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Dietary Intervention Study to Assess Estrogenicity of Dietary Soy among Postmenopausal Women

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ABSTRACT

We tested the hypothesis that postmenopausal women on a soy-supplemented diet show estrogenic responses. Ninety-seven postmenopausal women were randomized to either a group that was provided with soy foods for 4 weeks or a control group that was instructed to eat as usual. Changes in urinary isoflavone concentrations served as a measure of compliance and phytoestrogen dose. Changes in serum FSH, LH, sex hormone binding globulin, and vaginal cytology were measured to assess estrogenic response. The percentage of vaginal superficial cells (indicative of estrogenicity) increased for 19% of those eating the diet compared with 8% of controls ($P = 0.06$ when tested by ordinal logistic regression). FSH and LH did not decrease significantly with dietary supplementation as hypothesized, nor did sex hormone binding globulin increase. Little change occurred in endogenous estradiol concentration or body weight during the diet. Women with large increases in urinary isoflavone concentrations were not more likely to show estrogenic responses than were women with more modest increases. On the basis of published estimates of phytoestrogen potency, a 4-week, soy-supplemented diet was expected to have estrogenic effects on the liver and pituitary in postmenopausal women, but estrogenic effects were not seen. At most, there was a small estrogenic effect on vaginal cytology. (*J Clin Endocrinol Metab* **80:** 1685–1690,1995)

PHYTOESTROGENS ARE nonsteroidal plant compounds of diverse structure that produce estrogenic responses (1–5); they are found in many fruits, vegetables, and grains. Most are relatively weak estrogens, but they can have potent biological effects when ingested in large quantities. The most striking example occurred in the 1940s when an epidemic of infertility decimated the sheep breeding industry in southwest Australia. Red clover forage that contained large quantities of estrogenic isoflavones caused the outbreak [reviewed by Moule *et al.* (6)]. More recently, infertility and liver disease in captive cheetahs was explained by the presence of estrogenic isoflavones in soymeal contained in a commercial feline diet (7).

Hypothesized effects of dietary estrogens in humans include developmental changes (8–10), reduced fertility (11, 12), reduced severity of menopausal symptoms (13), cardiovascular reactions (3), and increased or decreased risk of hormonally related cancers, especially breast and endometrial cancer (3–5, 8, 14–16). However, few studies have been undertaken to measure biological effects of dietary phytoestrogen intake in humans. Two studies of premenopausal women reported alterations in menstrual cycle characteristics (17–18), and a study of postmenopausal women in Australia suggested estrogenic effects on vaginal epithelium (19).

Soybeans warrant particular interest because they are a widely used food source for humans and domestic animals. Concentrations of estrogenic isoflavones in most soy protein products reach levels of 0.1–0.2% (20), the major substances being daidzein and genistein. After ingestion of soy protein by humans, intestinal flora can convert the soy isoflavones to equol, a more potent estrogenic isoflavone that is absorbed along with the unconverted genistein and daidzein. The urinary excretion of equol in humans eating soy-supplemented diets can greatly exceed the concentration of urinary endogenous estrogens. Such high concentrations enhance the plausibility of human health effects (8).

We designed a dietary intervention study in postmenopausal women to assess estrogenic effects of a soybean-supplemented diet. Conjugated estrogens produce rapid biological changes in postmenopausal women; documented effects include reductions in LH and FSH, increases in sex hormone binding globulin (SHBG), and increased maturation of vaginal epithelium as reflected by vaginal cytology (21–22), with SHBG being the most sensitive (21). We hypothesized that these same biological changes, especially the increase in SHBG, would be detectable in postmenopausal women eating large quantities of soy protein.

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Materials and Methods

Study design

Ninety-seven women were randomly assigned (in approximately a 3:1 ratio) to a soy diet group or a control group after a 2-week period when baseline measurements were taken. During the 4 weeks after

randomization, the soy diet group ate daily portions of soy foods (provided by the study) as a substitute for approximately one third of their caloric intake. Members of the control group were instructed to eat as usual during the dietary intervention period. The following markers of estrogenicity were measured at baseline and again at the end of the dietary intervention: serum LH, serum FSH, serum SHBG, and cytology of the vaginal epithelium, as reflected by the maturation index or percentage of superficial cells in vaginal smears. The concentrations of serum estradiol and urinary soy estrogens (urinary daidzein, genistein, and equol) were also measured at baseline and at the end of the diet-intervention period. All laboratory analyses were conducted without knowledge of treatment status. The study began with a pilot phase (n = 8 women) and was then completed in two separate sessions, one in the fall (n = 40 women) and one in the spring (n = 49).

Study participants

Study participants were volunteers recruited through newspapers, fliers, and radio announcements in the three-county area of Research Triangle Park, North Carolina. Criteria for entry were age 65 yr or younger, at least 2 yr past last menses, no use of antibiotics or estrogen replacement therapy in the preceding 6 months, no use of prescription drugs known to affect outcome measures, *e.g.* corticosteroids. Women received $50 per week compensation for time and travel expenses. This study was approved by the Human Subjects Review Committee at the National Institute of Environmental Health Sciences, and informed consent was obtained from all participants.

Questionnaire data

Before randomization, the women completed an extensive self-administered questionnaire, which included an adaptation of the *Health Habits and History Questionnaire* (23) that collects information about dietary habits during the previous year. In addition, the women completed a short daily questionnaire that included body weight and a record of soy food intake for those in the soy diet group.

Soy foods

The major daily soy food was a main dish made from whole soybeans or texturized vegetable protein (dried defatted soybean flour). The whole soybeans were a single variety, organically grown, and purchased in a single batch. Soy splits (dried soybeans) were provided as a daily snack. The soy foods were analyzed for daidzein and genistein by high performance liquid chromatography mass spectrometry, as described previously (7). The daily intake of soy consisted of 38 g of dry texturized vegetable protein (2.1 mg/g daidzein, 0.6 mg/g genistein) or 114 g of dry whole soybeans (0.7 mg/g daidzein, 0.2 mg/g genistein). In addition, women ate 25 g of soy splits daily (1.8 mg/g daidzein, 0.7 mg/g genistein). Thus, daily intake of isoflavones was 165 mg/day. This is approximately equivalent on a molar basis to 0.3 mg/day of conjugated steroidal estrogen, assuming that the estrogenic activity of the phytoestrogens is about 0.1% that of conjugated estrogen.

Blood, urine, and vaginal smear collection

Participants visited 1 of 4 medical clinics 4 times during the study: twice in the prediet period, midway through the diet period, and at the end of the diet period. All appointments were scheduled between 0800 and 1000 h, and women were instructed to fast from 2400 h the previous night until after their appointment. At each clinic appointment, the women were weighed, and blood was drawn 4 times at 20-min intervals via venipuncture. Blood was centrifuged, and equal aliquots of serum from each sample were pooled and stored at −20 C. The pooled serum sample was used for assays in order to reduce the variability caused by the pulsatile release of LH and FSH from the pituitary. First morning urine specimens were collected and frozen daily after the first week of the study, and a 24-hour urine specimen was collected on the same day as each clinic appointment. At the second prediet and final clinic visits, samples of vaginal epithelial cells were taken from the left and right midlateral vaginal walls by making 5 to 10 scraping strokes with vaginal spatulae. A separate slide was prepared and fixed for each wall.

Measurement of urinary phytoestrogens

Urinary concentrations of soy isoflavones were measured to demonstrate compliance with the diet and to provide a crude measure of phytoestrogen dose for each participant. To minimize the effect of day-to-day variations in urinary isoflavone levels, we pooled first morning urine samples from before the diet (6-mL aliquots from each of the 7 days before randomization) and during the diet period (2-mL aliquots from each day of the last 3 weeks of the diet) and measured the phytoestrogens in the pooled sample. Concentrations were expressed relative to the creatinine concentration in the pooled sample. A pilot study of 20 paired specimens had been conducted to measure phytoestrogen concentrations in 24-h and first morning urine specimens from the same 24-h period to verify that first morning urine specimens (corrected for creatinine) were valid indicators of total urinary excretion.

Daidzein, genistein, and equol were extracted from urine by solid-phase extraction after addition of an internal standard 5α-androstane-3α,17α-diol (5 μg). Conjugates were hydrolyzed with β-glucuronidase and sulfatase enzymes. Unconjugated estrogens were extracted by liquid-solid extraction, and phenolic compounds were separated from neutral steroid hormone metabolites using an anion exchange gel, triethylaminohydroxypropyl Sephadex LH-20. Trimethylsilyl ethers were prepared, separated by gas chromatography on a DB-1 capillary column, and quantified by mass spectrometry using selected ion monitoring (8).

Measurement of serum LH, FSH, SHBG, and estradiol

LH, FSH, SHBG, and estradiol concentrations in sera were measured with commercial kits. Time-resolved fluoroimmunoassays for LH, FSH, and SHBG were performed with the appropriate LKB-Wallac DELFIA kits (Electronuclionics, Inc., Columbia, MD). Estradiol was measured by RIA (Leeco Diagnostics, Inc., Southfield, MI). All samples from an individual woman were assayed together. For all analytes, the intraassay coefficient of variation was less than 5%, and the interassay coefficient of variation was less than 10% on the basis of quality control standards.

Vaginal cytology

Specimens from each vaginal wall were read separately by a single trained technician who was unaware of which slides were paired. From each slide, 200 cells were examined to determine the percentage of parabasal, intermediate, and superficial cells (24). The values from the 2 walls were averaged. Of the 364 slides, 51 (14%) had too few cells to count and were not included in the calculations. This resulted in 4 women with no vaginal smear data and 34 women with vaginal smear data based on only 1 wall for at least 1 of the time periods. A maturation index was calculated as the percentage of superficial cells plus half the percentage of intermediate cells.

Statistical analyses

For each of the four dependent variables (change in FSH, LH, SHBG, and maturation index), we tested for the effect of dietary intervention by including treatment as a term in a basic linear regression model that also included season of study and the clinic that the woman attended. Thus, the null hypothesis was that the mean change for the soy diet group was not different from the mean change in the control group. FSH, LH, and SHBG concentrations were logarithmically transformed before calculating change variables. Baseline concentrations were estimated as the geometric mean of two prediet values. Change in serum estradiol level (difference in natural logarithms of end-of-diet and baseline concentrations), change in weight, and age (by chance, controls were younger on average than women in the soy diet group, although not significantly so) were also added one at a time to the basic model to adjust for possible effects of these factors. Change in percentage of superficial cells, the cells considered most indicative of estrogen stimulation (25), was examined separately with ordinal logistic regression. Because only 27 women exhibited a change in superficial cells during the study (most remained at 0%), we defined three levels of the dependent variable: decrease, no change, and increase. Adjusting for other variables was done as described above.

In further analyses to explore a possible dose response, we replaced

treatment as a term in the models with each of three different measures of urinary soy estrogens: equol concentrations alone, an unweighted sum, or a weighted sum of concentrations with weights of 4 for daidzein, 8 for genistein, and 100 for equol, on the basis of laboratory data on their relative estrogenicity (26–27).

To evaluate possible interaction effects, *i.e.* that subsets of the population responded differently to the dietary intervention, we added terms that represented interactions with treatment for age, length of time since menopause, smoking status, weight, Quetelet's index, and estradiol level at baseline to the models for FSH, LH, SHBG, and maturation index.

Results

Ninety-seven women began the study. Of these, 3 were found to be ineligible (1 was still premenopausal, 1 was taking corticosteroids, and 1 was taking medication for diabetes). Three others dropped out during the study (2 because of emergencies in their families, and 1 because she could not tolerate the soy foods). The remaining 91 women (66 in the soy diet group and 25 in the control group) completed all aspects of the study. First morning urine samples were more than 98% complete.

The participants were well educated women, ages 45–65 yr, about half of whom were employed outside the home. Demographic, lifestyle, and reproductive characteristics for the 91 participants are shown in Table 1. Dietary habits were similar for the soy diet and control groups as were baseline estradiol levels (mean ± SD for treatment and control groups were 41.85 ± 12.48 pmol/L and 44.79 ± 14.32 pmol/L, respectively).

Compliance with the soy diet appeared to be good. Most (73%) women reported that they ate all of their assigned foods. Eighteen women reported that they ate only part of their soy foods on at least one day, but only four women missed days completely (3 missed one day, 1 missed two days), and these occurred at the time of an illness. Consistent with these reports, urinary soy estrogens increased markedly for most women in the soy diet group (average of a 105-fold increase in the unweighted sum) but increased little for those in the control group (average of a 2-fold increase, which was not statistically significant). The distribution of changes in soy estrogens for the soy diet group and for the control group is shown in Table 2. As expected, there was little overlap between the control and the soy diet group, but the variation among the women in the soy diet group was broad, with some women showing extremely large increases, others showing more modest changes, and a few showing little change.

Women maintained fairly stable body weights through the diet intervention period. The average weight change was a gain of 0.5 pounds (0.4 for the diet intervention group and 0.9 for the control group). No one gained or lost more than 5 pounds, and most (82%) varied by no more than 2 pounds. Endogenous estrogen levels, as reflected by serum estradiol concentrations, decreased slightly during the study for both the diet intervention and the control group. The decline was slightly larger for the diet intervention group than for the control group (5.14 pmol/L *vs.* 3.30 pmol/L) but not significantly so. Weight change and change in estradiol level were both considered potential covariates in all analyses.

TABLE 1. Characteristics of the sample of 91 postmenopausal women participating in the soy study

	Total (n = 91)		Soy diet (n = 66)		Control (n = 25)	
	Number	%	Number	%	Number	%
Race						
White	88	97	63	95	25	100
Nonwhite	3	3	3	5	0	0
Age (yr)						
≤55	30	33	20	30	10	40
56–60	28	31	18	27	10	40
61–65	33	36	28	42	5	20
Years since menopause						
≤5	28	31	21	32	7	28
6–10	27	30	20	30	7	28
>10	36	40	25	38	11	44
Education (yr)						
≤12	16	18	11	17	5	20
>12 and <16	53	58	38	58	15	60
≥16	22	24	17	26	5	20
Smoking						
Yes	15	16	10	15	5	20
No	76	84	56	85	20	80
Alcohol						
None	23	25	20	30	3	12
Drink/wk (<1)	19	21	13	20	6	24
Drinks/wk (1–6)	29	32	20	30	9	36
Drinks/wk (≥7)	20	22	13	20	7	28
Quetelet's index						
<23	39	43	25	38	14	56
23–25.99	27	30	21	32	6	24
≥26	25	27	20	30	5	20
Recreational activity						
0 min/day	14	15	12	18	2	8
1–30 min/day	30	33	22	33	8	32
31–60 min/day	19	21	14	21	5	20
>60 min/day	20	31	18	27	10	40
Vegetarian						
Yes	4	4	2	3	2	8
No	87	96	64	97	23	92
Number of pregnancies						
None	8	9	6	9	2	8
1–2	34	37	22	33	12	48
>2	49	54	38	58	11	44
Prior use of replacement estrogen						
Yes	33	36	25	38	8	32
No	58	64	41	62	17	68

TABLE 2. Change in urinary soy estrogen concentration (ng/mg creatinine) during the diet intervention period of the soy study

Change	Control		Soy diet	
	Number	%	Number	%
Decreases				
≥1000	1	4	0	0
0–999	10	40	1	2
Increases				
0–999	13	52	3	5
1000–4999	1	4	11	17
5000–9999	0	0	10	15
10,000–14,999	0	0	14	21
15,000–19,999	0	0	11	17
20,000–24,999	0	0	11	17
≥25,000	0	0	5	8

Baseline and end-of-diet measurements for LH, FSH, SHBG, and maturation index are shown in Table 3. Changes in these outcomes during the 4-week diet period are shown

TABLE 3. Baseline and end-of-diet measurements of FSH, LH, SHBG, and maturation index for the controls and soy diet group of postmenopausal women

Outcome	Number	Observed mean[a] (SD)	
		Baseline	End-of-diet
FSH (U/L)			
Controls	25	63.4 (24.5)	61.6 (25.8)
Soy diet	66	61.1 (22.1)	58.4 (21.1)
LH (U/L)			
Controls	25	30.3 (14.4)	29.7 (14.0)
Soy diet	66	32.6 (14.0)	31.8 (14.0)
SHBG (nmol/L)			
Controls	25	57.9 (35.2)	54.6 (25.4)
Soy diet	66	51.5 (32.3)	48.0 (26.8)
Maturation index			
Controls	24	16.1 (18.8)	14.2 (18.4)
Soy diet	63	14.6 (18.7)	17.4 (22.2)

[a] Geometric mean is shown for FSH, LH, and SHBG; arithmetic mean for maturation index. Standard deviation (SD) gives variation about the arithmetic mean for all outcome measures.

TABLE 4. Changes in outcome measures during 4-week diet period for the control group and soy diet group of postmenopausal women

Outcome	Measure of change[a]		Effect of Soy Diet	
	Crude	Adjusted[b]	Test statistic[c]	*P* value
FSH (U/L)				
Controls	−0.028	−0.028	−0.017	
Soy diet	−0.045	−0.045	(−0.052, 0.018)	0.33
LH (U/L)				
Controls	−0.020	−0.025	0.004	
Soy diet	−0.024	−0.021	(−0.058, 0.067)	0.89
SHBG (nmol/L)				
Controls	−0.058	−0.073	0.007	
Soy diet	−0.071	−0.066	(−0.086, 0.010)	0.89
Maturation index				
Controls	−1.9	−0.9	3.3	
Soy diet	+2.8	+2.4	(−4.5, 11.1)	0.40

[a] For FSH, LH, and SHBG, change is measured as the natural logarithm of the end-of-diet geometric mean minus the natural logarithm of the baseline geometric mean. Exponentiating this difference gives the end-of-diet value as a proportion of the baseline value. For maturation index, change is measured as end-of-diet arithmetic mean minus baseline arithmetic mean.

[b] All outcomes are adjusted for season and clinic; additionally, SHBG is adjusted for estradiol change and FSH for both estradiol change and weight change.

[c] The test statistic is the adjusted change for the soy diet group minus the adjusted change for the controls. If the two groups were identical, the test statistic would be zero. Values in parentheses are 95% confidence limits.

in Table 4. Adjustment for season, clinic, age, change in body weight, or change in estradiol concentration had little effect on these relationships. LH and FSH were predicted to decrease with soy intervention, and both did tend to decrease slightly (FSH more so than LH). However, controls also showed small average decreases, and the soy diet and control groups did not differ from each other ($P = 0.33$ for FSH; $P = 0.89$ for LH). SHBG was predicted to increase with soy intervention, but SHBG tended to decrease for both the soy diet and the control group to a similar degree ($P = 0.89$). Maturation index was predicted to increase with soy intervention. Although there was a slight average increase in the soy group compared with a slight decline in the control group, the groups did not differ significantly ($P = 0.40$).

When we examined vaginal epithelium data further by focusing on superficial cells (the cells most indicative of estrogen stimulation), the changes were again in the predicted direction (increased superficial cells with dietary intervention). In unadjusted data, 43 (68%) women in the soy diet group showed no change in percentage of superficial cells during the diet, 8 (13%) showed a decrease, and 12 (19%) showed an increase compared with 17 (71%), 5 (21%), and 2 (8%) in the respective categories of the controls ($\chi^2_{2d.f.} = 2.03$; $P = 0.36$). We found the same trend after adjustment for other factors with ordinal logistic regression. In comparison with women in the control group, women in the soy diet group tended to have no change or to have their proportion of superficial cells increase rather than decrease during the diet period [odds ratio = 2.5 (0.8, 7.8)], but the difference was consistent with chance ($P = 0.06$).

Concentration of urinary soy estrogens was not a better predictor of the outcome measures than was treatment alone, whether the change in soy estrogens was modelled as a linear term or as four separate categories corresponding to quartiles of change. Adjustment for other variables did not affect this result.

We also examined potential interactions to determine whether subsets of the study participants may have responded to the diet as predicted even though the soy diet group as a whole showed no clear estrogenic responses. We systematically looked for effects that might depend on age, length of time since menopause, smoking status, weight, Quetelet's index, and estradiol level at baseline. Of the 24 tests conducted, 2 showed significant interaction effects ($P < 0.05$), but neither were consistent with biological predictions. The first suggested that the decrease in FSH was similar in magnitude at all ages among dieters, but among controls the decrease tended to be larger in older women. The other suggested that SHBG decreased slightly for both smoking and nonsmoking dieters, whereas among controls, SHBG decreased slightly for nonsmokers but increased slightly for smokers. Considering the multiple tests conducted, the significant interactions probably resulted from chance.

Discussion

A diet high in soy resulted in significantly increased urinary isoflavone excretion in postmenopausal women, indicating that large quantities of soy estrogens were being ingested and absorbed. However, after 4 weeks of soy-supplemented diet estimated to have estrogenicity similar to a 0.3 mg/day dose of Premarin (Wyeth-Ayerst brand of conjugated estrogens), there was little evidence of estrogenic effects from the plant estrogens. Geola *et al.* (21) reported that Premarin at 0.15 mg/day resulted in significant increases in SHBG (n = 21 postmenopausal women). In their study, SHBG was a more sensitive indicator of estrogenic response than FSH (which decreased significantly at 0.3 mg/day), LH (which showed no significant decrease below a dose of 0.625 mg/day), or the percentage of superficial cells in vaginal epithelium samples (which showed no significant increase

below a dose of 1.25 mg/day). In our study of soy estrogen, the order of sensitivity seems to be reversed if the increase in superficial cells of the vaginal epithelium is real. The overall maturation index did not differ between women in the soy diet and the control group, but the percentage of superficial cells tended to increase with dietary intervention ($P = 0.06$). We reported similar preliminary analyses (based on examination of cells from only one vaginal wall by a commercial laboratory) at a soy workshop sponsored by the National Cancer Institute (28) and then had slides of both vaginal walls read by a research laboratory. The more complete data are reported here. FSH and LH decreased during the intervention period, but the changes were small and occurred in both the soy diet and the control group.

Contrary to expectation, SHBG tended to decrease in both the control and the soy diet group. Adlercreutze *et al.* (16, 29–30) reported an association between urinary phytoestrogen concentrations and increased SHBG in observational studies, and genistein was reported to stimulate SHBG production in cultured human liver cancer cells (31). However, we saw no evidence of such effects *in vivo* nor did Cassidy *et al.* (17) in premenopausal women. The association in observational studies may not have been causal, or the differences in results could be a result of differences in length and type of dietary intake. Premarin may be effective when phytoestrogens are not because Premarin increases levels of estradiol (32), a much stronger estrogen, whereas the phytoestrogens remain weak estrogens, albeit in high concentrations.

Urinary concentrations of soy isoflavones were measured in order to document intake and to provide a possible measure of dose. Equol, the most potent soy-related estrogen, is produced by bacterial conversion in the gut, and the conversion rates are extremely variable (8). Thus, we hypothesized that the urinary equol level could be a more precise measure of soy estrogen exposure than treatment status could. However, when we looked for dose-response effects in relation to urinary equol level or other measures of soy estrogen dose, no stronger relationships emerged between the soy diet and outcomes. In addition, we looked for differential effects in different women (*e.g.*, stronger effects in women with low estradiol levels or in women with low body mass index), but no such interactions emerged.

One other study looked for estrogenic effects of phytoestrogens in postmenopausal women and reported significant increases in vaginal cell proliferation during dietary intervention, as measured by the maturation index (19). In this Australian study, baseline measures were compared with measures taken during a 6-week diet period when 25 women ate soy flour, red clover sprouts, or linseed, each for a 2-week period. The Australian study included no control group so that effects of diet could not be separated from other changes over time, but vaginal smears taken 8 weeks after return to a normal diet showed maturation index values similar to those at baseline. The other difference between this study and ours is that they made no mention of laboratory personnel performing analyses in a blinded fashion, which may be important for somewhat subjective measures such as cytology. The daily amount of soy estrogen eaten by the North Carolina women should have been more than that ingested by the Australian women (isoflavone content of various soy flours is reported to be from 178–306 mg per 100 g) (20); therefore, the 45 g/day ingested by the Australian women would have included from 80–138 mg/day of isoflavones compared with the 165 mg/day for the North Carolina women. Possibly, the linseed and clover sprouts may have been more highly estrogenic than soy, and the effects on the Australian women that appeared to be soy-related may have resulted from residual effects of the other foods.

The Australian and North Carolina studies did not seem to differ appreciably in enrollment criteria, but despite the similarity in the ages of participants, their baseline maturation index was much higher than ours (31 *vs.* 16), suggesting that they had fewer women with completely atrophic smears. Atrophic vaginal epithelia may be less likely to respond to the dietary estrogen. However, when we limited analysis to the 52 women whose vaginal epithelia were not atrophic at baseline, there was still limited evidence for increased proliferation.

The absence of any clear estrogenic effects in our study was surprising, but several factors may have played a role. Phytoestrogens do not bind well to SHBG (33), and the vast majority of molecules may be conjugated before they reach target organs. Competitive binding at the estrogen receptor (27) could reduce estrogenicity of the more potent endogenous estrogens, thus counteracting predicted estrogenic effects; direct inhibition of aromatase has been reported (34–35), which would lower endogenous estrogen production. We saw little direct evidence of antiestrogenic responses, but such effects would be more easily observed in premenopausal women with high estrogen levels. Finally, effects of weak, nonsteroidal estrogens may also be biphasic, as observed in some laboratory studies of phytoestrogens (36–38). Biphasic responses would have been difficult to evaluate in this protocol.

In summary, this study showed no clear estrogenic effects in postmenopausal women eating a soy-supplemented diet for 1 month, despite evidence of absorption of high quantities of estrogenic isoflavones. Reproductive tract epithelial cell proliferation may have increased with the diet, but the effect was weak. A longer dietary exposure may be required for estrogenic effects. This was the first intervention study of postmenopausal women to measure urinary levels of phytoestrogens. The lack of clearly detectable effects of the diet suggests that more information about the metabolism of these compounds is needed, as well as more sensitive, site-specific markers of estrogenic and antiestrogenic effects.

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