Effects of a 2-Year Randomized Soy Intervention on Sex Hormone Levels in Premenopausal Women

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Abstract

Objective: **Several epidemiologic studies have described protective effects of soy consumption against breast cancer. The goal of this trial among premenopausal women was to examine the effect of soy foods on menstrual cycle length and circulating sex hormone levels.**

Methods: **This 2-year dietary intervention randomized 220 healthy premenopausal women. The intervention group consumed two daily servings of soy foods containing ~50 mg of isoflavones; the control group maintained their regular diet. Five blood samples (obtained in months 0, 3, 6, 12, and 24) were taken 5 days after ovulation as determined by an ovulation kit. The serum samples were analyzed for estrone, estradiol, sex hormone binding globulin, androstenedione, and progesterone by immunoassay.**

Results: **At baseline, both groups had similar demographic, anthropometric, and nutritional characteristics. The dropout rates of 15.6% (17 of 109) in the intervention group and 12.6% (14 of 111) in the control group did not differ significantly. According to soy intake logs, 24-hour recalls, and urinary isoflavone excretion, the women closely adhered to the study regimen. Menstrual cycles became slightly shorter in both groups but did not differ by group. Mixed general linear models indicated no significant intervention effect on any of the serum hormones. However, androstenedione and progesterone decreased significantly over time in both groups.**

Conclusions: **The results of this study suggest that the preventive effects of soy on breast cancer risk in premenopausal women may not be mediated by circulating sex hormone levels. Different mechanisms of actions or effects of exposure earlier in life are alternate hypotheses that require further investigation.** (Cancer Epidemiol Biomarkers Prev 2004;13(11):1736–44)

Introduction

The fact that breast cancer rates have been four to six times lower in Japan and China than in Western countries and that consumption of soy products is high in Asian countries has led to the investigation of soy foods as a possible protective factor against breast cancer (1). Several case-control studies offer strong support for this hypothesis (2-5) despite some conflicting results (6, 7). In particular, recent reports from Shanghai (4, 8), Asian Americans in California (9), and Japan (5) observed significant protective effects in women with high soy intake. When the possible estrogenic and antiestrogenic effects of isoflavones, phytoestrogens contained in soy foods, were identified, the theory seemed more plausible (10), especially because it had been discovered that estrogens and possibly other sex steroids are involved in the development of breast cancer (11). Although the evidence for an association between endogenous hormones and breast cancer is stronger for postmenopausal (12) than for premenopausal (13, 14) women, estrogen levels seem to be a biomarker for breast cancer risk (15). Several interventions have examined the effects of soy supplementation with either foods or protein isolates on circulating estrogen levels. Reports from a few small studies (16, 17) have also suggested changes in menstrual cycle length after increased soy intake. The Breast, Estrogen, and Nutrition study was a 2-year nutritional intervention project investigating the possible effects of soy on breast cancer risk. In a previous report (18), we have shown the feasibility of introducing soy foods into the diet of premenopausal women and their high compliance with the nutritional intervention strategy over the relatively long period. The objective of this analysis is to examine the effect of two daily servings of soy foods on menstrual cycle length and circulating sex hormone levels among premenopausal women. Specifically, we assessed serum concentrations of estrone (E_1), estradiol (E_2), free E_2, androstenedione (Adione), progesterone (Prog), and sex hormone binding globulin (SHBG) during the luteal phase.

Methods

Study Design and Recruitment. The protocol of this randomized clinical trial of 2 years' duration was approved by the University of Hawaii Committee on Human Studies and by the institutional review boards

Received 3/9/04; revised 4/29/04; accepted 5/12/04.

Grant support: National Cancer Institute grant R01 CA80843.

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of the three hospitals where women were recruited. All women signed an informed consent form before entry into the trial. A Data Safety Monitoring Committee reviewed the progress of the study, reasons for dropouts, and any reported symptoms annually. After sending out 10,022 invitations to women who had received a screening mammogram that was found to be normal, 975 (9.73%) interested women replied (Fig. 1). During a telephone screening interview, 352 eligible women were identified and invited for a visit. We excluded women who were taking oral contraceptives or other sex hormones, have ever been diagnosed with cancer, did not have a uterus, have one intact ovary, have regular menstrual periods, or consumed ≥6 weekly servings of soy as assessed by a soy food frequency questionnaire (19, 20). After the baseline assessment and sample collection, 245 women agreed to a run-in period of 1 week during which they tasted all soy foods to be used for the intervention; 220 of these (62.3% of eligible) subjects were randomized using a blocked randomization scheme to balance the groups by age and ethnicity. The number of dropouts and the reasons for leaving the study did not differ significantly by group (Fig. 1). Altogether, 17 (15.6%) women in the intervention group and 14 (12.6%) controls left the study prematurely (P = 0.53).

Study Procedures. The intervention was designed to provide two daily servings of soy. The size of one serving was calculated to supply ~25 mg of isoflavones (Table 1; refs. 21, 22) as determined by high-pressure

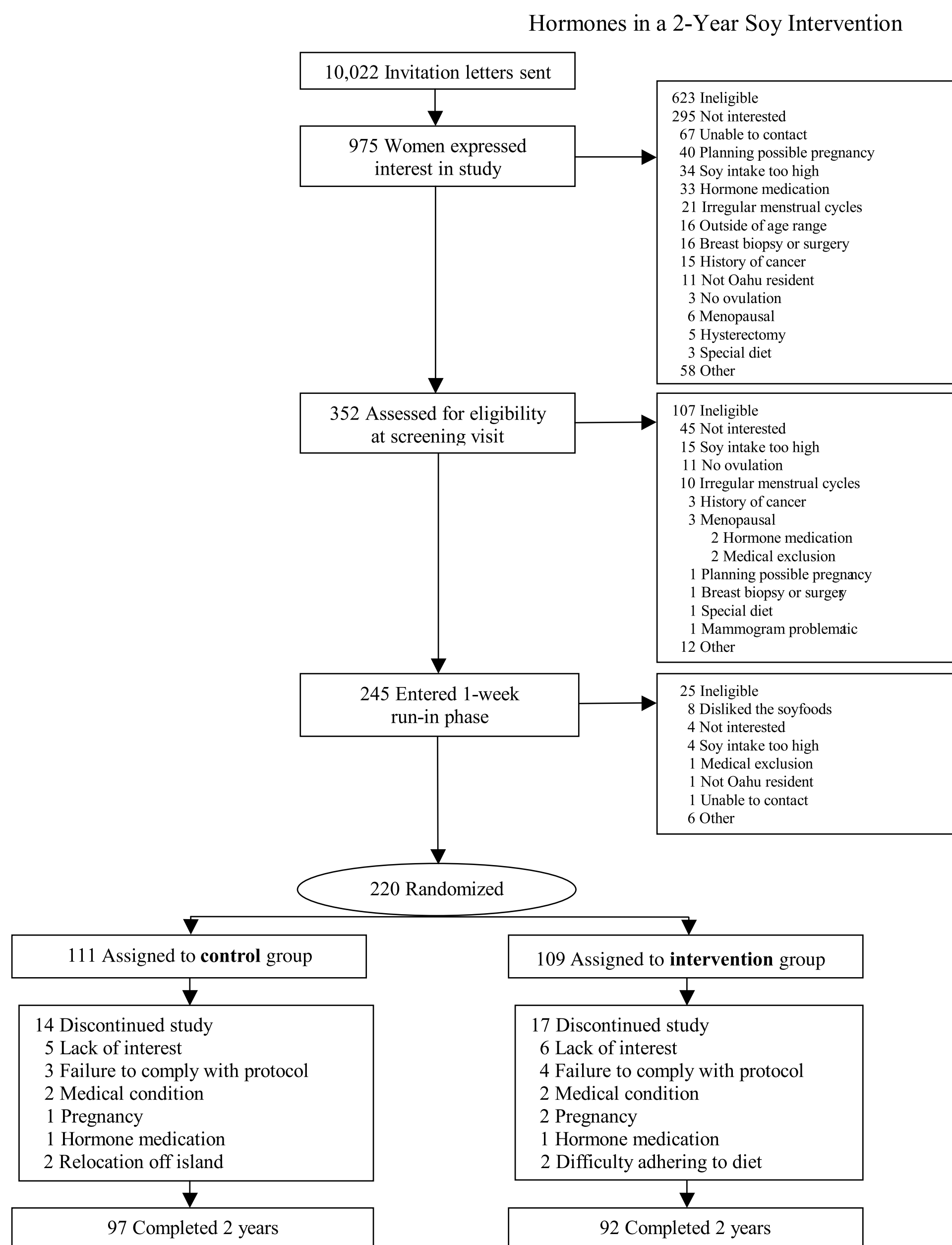

Figure 1. Flow chart for recruitment and study population.

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Table 1. Nutritional content of soy foods for the intervention

Soy food	Serving size (g)	Energy (kcal)	Protein (g)	Isoflavones (mg)*
Tofu	126	74	7.5	25.1
Soymilk	180	97	4.7	27.2
Roasted soy nuts	23	110	8.2	25.6
Soy protein powder	31	100	21.9	26.4
Soy protein bars	58	160	10.9	19.8

*Analyzed by the Analytical Laboratory at the Cancer Research Center of Hawaii.

liquid chromatography with photodiode array detection (23, 24). Soy foods of the same brand were used throughout the intervention. To achieve long-term compliance, a choice of fresh and silken tofu was offered along with roasted soy nuts, soy bars, and soy protein powder. Our goal was to recommend primarily traditional Asian foods such as tofu and soymilk. Absorption and metabolism of isoflavones derived from different soy foods and supplements are very similar and lead to comparable patterns of plasma levels and urinary excretion rates (23, 25, 26). Women in the intervention group received supplies of nonperishable foods and grocery store coupons for perishable foods and soymilk at regular intervals. As described previously (18), dietitians provided nutritional counseling and group meetings to women in both groups. The women in the control group maintained their regular diet and were counseled about healthy nutrition according to the Food Guide Pyramid (27).

Dietary Intake and Compliance Measures. At baseline, all subjects completed a validated food frequency questionnaire (28), a one-page validated soy food frequency questionnaire assessing intake during the last year (20), and a lifetime soy questionnaire asking about soy food intake during different phases in life. Adherence to the intervention strategy was assessed by four measurements of urinary isoflavone excretion using high-pressure liquid chromatography (29), self-reported soy intake logbooks, a one-page soy food frequency questionnaire (20, 30) at the end of each study year, and seven randomly repeated 24-hour recalls.

Sample Collection. The goal was to obtain blood and urine samples 5 days after ovulation as determined by an ovulation kit, corresponding approximately to day 19 in a 28-day menstrual cycle. In addition to baseline, samples were collected at 3, 6, 12, and 24 months after randomization. Average cycle length and the dates of the two most recent periods were recorded at the screening visit. The first day of full flow was considered to be the first day of the menstrual cycle; days of spotting were not counted. Menstruation dates were updated in the database at each contact. If a participant forgot to record the exact date of menstruation, a date based on the average cycle length or the number of days between known menstruation dates for this individual was entered into the database. If a period was skipped, no dates were entered. Ovulation was tested by the subjects using the SureStep LH Ovulation test (Applied Biotech, Inc., San Diego, CA, catalogue no. 6108). This test detects luteinizing hormone in a urine sample, contains a built-in procedural control, and complies with the WHO Second International Standard (IS 80/552). In addition, 10% of women used the more sensitive OvuQuick One-Step ovulation prediction kit (Quidel, San Diego, CA, catalogue no. 0489800). On the first day of menstruation, participants notified study staff that determined the presumptive first day for ovulation testing using estimated average cycle length. After a reminder call, participants tested daily for up to 7 days between 10:00 a.m. and 8:00 p.m. at approximately the same time each day without excess consumption of liquids within 4 hours prior to testing. After testing positive, participants arranged for blood collection 5 days later or if necessary on day 6 or 4. Blood was collected before 9:30 a.m. at our center or at a commercial laboratory. If a positive test result did not occur after three cycles, blood was drawn on either day 19 of the cycle or on a day approximating an average ovulation date, but this approach was only necessary for 16 blood collections. Participants who had not menstruated within 40 days or who discontinued the study were assigned a random day for blood collection. Serum was allowed to clot for 30 minutes and then was centrifuged at 3,000 rpm for 15 minutes. The samples were delivered to a central processing location on the same day, aliquoted into 1 mL cryovials, stored at −80°C, and shipped to Los Angeles on dry ice.

Serum Analysis. Hormone assays were done in the Reproductive Endocrine Research Laboratory at the University of Southern California Keck School of Medicine over a 6-month period. The analyses were conducted in batches of 30 or 40 samples. Each batch contained all five samples collected at baseline and at months 3, 6, 12, and 24 from the same woman. The batches were assembled with an equal number of intervention and control women in each batch. Whenever possible, we balanced the batches according to our block randomization scheme described above. A complete set of samples was available for 156 women. For 15 women who donated only baseline samples, no hormone analysis was done. In addition, 61 samples were missing because women had either left the study or failed to provide a sample. The missing blood samples were distributed equally between groups: 10 and 8, 7 and 6, 6 and 7, and 9 and 8 were unavailable in the control and intervention groups for the four blood draws, respectively. Therefore, there were only four specimens available for 41 women, three specimens for 4 women, and two specimens for 4 women. For quality control, we included two or three blind samples obtained from a pooled blood sample into each batch. These samples were donated by 10 premenopausal employees at our center during the luteal phase of their menstrual cycle.

Blood samples were assayed in serum for Adione, E_2, E_1, Prog, and SHBG. Adione, E_1, and E_2 were quantified by specific and sensitive RIA after an extraction and a purification step (31-33). Prog and SHBG were measured by direct immunoassays on the Immulite system (Diagnostic Products Corp., Inglewood, CA). Free E_2 was determined by calculation using a computerized algorithm described previously (34). Based on the 60 blind samples (two to three samples per batch), we obtained the following interassay coefficients of variation

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(%) for the 28 batches: E_1, 17.7; E_2, 11.2; Adione, 14.2; SHBG, 6.2; and Prog, 8.6. The respective mean (range) intraassay coefficients of variation (%) were as follows: E_1, 9.2 (0.5-29.3); E_2, 6.5 (1.2-14.8); Adione, 9.1 (0.28-22.1); SHBG, 2.9 (0.12-6.4); and Prog, 7.3 (0-18.9).

Statistical Analysis. The SAS statistical software package version 8.2 (SAS Institute, Inc., Cary, NC) was used for all analyses. Menstrual cycle length was calculated as the number of days from the first day of full menstrual flow to the day before the next full flow. We considered only cycles with a length of >20 days because shorter cycles are biologically unlikely. Based on the lifetime soy questionnaire, we calculated an estimated number of soy servings during childhood and during the entire life.

The *t* test procedure (35) was used to compare the means of the two groups at baseline and the mean difference in change between groups. To test for an intervention effect according to the original assignment, we examined overall group mean differences and the effect of time. This examination was carried out using maximum likelihood estimation of a mixed general linear model that takes into account the covariance structure of the repeated measures within subjects (36, 37). The repeated measurements were included as a random effect into the model testing for a change in hormone level over time in both groups combined. An interaction effect for group assignment with time was also added to the model. Analyses for all serum levels were repeated with data restrictions or model additions to account for presumed anovulatory status (<5 ng/mL Prog), excluding flagged blood draws (not timed with ovulation kit or other violation), compliant subjects according to urinary isoflavone excretion only (<4 nmol/mg creatinine for controls and $\geq$4 nmol/mg creatinine for intervention subjects), compliant subjects according to 24-hour recalls only (<10 mg/d isoflavones for controls and $\geq$10 mg/d isoflavones for intervention subjects), Asian ethnicity, body mass index (<25 and $\geq$25), and high soy consumption during childhood and during life. Based on a sample size of 100 subjects per arm (α = 0.05, two-sided; β = 0.2), our analyses had a power of 0.80 to detect a significant difference between groups of 23 pg/mL for E_1 and 35 pg/mL for E_2. Our a priori power calculations were based on the somewhat lower SDs in the Japanese trial (38) and had suggested minimum detectable differences of 12 and 25 pg/mL for E_1 and E_2, respectively.

Results

After randomization, the control group of 111 women and the intervention group of 109 women did not show any significant differences in demographic and anthropometric characteristics. Women were between 35 and 47 years old at entry with a mean age of 42.8 $\pm$ 2.9 and 43.2 $\pm$ 2.7 years (P = 0.29) for the control and intervention groups, respectively. At the time of randomization, 44 controls and 41 intervention women (P = 0.76) were of Asian descent (Chinese, 8 versus 8; Japanese, 29 versus 27; and Filipino, 7 versus 6). We also had 13 Native Hawaiians in each group. The respective numbers for Caucasians and Others were 43 versus 40 and 11 versus 14). The average body mass index at baseline was 25.9 $\pm$ 5.9 kg/m^2 in the control group and 26.2 $\pm$ 5.5 kg/m^2 in the intervention group (P = 0.67). Within 2 years, it increased slightly in both groups: 0.26 kg/m^2 in the control women and 0.39 kg/m^2 in the intervention women (P for paired t test = 0.54). At baseline, mean self-reported intake and urinary excretion of isoflavones (Table 2) as well as serum measurements (Table 3) were very similar in both groups.

Good adherence to the intervention diet (Table 2) was shown by an increase in self-reported isoflavone intake from 4.3 $\pm$ 4.9 to 59.0 $\pm$ 24.5 mg/d at 24 months (P < 0.0001), which was confirmed by an increase in urinary isoflavone excretion from 9.2 $\pm$ 20.0 to 64.1 $\pm$ 67.8 nmol/mg creatinine (P < 0.0001). There was little change in soy food intake among controls. The six to seven unannounced 24-hour recalls showed a mean isoflavone intake of 5.0 and 58.4 mg/d (P < 0.0001) in the control and intervention groups, respectively. Tofu and soymilk were chosen as the primary foods: soymilk was the most popular (38%) followed by tofu (31%), soy nuts (14%), soy bars (13%), and soy powder (4%). There was no evidence for adverse effects due to soy. The control group reported 157 symptoms and conditions and the intervention group reported only 126 throughout the 2 years. Of these, 34% were upper respiratory infections, 11% were musculoskeletal conditions, 8% each were gynecologic or gastrointestinal disorders, 4% each were headaches and psychological symptoms, and the rest included skin conditions, bladder infections, asthma, dental problems, high blood pressure, and diabetes. Three women in the control group were diagnosed with breast cancer during the study, whereas only one woman in the intervention group was found to have breast cancer after she had completed the study.

Self-reported menstrual cycle length at baseline was 27.6 $\pm$ 3.7 days in the control group and 28.0 $\pm$ 2.2 days in the intervention group (P = 0.34). This agreed well with the respective values of 27.7 $\pm$ 3.2 and 27.8 $\pm$ 3.0 measured before randomization. During the entire intervention, the former group experienced 23.0 $\pm$ 6.3 cycles and the latter experienced 22.7 $\pm$ 7.1 cycles (P = 0.72). The slight decline in cycle length (−0.29 and − 0.38 days;

Table 2. Compliance with the nutritional intervention

Compliance measure	Time (mo)	Control group			Intervention group		
		n	Mean	SD	*n*	Mean	SD
Urinary isoflavone excretion (nmol/mg creatinine)	Baseline	110	5.9	15.8	108	9.2	20.0
	3	99	8.5	27.8	94	44.1	36.2
	6	102	5.2	14.6	94	35.7	43.0
	12	100	7.2	19.3	98	32.2	34.9
	24	78	9.9	18.6	72	64.1	67.8
Isoflavone intake from soy questionnaire (mg/d)	Baseline	111	4.8	7.0	109	4.3	4.9
	12	102	7.2	8.8	98	57.1	22
	24	90	4.3	4.4	92	59.0	24.5
Isoflavone intake from 24-h recalls (mg/d)	6.6 recalls	107	5.0	6.0	103	58.4	15.9

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Table 3. Serum hormone levels at baseline and during intervention

Hormone	Time (mo)	Control			Intervention			Mean difference in change (95% confidence interval)*	Test of overall effects†	
		n	Mean	SD	*n*	Mean	SD		Group	Time
E_1 (pg/mL)	Baseline	105	97	59	100	90	44	−9 (−30 to 13), *P* = 0.44	*F*(1,203) = 0.11, *P* = 0.74	*F*(4,203) = 0.25, *P* = 0.91
	3	95	91	38	92	106	88			
	6	98	88	38	94	94	58			
	12	99	93	62	93	93	81			
	24	95	92	61	91	88	47			
E_2 (pg/mL)	Baseline	105	150	88	100	136	66	−30 (−70 to 11), *P* = 0.15	*F*(1,203) = 0.01, *P* = 0.93	*F*(4,203) = 0.69, *P* = 0.60
	3	95	148	65	92	165	175			
	6	98	133	55	94	152	104			
	12	99	147	114	93	144	135			
	24	96	136	74	91	138	95			
Free E_2 (pg/mL)	Baseline	105	3.3	1.7	100	3.1	1.4	−0.6 (−1.3 to 0.2), *P* = 0.16	*F*(1,203) = 0.02, *P* = 0.90	*F*(4,203) = 0.91, *P* = 0.46
	3	95	3.3	1.4	92	3.8	3.3			
	6	98	3.1	1.3	94	3.6	2.8			
	12	99	3.4	2.6	93	3.3	2.5			
	24	96	3.0	1.6	91	3.2	2.3			
SHBG (nmol/L)	Baseline	104	58	31	99	57	30	0.6 (−4.7 to 5.9), *P* = 0.81	*F*(1,203) = 0.60, *P* = 0.44	*F*(4,203) = 2.06, *P* = 0.09
	3	94	58	28	92	54	27			
	6	97	56	26	94	53	27			
	12	99	56	26	93	54	27			
	24	96	59	31	91	56	31			
Prog (ng/mL)	Baseline	105	11.5	4.3	100	10.7	4.7	−0.7 (−2.3 to 1.0), *P* = 0.44	*F*(1,203) = 2.36, *P* = 0.13	*F*(4,203) = 2.49, *P* = 0.04
	3	91	11.5	5.1	91	10.2	4.2			
	6	98	10.2	5.0	94	10.8	5.0			
	12	99	10.5	5.1	91	9.8	4.5			
	24	95	10.1	5.9	88	10.2	6.0			
Adione (pg/mL)	Baseline	105	987	384	99	987	414	−34 (−131 to 62), *P* = 0.48	*F*(1,203) = 1.04, *P* = 0.31	*F*(4,203) = 8.07, *P* ≤ 0.0001
	3	95	972	387	91	1,054	396			
	6	98	1,034	400	93	1,056	375			
	12	98	960	367	93	1,014	360			
	24	96	903	338	92	941	376			

**P*s were obtained from paired *t* tests comparing the mean change (last minus baseline measurement) in hormone levels between the intervention group and the control group.
†*P*s were obtained from mixed general linear models. The first *P* evaluates the difference between the two groups as a fixed effect; the second one assesses the effect of time as a random effect for both groups combined.

P = 0.79) was similar in both groups, resulting in a mean cycle length of 27.4 days in both groups during the 2-year study period. Despite the use of ovulation kits, women in both groups sometimes donated blood when an ovulation had not occurred as indicated by a measured Prog value of <5 ng/mL, the minimum level after a successful ovulation. This could be the result of either misreading the result of the ovulation kit from an insufficient development of the corpus luteum or from a blood draw that was timed without an ovulation kit. At baseline, ovulation had not occurred for 5 control and 10 intervention women (*P* = 0.15) whose blood samples were included in the analysis, whereas during the entire intervention period the respective numbers were 50 and 56 (*P* = 0.36). Blood and urine samples were obtained during the intervention period as planned; the four blood draws occurred at 2.8 ± 0.7, 6.0 ± 1.2, 12.3 ± 1.5, and 22.7 ± 1.7 months after randomization.

We did not observe an effect of the intervention on any of the hormones measured (Table 3). Although the levels of E_1, E_2, and free E_2 increased by close to 15% in the intervention group during the first 3 months and remained slightly higher at the 6-month blood draw, this difference disappeared after 6 to 12 months. In addition, after excluding the data for 8 control and 10 intervention subjects with Prog levels below 5 ng/mL, the difference in estrogen levels at 3-month became smaller: 89 and 94 pg/mL for E_1 and 142 pg/mL for E_2 in both groups. Whereas mean E_1, E_2, and free E_2 levels remained constant during the 2-year study period, Prog (*P* for time effect = 0.04) and Adione (*P* for time effect < 0.0001) decreased significantly over time and SHBG showed some decline (*P* for time effect = 0.09). The decrease in hormone levels did not differ by group.

A series of post hoc analyses exploring a possible intervention effect in women with different characteristics (Table 4) suggested no effects on E_1 and E_2 in any subgroup. Free E_2 showed a small decline in women younger than 43 years and in women who reported soy intake during childhood. Mean SHBG levels varied in a nonlinear fashion over time when ovulatory cycles only were considered, in women with a body mass index <25 kg/m^2, and in compliant women according to urinary isoflavone excretion. In women with high urinary isoflavone excretion at baseline, the intervention group experienced a significant decrease in SHBG (*P* = 0.03). For Adione, we observed a decrease over time in all subgroups. Among younger women and among subjects who did not consume soy during childhood, Prog levels declined significantly in the intervention group.

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In older but not in younger women, Prog decreased significantly over time and women with higher body mass index experienced significant fluctuations of Prog over time.

Discussion

The results of this 2-year nutritional trial with two daily servings of soy foods indicated no significant intervention effect on E_1, E_2, SHBG, Prog, Adione, and menstrual cycle length. The slight increase of E_1 and E_2 in the intervention group at the time of the second and third blood draws disappeared after restricting the analysis to women with a confirmed ovulation (Prog ≥5 ng/mL). The slight decline in Prog, Adione, SHBG, and menstrual cycle length over time that occurred in both groups probably reflects hormonal changes due to aging. Several post hoc analyses do not suggest a significant change in hormone levels during the intervention in any particular subgroup. Given the large number of comparisons in the subgroup analyses, the few suggestive findings are most likely false-positive results.

Among previous interventions in premenopausal women (Table 5), decreases in estrogen levels were observed in four short studies (17, 38-40), all of which used soymilk or soy foods. Only the Japanese study (38) had >20 subjects and a control group, but the change in estrogens was not statistically significant. The lack of a control group casts considerable doubt on the findings of some studies (17, 39) despite the tightly controlled and monitored conditions in metabolic units. A large number of unmeasured factors (e.g., psychological stress, change in living environment, and physical activity) may influence the frequency of menstruation and related hormonal patterns (41). In the intervention study from California (40), the estrogen lowering effect of soy foods was restricted to Asian women. The other interventions observed either no change in serum E_1 and E_2 levels (33, 42-44) or a slight increase (16, 45, 46). Interestingly, a decrease in E_2 and Prog levels was observed in an intervention with soymilk that had been depleted of isoflavones (47). The results regarding menstrual cycle length are as inconsistent as the estrogen results. Whereas five studies (16, 17, 38, 44, 46) reported a small delay in menstruation during the soy intervention, four studies (33, 40, 42, 43) did not detect a similar effect.

This study has several strengths. An intervention of this length introducing a variety of soy foods into the diet of free-living women who prepare their own food is quite unique. In contrast, previous soy feeding studies often provided high doses of soy protein isolate or supplements (16, 33, 42-46) or administered a defined amount of one soy product in a metabolic unit (17, 39, 47). Although feeding controlled levels of isoflavones eliminates variation in isoflavone levels due to different crops and food processing (21, 22), there are several reasons why the food-based approach is a more realistic test of the hypothesis proposed for this intervention. Given an estimated daily isoflavone intake of 17 mg (5) to 40 mg (4) in Asian countries, high-dose trials do not replicate nutritional conditions of women in Japan and China. To consider the beneficial effects of soy foods for public health interventions, it is essential to determine whether soy foods can be introduced into the usual diet of women who have not regularly consumed soy foods. Furthermore, it is quite possible that the effects of soy are the result of different macronutrients and

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Table 4. Subgroup analyses for hormone levels during intervention (*P*s for ANOVA*)

Variable and condition	*n*	E_1		E_2		Free E_2		SHBG		Adione		Prog	
		Group	Time	Group	Time	Group	Time	Group	Time	Group	Time	Group	Time
Prog ≥5 ng/mL†	205	0.93	0.37	0.73	0.71	0.73	0.75	0.39	0.008	0.71	0.0009	0.27	0.63
Correct blood draws No flag	220	0.66	0.97	0.31	0.90	0.43	0.74	0.41	0.16	0.41	0.0004	0.09	0.17
Age (y)													
<43	108	0.35	0.25	0.92	0.09	0.65	0.01	0.36	0.32	0.19	0.02	0.05	0.86
≥43	112	0.55	0.30	0.28	0.29	0.37	0.49	0.70	0.14	0.52	<0.0001	0.77	0.02
Body mass index (kg/m^2)													
<25	114	0.80	0.65	0.59	0.61	0.82	0.74	0.80	0.03	0.35	0.002	0.15	0.10
≥25	106	0.91	0.51	0.63	0.38	0.94	0.17	0.28	0.14	0.66	0.002	0.40	0.01
Ethnicity													
Asian	85	0.24	0.26	0.91	0.25	0.72	0.14	0.84	0.08	0.89	0.0001	0.87	0.14
Non-Asian	135	0.38	0.65	0.91	0.71	0.95	0.60	0.36	0.61	0.22	0.002	0.11	0.29
Soy intake during childhood													
No	126	0.53	0.45	0.55	0.76	0.66	0.76	0.20	0.14	0.28	0.001	0.05	0.22
Yes	94	0.50	0.81	0.31	0.19	0.60	0.04	0.91	0.26	0.99	0.004	0.92	0.33
Lifetime soy intake													
Low	110	0.54	0.30	0.54	0.66	0.86	0.66	0.16	0.21	0.12	0.02	0.11	0.51
High	110	0.38	0.19	0.11	0.47	0.18	0.37	0.98	0.18	0.83	<0.0001	0.49	0.12
Basis for compliance													
Urinary isoflavones	193	0.52	0.60	0.53	0.41	0.47	0.38	0.41	0.04	0.15	<0.0001	0.38	0.58
24-h recalls	188	0.63	0.85	0.46	0.69	0.62	0.55	0.97	0.12	0.31	<0.0001	0.54	0.19
Baseline urinary isoflavones													
Low	129	0.73	0.85	0.81	0.36	0.92	0.36	0.71	0.47	0.30	0.004	0.10	0.21
High	89	0.27	0.92	0.20	0.74	0.35	0.76	0.03	0.22	0.58	0.003	0.32	0.27

*Mixed general linear models with group as a fixed effect and time as a random effect that applies to both groups.
†A Prog level of <5 ng/mL suggests that ovulation had not taken place.

Table 5. Interventions with soy foods or supplements in premenopausal women

Author	Isoflavone dose; type of food	Length (mo)	Type	*n*	Cycle length (d)	E_1 (%)	E_2 (%)	Prog (%)	SHBG (%)
Cassidy et al. (16)	45 mg; textured soy protein	1	CO	6	+1.5	NA	**+47** (F), −0.3 (L)	−14 (L)	−2.6 (T)
Petrakis et al. (45)	75 mg; soy protein isolate	6	CO	14	NA	NA	+28 (T)	−**33** (T)	−**38** (T)
Lu et al. (17)	200 mg; soymilk	1	CO	6	+3.5	NA	−**62** (T)	−**35** (L)	NA
Nagata et al. (38)	109 mg; soymilk	2	RI	31	+1.8	−23 (F)	−27 (F)	NA	−0.7 (F)
			RC	29	−1.2	+0.6 (F)	+4 (F)		+3.5 (F)
Duncan et al. (42)	10, 65, 129 mg; soy protein isolate	3	CO 10 vs. 129	14	+0.4	−7 (L)	−0.2 (L)	−13 (L)	−0.9 (F)
Martini et al.* (43)	38 mg; soy beverage	2	CO	16	+0.2	−2.2 (L)	+6.4 (L)	−7.2 (L)	−2.4 (F)
Wu et al. (40)	32 mg; tofu, soymilk, soy bean	3	CO	20	0	NA	−**9.3** (L)	−14 (L)	+2 (L)
			Asians	(10)			(−17)		
			Other	(10)			(−1)		
Lu et al. (39)	mean 154 mg; soymilk	1	CO	10	−0.6	NA	−24 (T)	−45 (T)	NA
Lu et al. (47)	<5 mg; soymilk	1	CO	9	−1.1	NA	−20 (T)	−33 (T)	−4 (T)
Brown et al. (44)	40 mg; soy protein isolate	2	CO	14	−1.8	−1 (L)	+2.2 (L)	+45 (L)	+2.5 (L)
Maskarinec et al. (33)	100 mg; isoflavone supplement	12	RI	34	−1.4	−27 (L)	−19 (L)	−15 (L)	−2 (L)
			RC		+0.1	−32 (L)	−15 (L)	−9 (L)	−13 (L)
Kumar et al. (46)	40 mg; genistein soy protein isolate	3	RI	33	**+3.52**	−6.7 (F)	+6.0 (F)	NA	−6.9 (F)
			RC	33	−**0.06**	+1.7 (F)	+9.8 (F)		−3.8 (F)

NOTE: Abbreviations: CO, crossover; R, randomized; RI, intervention; RC, control; F, follicular phase; L, luteal phase; T, total cycle; NA, not available. Bold numbers indicate statistical significance.

*Only data for the 16 nonusers of oral contraceptives are presented.

micronutrients in the soybean, not just of the isoflavonoid-related activity. The similar dropout rates of 15.6% and 12.6% in the intervention and control groups support the feasibility of implementing the free-living diet approach over a long period required for cancer prevention. Other strengths of the study include its relatively large size, its long duration, and its high level of compliance with the dietary strategy.

We did not have complete sets of blood samples for all women, but we obtained at least four specimens for 197 (90%) of the 220 randomized subjects. Although the timing of the blood draws was not perfect, 87% of the control and 85% of the intervention blood samples were collected after an ovulation had occurred. The strongest limitation of this type of study is the lack of more frequent hormone measurements. Ideally, daily samples would allow the calculation of hormone exposure over an entire cycle, but logistic and budgetary constraints as well as the burden on the subjects did not permit such an approach. There is a concern that the variability in hormone levels and cycle length in women who are approaching perimenopause may mask a small intervention effect. However, our menstrual data indicate fairly regular cycles and little variation. The SDs for cycle lengths were close to 3 days, and 91% of all menstrual cycles recorded during the study period lasted between 26 and 30 days. One of the previous interventions (48) described lower E_1 and higher SHBG levels for women excreting equol than for nonexcretors. It was not possible to test the hypothesis proposed by Setchell et al. (49) that beneficial effects of soy are limited to individuals who have the ability to produce equol from dadzein, an estimated 30% of the population, because the subjects of our trial did not undergo a soy challenge to determine their equol excretor status.

Given the results of this randomized nutritional trial, it seems likely that the putative effects of soy on breast cancer prevention, if they exist, are mediated by mechanisms other than the lowering of circulating estrogen levels in premenopausal women. These negative findings introduce a critical uncertainty into the soy-breast cancer field. Given the epidemiologic evidence in support of a protective effect of soy during adolescence (9, 50) and in populations with habitual soy intake (38) and the repeated observation that prepubertal isoflavone exposure in animals (51, 52) is more effective in suppressing the growth of mammary tumors than adult soy feeding, future investigations may consider earlier phases of life to explore possible effects of soy on hormonal variables. Alternatively, circulating hormones and isoflavones may not accurately reflect tissue exposure (53) and the hypothesis of a direct competitive effect of isoflavones in breast tissue through binding to estrogen receptors deserves future research efforts (54). It is also possible that isoflavones modulate estrogen metabolism via an effect on the activity of specific cytochrome *P*450 isoenzymes responsible for estrogen hydroxylation (55, 56). Thus, the relative proportion of estrogen metabolites may be altered toward a more favorable metabolite pattern as suggested by two reports (55, 57). Most importantly, mechanisms through estrogen-independent pathways have to be considered as mode of action.

Acknowledgments

We thank the dedicated study participants; Aloha Tofu, Dr. Soy, and The Solae Company for support through food donations; the staff in Hawaii for assisting with the Breast, Estrogen, and Nutrition study, including Casey Robbins, Lauren Kane-Sample, Sushama Acharya, Allison Chan, Yvonne Tatsumura, Laurie

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Custer, Lea Crosetti, Bernard Riola, Yumie Takata, Kristine Wallerius-Cuthrell, Pia Jensen, Deborah Ishiyama, Carol Tanaka, Traci Murakami, Saki Miwa, Leila Martin, and Jill Harrison as well as Elizabeth Gentzschein for assisting in the hormone assays in Los Angeles; Data Safety Monitoring Committee, including Johanna Lampe, PhD, Bruce Kessel, MD, and Hazel Beh, JD; and Dr. Kessel for teaching us about endocrinology.

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