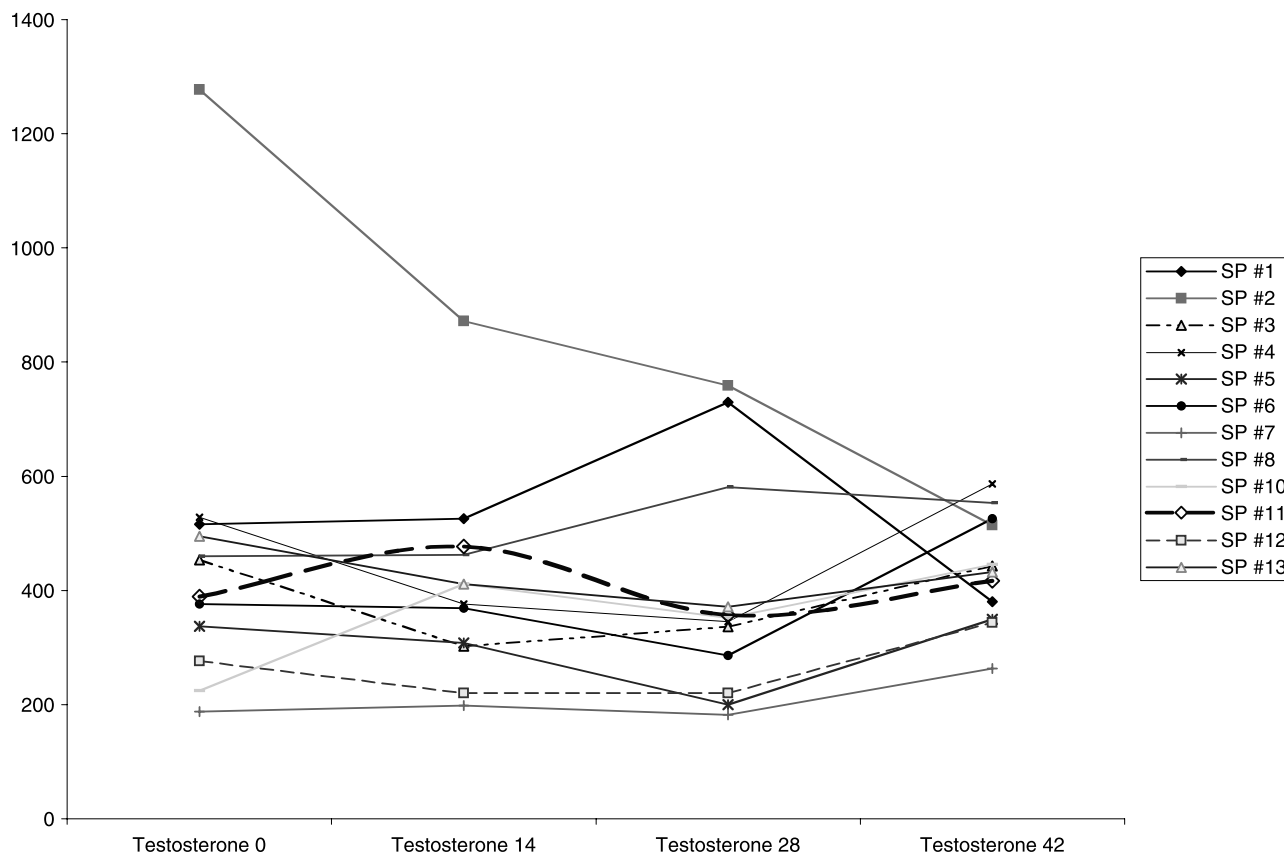


Figure 1. The mean testosterone levels at baseline, day 14, day 28 (at the completion of 4 wks of soy protein powder supplementation), and at day 42 (2 wks after the completion of the 4-wk therapy).

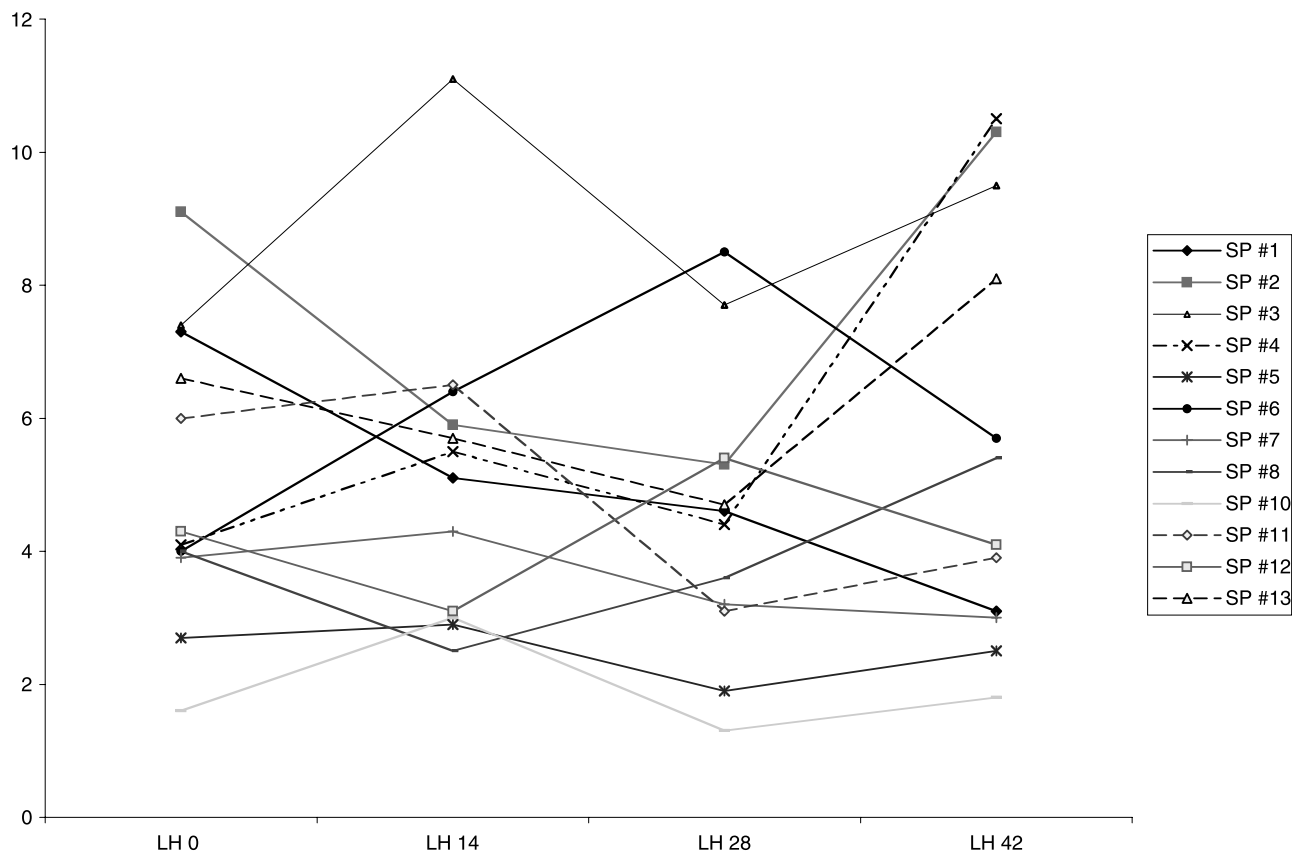


Figure 2. The mean LH levels at baseline, day 14, day 28 (at the completion of 4 wks of soy protein powder supplementation), and at day 42 (2 wks after the completion of the 4-wk therapy).

serum testosterone did not decrease below the normal reference range in any subject.

The serum LH was assessed at the same time points as the testosterone to determine if changes in plasma LH could account for any changes in testosterone. Serum LH concentrations decreased during the 4-week use of soy protein powder and then increased within 2 weeks after we stopped the soy protein powder. However, the changes did not reach statistical significance ($P > 0.20$). The mean \pm SD of LH was 5.2 ± 2.36 mIU/mL (range 2.5 to 11.1 mIU/mL) at the start of therapy, 4.5 ± 2.11 mIU/mL (range 1.3 to 8.5 mIU/mL) at day 28, and 5.7 ± 3.16 mIU/mL (range 1.8 to 10.5 mIU/mL; Fig. 2) at day 42. The LH did not decrease below the normal reference range for adult males (1.3 to 13 mIU/mL) in any subject.

The effect of soy protein powder on LH was inversely correlated with age ($r = -0.41$ to -0.53 ; $P = 0.026$); the greater the age, the smaller the effect. However, the same relationship was not shown with testosterone ($r = -0.03$ to -0.27 ; $P = 0.663$). There were no adverse effects reported by any subject.

Estrogenic Activity *In vitro*. The estrogenic activity of soy protein powder in the *S. cerevisiae* yeast strain PL3 is outlined in Table 1. Soy protein powder was found to be specific to the ER- β receptor. The growth of estrogen-dependent yeast was present when both a 1,000 \times and 10,000 \times dilution of soy protein were added to a medium lacking uracil but containing the ER- β ligand. When soy protein was added to the uracil-lacking medium with an ER- α ligand, no growth of the yeast was seen. Both lots of soy protein powder displayed similar behavior specific to the ER- β receptor. Two controls, both positive and negative, were also planned within this experiment. Ethanol served as a negative control, and 17 β -estradiol was the positive control.

Discussion

These data show that soy protein powder decreases serum testosterone levels in healthy men and acts as an ER- β agonist

Table 1. Estrogenic activity of soy protein powder in *S. cerevisiae* yeast strain PL3

Treatment	Dilution	ER- β vector control	ER- β	ER- α vector control	ER- α
Pure soy protein powder lot 1	1:1,000	—	+	—	—
	1:10,000	—	+	—	—
Pure soy protein powder lot 2	1:1,000	—	+	—	—
	1:10,000	—	+	—	—
Ethanol control*	N/A	—	—	—	—
Estradiol control [†]	1:10,000	+	+	+	+

Abbreviation: N/A, not applicable.

*Ethanol served as a negative control.

[†]17 β -Estradiol served as a positive control.

in vitro. These findings support the rationale for further studies of this effect on human physiology, including prostate health, fertility, and reducing the risk factors for cardiovascular disease, including triglycerides and cholesterol.

Our study shows a small but statistically significant decrease in serum testosterone in healthy men who consume an over-the-counter soy protein product. As shown in Fig. 1, testosterone levels decreased on therapy and began to increase on day 42, 2 weeks following discontinuation of the soy supplement. Few prior studies report similar effects of consumption of soy on this hormone in either men with prostate cancer or healthy men. Hussain et al. (9) studied the effect of soy isoflavone in 41 patients with prostate cancer and showed stabilization of the prostate-specific antigen (PSA), but no significant changes in testosterone. deVere White (10) also found no definite decrease in serum testosterone with the consumption of a genistein-rich extract in men with prostate cancer, whereas Dalais (11) showed the effect of a phytoestrogen-rich diet in decreasing serum PSA. Finally, in men with early-stage prostate cancer, soy isoflavones did not reduce either PSA or serum testosterone levels (12-14).

In healthy males, various soy formulations have been evaluated in several studies to determine its effectiveness in decreasing testosterone and ultimately its role in reducing the risk of prostate cancer (15-22). Most of these studies did not document a significant reduction in circulating testosterone, with the exception of the study by Gardner-Thorpe (18), which used a high concentration of soy isoflavone, and the study by Dillingham (22), which showed a minor reduction of hormone levels. Several possibilities may explain why some studies did not show a change in testosterone levels in healthy volunteers, whereas the trials in prostate cancer revealed some changes in serum PSA levels. First, some of the studies did not specify the control for the heterogeneity of the soy products. Consequently, the phytoestrogen content or the selectivity for the receptor type may have varied the results. Second, some of the studies failed to control for the daily testosterone variation, which could likewise affect the measurements. In contrast, our current study rigorously controlled for the soy powder used, as well as the timing of the end point measurements (testosterone and LH levels) during and after stopping supplementation.

The mechanisms by which soy intake may influence serum hormones, including testosterone as shown in our study, are unclear. Because soy isoflavones possess some estrogenic activities, it is postulated that soy may exert an effect on of the hypothalamic-pituitary-gonadal axis to down-regulate estrogen and androgen synthesis. However, the lack of significant treatment effects on serum LH in the current study is consistent with previous studies using soy protein isolate (19, 22, 23) and suggests that the effects on testosterone in our study may not be a result of isoflavone influences on the hypothalamic-pituitary-gonadal axis. Another possible mechanism for the results of our trial, although not assessed in the current study, may be the inhibition of enzymes involved in hormone metabolism by phytoestrogens such as soy, including 17 β -hydroxysteroid dehydrogenase (24) or 5 α -reductase (18, 25-27), which could ultimately lead to lower levels of testosterone. Future studies are needed to further elucidate the potential mechanisms by which soy may influence serum testosterone.

Our *in vitro* data show specific effects on ER- β compared with ER- α . This is a potentially important observation because prior studies showed the importance of ER- β in prostate cancer. Bardin (7) showed that the loss of ER- β correlated with tumor progression, whereas Cheng et al. (6) showed that ER- β induces apoptosis in prostate carcinoma cells. Finally, Maggiolini (28) showed that ER- β was required for androgen-dependent proliferation of LNCaP prostate cancer cells. Taken together, these and our current data support the

assessment of ER- β targeting in prostate cancer therapy and prevention.

In summary, these data suggest that a common soy protein supplement exerts a clinical and biological activity when used by men and provides additional rationale for testing soy phytoestrogens in the prevention of prostate cancer. These data also add further rationale for following the specific effects on ER- β of phytoestrogens, which target this receptor. Finally, our data also raise concerns about the effects of soy phytoestrogens on normal physiology, especially for over-the-counter products that are unregulated and may have higher concentrations of phytoestrogens. These preliminary data are hypothesis generating, and further studies to determine the effects on cancer prevention and normal health seem warranted.

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