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ORIGINAL ARTICLE

Serum prostate-specific antigen but not testosterone levels decrease in a randomized soy intervention among men

G Maskarinec[1], Y Morimoto[1], S Hebshi[1], S Sharma[1], AA Franke[1] and FZ Stanczyk[2]

[1]*Cancer Research Center of Hawaii, University of Hawaii, Honolulu, HI, USA and* [2]*Department of Obstetrics and Gynecology, School of Medicine, University of Southern California, Los Angeles, CA, USA*

Background: Low prostate cancer incidence and high soy intake in Asian countries suggest a possible protective effect of soy foods against prostate cancer. The goal of this pilot study was to evaluate the feasibility of a randomized, crossover soy trial among men and to investigate the effects of daily soy intake on serum prostate-specific antigen (PSA) and testosterone levels.
Methods: We randomized 24 men to a high or a low soy diet for 3 months. After a 1-month washout period, the men crossed over to the other treatment. During the high soy diet, the men consumed two daily soy servings; during the low soy diet, they maintained their usual diet. During the entire study each man donated four blood samples and five overnight urine samples. Dietary compliance was assessed by soy calendars, 24-h dietary recalls, and urinary isoflavone excretion measured by high-pressure liquid chromatography with photodiode array detection. Blood samples were analyzed for serum testosterone and PSA by radioimmunoassay. When necessary, variables were log transformed. Two sample *t*-tests compared the two groups before each study period. Mixed models incorporating the repeated measurements were used to evaluate the effect of the soy diet on urinary isoflavone excretion and serum analytes.
Results: Twenty-three men aged 58.7 ± 7.2 years completed the study. The compliance with the study regimen was high according to self-reported soy food intake and urinary isoflavone excretion. No significant between-group and within-group differences were detected. During the high soy diet, dietary isoflavone intake and urinary isoflavone excretion increased significantly as compared to the low soy diet. A 14% decline in serum PSA levels ($P = 0.10$), but no change in testosterone ($P = 0.70$), was observed during the high soy diet in contrast to the low soy diet.
Conclusion: The high adherence as shown by three measures of compliance in this pilot trial demonstrated the feasibility of an intervention based on soy foods among free-living men.
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Introduction

High soy intake and low incidence rates in Asian countries suggest a possible protective effect of soy foods against prostate cancer. Furthermore, Japanese and Chinese men who migrated to the US, where soy consumption is low, have experienced increasing rates of prostate cancer over consecutive generations (Shimizu *et al*., 1991; Ferlay *et al*., 2001). A study in a multiethnic population showed a nonsignificant, protective effect of soy food consumption across Asian and non-Asian populations including Japanese, Chinese, African-American, and Caucasian men (Kolonel *et al*., 2000). A case–control study suggested a protective effect of soy isoflavones (Strom *et al*., 1999). It was proposed that the

Correspondence: Dr G Maskarinec, Cancer Research Center of Hawaii, 1236 Lauhala St Honolulu, HI 96813, USA.
E-mail: gertraud@crch.hawaii.edu
Guarantor: G Maskarinec.
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estrogen-like structure of isoflavones may interact with male steroid hormones and alter circulating sex hormone levels (Makela *et al*., 1991). A randomized double-blind intervention study of a dietary supplement containing soy isoflavones, phytosterols, and other nutrients noted a significant reduction in circulating testosterone, dihydrotestosterone (DHT), and free prostate-specific antigen (PSA) levels among prostate cancer patients (Kranse *et al*., 2005). An intervention with soy protein isolate showed significantly lower serum DHT levels and a decreased DHT/testosterone ratio at the end after a low soy and a high soy diet as compared to the control diet (Dillingham *et al*., 2005). Few nutritional interventions have investigated the influence of soy foods rather than supplements on sex hormones in men although whole foods are more likely to avoid toxicities connected with extremely high isoflavone doses (Ryokkynen *et al*., 2005). Also, little evidence exists for the potential effects of soy foods on total PSA, a commonly used biomarker of prostate cancer risk (Lieberman, 2004; Troyer *et al*., 2004). In the present pilot study, we evaluated the feasibility of conducting a randomized soy intervention among free-living men and collected urine and blood samples to investigate the possible effects of soy foods on serum levels of testosterone and PSA.

Materials and methods

Study design and population

We conducted a randomized, crossover soy intervention consisting of two 3-month study periods separated by a 1-month washout period (Table 1). The University of Hawaii Committee on Human Studies and the Institutional Review Board of the participating hospital approved the study protocol. All subjects signed an informed consent form. We recruited free-living men between the ages 44 and 69 years from Kaiser Permanente Hawaii, a large health maintenance organization. An invitation letter was initially sent to 1000 men who had received normal PSA test results within the past 6 months (Figure 1); 90 men (9%) expressed interest in the study. During a telephone-screening interview, 25 eligible men were identified and invited for a screening visit. We excluded men who traveled off-island extensively, had ever been diagnosed with cancer, took finasteride or sex steroids, had dietary restrictions, or consumed six or more servings of soy foods per week.

Table 1 Study design for a crossover soy intervention among men

Month	*0*	*1*	*2*	*3*	*0*	*1*	*2*	*3*
	Baseline	*Period 1*			*Wash-out*	*Period 2*		
Diet group 1		Low soy				High soy		
Diet group 2		High soy				Low soy		
24-h diet recalls	x	x		x		x		x
Soy questionnaires[a]	x							
Urine collection	x	x		x	x			x
Blood collection	x			x	x			x
Body weight	x			x	x			x
Soy calendars		x	x	x		x	x	x

[a]1-year and lifetime soy consumption.

Study procedures

All 25 interested men proceeded to the screening visit and completed a one-page soy food frequency questionnaire (FFQ) to report soy intake during the past 12 months (Williams *et al*., 2003) and a lifetime soy FFQ for different phases in life. At a subsequent run-in visit, a research dietitian introduced soy food choices (tofu, soymilk, soy nuts, soy protein powder, and soy protein bars) and provided a 1-week supply of soy foods to consume two servings per day. After 1 week, the dietitian discussed the feasibility of continuing the study with each participant; 24 men (92% of eligible) were randomly assigned to either the high soy or the low soy diet. One man was excluded from the study due to medical conditions that required dietary restrictions. During the high soy diet, the men consumed two daily soy servings for 3 months while they were instructed to maintain their usual diet during the low soy diet (Table 1). To maximize compliance, the men were given a choice at which meal to consume the soy foods. Following a 1-month washout period, the two groups crossed over to the other diet (Figure 1). A urine sample collected at the end of the washout period confirmed that soy food intake was minimized during that time. To achieve adherence with the study protocol, a registered dietitian provided nutritional counseling and maintained regular contact with the study subjects.

The serving sizes were designed to provide approximately the same amount of isoflavones as consumed in Asian countries (Adlercreutz *et al*., 1991; Wakai *et al*., 1999). The food choices included 3/4 cup fresh tofu (38 mg isoflavones; Aloha Tofu, Aloha Tofu Factory, Honolulu, HI, USA), 1 cup of soymilk (38 mg isoflavones; WestSoy Soymilk Plus Plain, The Hain Celestial Group, Melville, NY, USA), 1/2 cup roasted soy nut trail mix (35 mg isoflavones; DrSoy Trail Mix, DrSoy Nutrition, Irvine, CA, USA), and 29 g of soy protein powder in plain, vanilla, and chocolate flavors (40 mg isoflavones; The Solae Company, St. Louis, MO, USA) (Franke *et al*., 1999; United States Department of Agriculture, 2002). In comparison to a previous study among women (Maskarinec *et al*., 2003), we increased the serving sizes by 50% to provide for the higher energy needs of men. Despite its lower isoflavone content, a 50 g soy protein bar (20 mg isoflavones; DrSoy Soy Protein Bar) was offered to maintain compliance with the study protocol when there was little time to prepare a meal. The isoflavone content of the chosen products had been determined by high-pressure liquid chromatography (HPLC) with photo diode array detection without hydrolysis and using flavone as internal standard. This method had been validated in a comparison with LC/MS-based assays (Franke

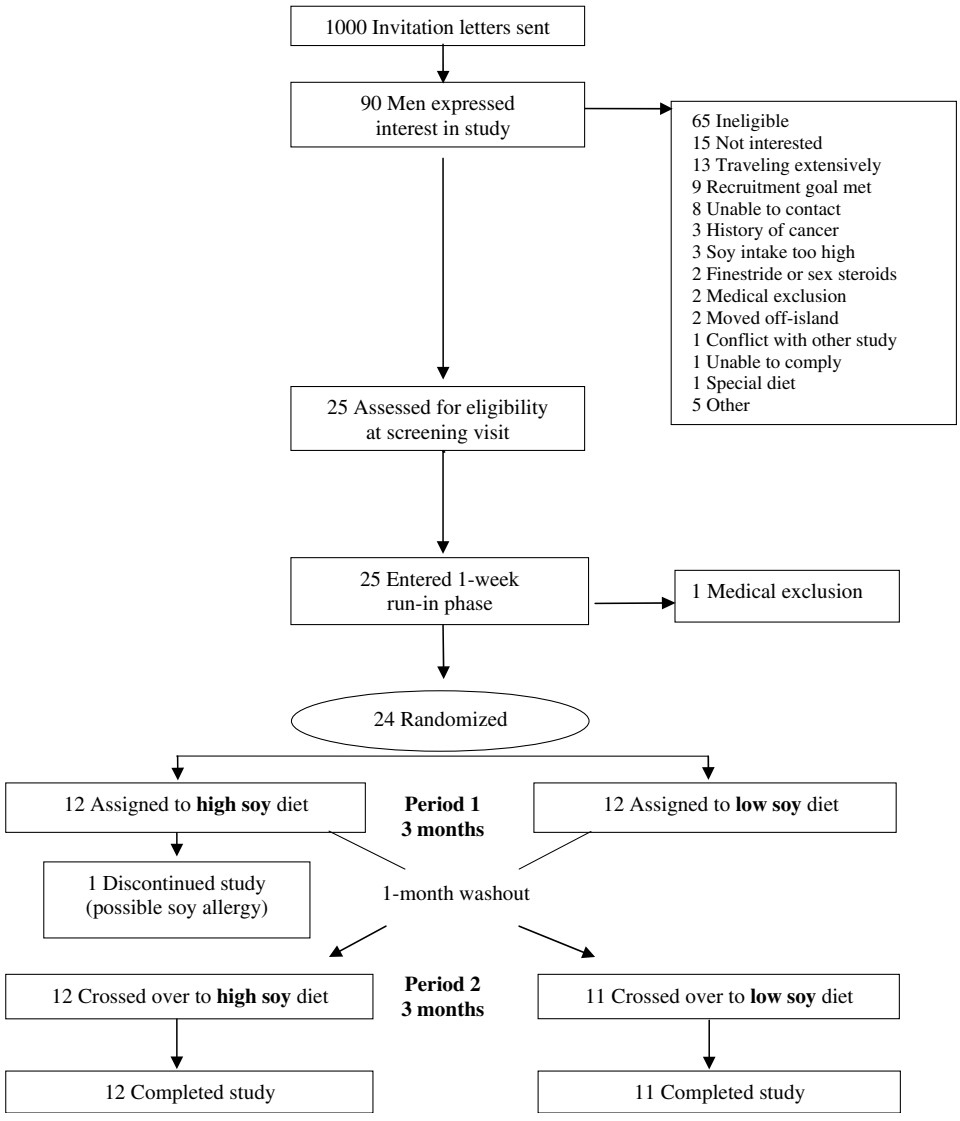

Figure 1 Recruitment and study population of a soy trial among men.

et al., 1999). To account for variations in isoflavone content by crop and manufacturing batches, we measured isoflavones in the intervention foods repeatedly.

Compliance measures

Adherence to the intervention strategy was assessed by three different methods that were found to be useful in a previous trial (Maskarinec *et al*., 2003): soy intake calendars, five randomly repeated 24-h dietary recalls, and five urinary isoflavone assessments (Table 1). On the soy intake calendars, participants entered the types of soy food consumed daily according to defined portion sizes as described above. The calendars were collected at the end of each study period, but they were not required during the washout period to lower the burden on the subjects. The dietary recalls were collected by trained staff members during visits and telephone interviews at baseline and in the beginning and toward the end of each study period. They were analyzed for isoflavones (daidzein, genistein, and glycitein) and all major nutrients and foods using the Food Composition Database (FCD) and the Food Groups Servings Database (FGSD) at our center (Murphy, 2002; Sharma *et al*., 2003). The FCD contains information for more than 2500 food items and is based on nutritional data from the US Department of Agriculture, (2003), as well as on laboratory analyses and existing professional and commercial publications. The FGSD was developed according to the USDA's Pyramid serving sizes (Center for Nutrition Policy and Promotion, 1996).

Sample collection and analysis

Fasting blood and overnight urine samples were collected at the beginning and at the end of each study period; an additional overnight urine sample was collected 1 month after randomization during the first study period (Table 1). Participants voided their bladders before going to bed and then collected all urine during the night and the first morning urine. Therefore, collections covered approximately 8 h. A mixture of boric and ascorbic acid was added to the air-tight plastic containers to reduce the pH and to stabilize the isoflavones without interfering with the assay. The urine samples were analyzed for isoflavone levels (daidzein, genistein, glycitein, equol, *O*-desmethylangolensin, dihydrodaidzein and dihydrogenistein). After hydrolysis by incubation with glucuronidase and sulfatase and extraction with diethylether, we used HPLC with photodiode array detection and flavone as internal standard (Franke *et al*., 1995; Franke *et al*., 1998; Blair *et al*., 2003). Isoflavones are specific to soy foods and are excreted in urine within 24–36 h of consumption (Franke *et al*., 1995; Franke *et al*., 1998). Positive relations between urinary excretion and dietary intake of isoflavones were first reported from a study in Japan (Adlercreutz *et al*., 1991). Later, the use of urinary assessment of isoflavones in overnight urine as a marker for soy intake was confirmed in different populations (Maskarinec *et al*., 1998; Chen *et al*., 1999; Yamamoto *et al*., 2001; Atkinson *et al*., 2002) although 24-h urine collections would be ideal (Ritchie *et al*., 2004).

We measured urinary creatinine in 0.01 ml of urine using a test kit from Sigma Chemical Company (St Louis, MO, USA), expressed urinary isoflavonoid excretion rates per mg of creatinine (Zheng *et al*., 1999; Dai *et al*., 2002), and adjusted these for body weight and age (mg/kg/day) using published reference values (Kampmann *et al*., 1974) to produce final isoflavone excretion values in nmol per hour, a superior unit than the creatinine-based measure (Knudsen *et al*., 2000). Blood samples were allowed to clot for 30 min and subsequently centrifuged at 2200 r.p.m. for 20 min. Serum was aliquoted into 1 ml cryovials and frozen at –80°C. These serum samples were then shipped on dry ice via overnight courier to the Reproductive Endocrine Research Laboratory at the University of Southern California, School of Medicine, Department of Obstetrics and Gynecology (Los Angeles, CA, USA), for analysis. Serum PSA and testosterone assays were performed in three batches; each batch contained all four serum samples for one subject. Testosterone was quantified in serum by a highly specific immunoassay (RIA) after extraction using ethyl acetate: hexane (3:2) and Celite column partition chromatography (Goebelsmann *et al*., 1979). Separation of antibody-bound steroid from inbound steroid was achieved by the second antibody method. PSA was measured by direct immunoassay using the Immulite analyzer (Diagnostic Products Corporation, Inglewood, CA, USA). The RIA had been validated with respect to sensitivity, accuracy, precision, and specificity. In the extraction/chromatographic assays, internal standard (^{3}H-testosterone) was utilized to follow procedural losses. Low, medium, and high level quality control samples were used in duplicate to assess assay reliability and showed interassay coefficients of variation (CVs) between 6.4 and 9.4% for testosterone. Also, eight blind samples from a pooled blood sample were tested. The CVs calculated on these samples were 7.8 and 3.6% for PSA and testosterone, respectively.

Statistical analysis

The statistical analysis was performed using the SAS statistical software package version 8.2 (SAS Institute Inc., Cary, NC, USA). Based on the soy FFQs, we estimated isoflavone intake during the past 12 months (mg/day) and soy food consumption before age 20 years and since age 20 years in servings per year. Given the non-normal distributions of PSA and the isoflavone excretion and intake variables, we performed log transformations when necessary. We conducted two-sample *t*-tests to compare demographic characteristics and measured analytes between the two groups after randomization and after the washout period. Paired *t*-tests were used to detect any within-group changes from the first to the second study period. Unadjusted means and standard deviations were calculated for dietary isoflavone intake, urinary isoflavone excretion, serum PSA, and testosterone by group and treatment. To account for the repeated measurement design, we applied mixed models using the PROC MIXED procedure to evaluate the effect of the soy intervention on isoflavone intake, urinary isoflavones, serum testosterone, and PSA levels (Littell *et al*., 1996). The models included the type of diet (low soy vs high soy), the group assignment (the group that started with the high soy diet first vs the group that started with the low soy diet), and the study period (first vs second) as independent variables.

Results

A total of 23 men completed both study periods; 10 were of Asian ethnicity (primarily Japanese) and 13 were Caucasian or mixed ethnicities including Native American and Hawaiian. One non-Asian participant who started with the high soy diet withdrew from the study after 1 month due to possible allergic reactions (stomach discomfort) to soy foods. No acute adverse effects related to soy were reported. At baseline, the mean age and standard deviation for all men was 58.7 ± 7.2 years; the mean body mass index (BMI) was $28.4 \pm 5.0\,kg/m^2$. After randomization, there were no statistically significant differences between the two groups in ethnicity, age, soy intake before age 20 years (22 ± 42 and 64 ± 157 servings/year; $P = 0.43$), soy intake since age 20 years (51 ± 53 and 37 ± 48 servings/year; $P = 0.70$), and soy intake during the past 12 months (3.8 ± 3.9 and 6.9 ± 9.2 mg isoflavones/day, $P = 0.91$). Moreover, mean BMI, urinary isoflavone excretion, serum PSA, and serum testosterone were similar in both groups at baseline and after the

Table 2 Compliance and serum measures by treatment[a]

Variable	*Month*	*N*[b]	*Low soy*	*High soy*	P *value*[c]
Dietary isoflavone intake (mg/day)	0	23	10.1±17.0	9.2±24.0	
	1	12	4.3±8.3	69.1±37.7	
	3	23	0.3±0.4	68.5±26.0	<0.001
Urinary isoflavones (nmol/h)	0	23	1227±2429	889±1263	
	1	12	715±1126	3703±3018	
	3	23	772±1969	4039±4763	<0.001
Serum PSA (ng/ml)	0	23	1.83±1.45	2.02±1.85	
	3	23	1.95±1.60	1.74±1.40	0.10
Serum testosterone (ng/dl)	0	23	481±152	454±121	
	3	23	467±144	466±154	0.70

[a]Unadjusted means±standard deviations.
[b]Same number for both diet groups.
[c]Based on mixed models and log transformed values.
Abbreviation: PSA, prostate-specific antigen.

1-month washout period. Likewise, no significant within-group changes were observed from baseline to the beginning of the second study period.

The daily soy calendar was completed by most men during the entire intervention; 22 men kept the calendar in the initial study period and 20 men in the second study period. During the high soy diet, 20 men reported mean intakes of 12 soy servings per week or more, only one man reported a lower intake and two men had missing information. When the subjects were advised to follow their regular diet, 16 men reported a mean intake of one or less soy servings per week, four men reported a higher intake, and three men had missing information. According to the 24-h dietary recalls, the mean intake of isoflavones during the high soy diet was seven-fold higher than during the low soy diet (Table 2), while the estimated isoflavone intake during the low soy diet was even lower than at baseline ($P<0.001$). Similarly, the mean urinary isoflavone excretion for men on the high soy diet was 4.5-fold higher than for men on the low soy diet (Table 2). In a mixed model, the soy treatment showed a highly significant effect on urinary isoflavone excretion ($P<0.001$), but there was no statistically significant effect of initial group assignment ($P=0.26$) or study period ($P=0.67$).

Mean serum PSA levels for men on the high soy diet were 0.28 ng/ml lower at the end of the high soy diet as compared to baseline, whereas PSA levels were 0.12 ng/ml higher after the low soy diet (Table 2). The differences in serum PSA level by treatment were close to statistical significance in a mixed model ($P=0.10$). PSA levels decreased by more than 0.1 ng/ml during the high soy diet for six men in each group, whereas PSA declined among four men in one group and one man in the other group during the low soy diet. There was no significant effect of group ($P=0.72$), but overall PSA levels were 0.16 ng/ml higher during the second study period than during the first period (1.97 vs 1.81 ng/ml, $P=0.16$). We observed no significant changes in serum testosterone levels related to soy treatment ($P=0.70$). Moreover, there was no difference in testosterone levels by randomization group ($P=0.34$) and study period ($P=0.31$).

Discussion

The very low dropout rate and the high adherence as shown by three measures of compliance in this pilot study demonstrated that it is feasible to conduct a soy intervention among free-living, middle aged men using a choice of soy foods. The similar change in dietary isoflavone intake and urinary isoflavone excretion according to diet indicated a high compliance with the study protocol during both diet periods. Previous studies have demonstrated the validity of urinary isoflavone excretion from single-urine samples as a compliance measure of regular soy consumption in a variety of populations (Kelly *et al*., 1993; Karr *et al*., 1997; Maskarinec *et al*., 1998; Maskarinec *et al*., 2003). Although a soy intake calendar was completed by most men, the completion rate declined in the second study period, which questions its usefulness as a dietary compliance measure in longer studies.

A modest reduction in circulating PSA levels, but not in testosterone levels, was detected as a result of the soy treatment. These findings suggest a potential protective effect of soy foods against prostate cancer, possibly through a non-hormonal mechanism, but it is always possible that the changes were due to random fluctuations in PSA levels. The overall increase in PSA over time was most likely to aging of the participants. Unlike previous studies (Gardner-Thorpe *et al*., 2003; Dillingham *et al*., 2005), the present study observed no effect on serum testosterone levels. The short duration and limited sample size may have prevented a substantial effect due to soy foods. It is also possible that timing of soy exposure in the development of prostate cancer is crucial and that interventions in adults cannot observe major changes in hormonal parameters. In animal studies, evidence has emerged that prepubertal soy exposure may be more protective than adult intake (Lamartiniere *et al*., 2002). Some studies have suggested that soy may also affect other sex hormones, such as DHT, estrone and estradiol (Habito *et al*., 2000; Nagata *et al*., 2000; Dillingham *et al*., 2005). Moreover, isoflavones may protect against

prostate cancer through non-hormonal mechanisms (Wang *et al*., 2003; Hedlund *et al*., 2005). For example, *in vitro* effects against insulin-like growth factor-1 and subsequent prostate cancer cell growth have been observed (Wang *et al*., 2003). As soy isoflavones have been detected in much higher concentrations in prostatic fluid than in the serum, they may also act locally (Hedlund *et al*., 2005). The small (14%) decline in mean serum PSA level on the high soy diet is consistent with an intervention using a soy protein supplement among prostate cancer patients that showed nonsignificant declines in serum PSA and free testosterone in the soy isoflavone supplemented group (Kumar *et al*., 2004). PSA is thought to be an indicator of cell proliferation and commonly used during prostate cancer risk screening. However, its use as a surrogate marker has been controversial (Lieberman, 2004; Troyer *et al*., 2004) because the ability of PSA to discriminate between neoplastic and hyperplastic changes is limited (Thompson *et al*., 2004). Thus, it is uncertain whether the small decrease in mean PSA level during the high soy diet can be translated into a potentially protective effect. Alternate PSA measures, such as free-vs-attached PSA and PSA velocity appear to be important and need to be included in future investigations (Lieberman, 2004).

The study had several strengths. We offered a variety of foods that enabled the men to make their own choices of how to incorporate soy foods into their daily diet and appeared to facilitate adherence to the study protocol. The dropout rate was very low; all men, except one participant with possible soy allergies, completed the present study. On a poststudy questionnaire, 19 of 23 men expressed willingness to participate in a similar soy trial if it were to be twice as long (6-month high soy diet). The population-based recruitment strategy using a large health care provider that serves 19% of the population of Hawaii (Hawaii Health Information Corporation, 2004) allowed us to enroll a diverse population. The inclusion of some traditional soy foods consumed in Asian countries in commonly consumed amounts and the choice between different types of soy foods made our study a more realistic test of the soy hypothesis than supplement trials. The crossover design was efficient in that it decreased recruitment and study costs. Nevertheless, the limited sample size and duration of this pilot study restricts our ability to generalize the findings to a population level. The fact that, due to budgetary constraints, we were not able to assess other sex steroids is a serious limitation of our project. Given the success of the current pilot study, future investigations should be expanded in duration and consider evaluation of additional sex hormone and PSA measurements to explore both hormonal and non-hormonal mechanisms of regular soy intake against prostate cancer.

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