Urinary Phytoestrogen Excretion and Breast Cancer Risk: Evaluating Potential Effect Modifiers Endogenous Estrogens and Anthropometrics[1]

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Abstract

Steroid sex hormones play a central role in breast carcinogenesis. Evidence from *in vitro* and animal studies suggests that phytoestrogens may inhibit the development of mammary tumors through their role in regulating the synthesis, metabolism, and signal transduction of steroid hormones. In a study of 117 case-control pairs of postmenopausal women in Shanghai, we investigated whether the association between urinary phytoestrogen excretion and breast cancer risk may differ by levels of endogenous steroid sex hormones, sex hormone binding globulin (SHBG), body mass index (BMI), and waist:hip ratio (WHR). Fasting morning blood and urine samples were collected for the analysis of urinary isoflavonoids and mammalian lignans, as well as blood levels of SHBG and selected steroid hormones. For cancer patients, samples were collected before any cancer therapy. Conditional logistic regression models were used to estimate odds ratios and 95% confidence intervals after adjusting for potential confounding factors. The inverse associations between urinary phytoestrogens and breast cancer risk were found to be more evident among women with a high BMI or WHR than those with a low level of these anthropometric measurements. Although a reduced risk of breast cancer was observed among women with a high excretion rate of urinary isoflavonoids in all of the strata defined by blood SHBG and steroid hormones, the inverse association was more pronounced among women with a high blood concentration of estradiol, a low level of estrone sulfate, or a low level of SHBG. The risks of breast cancer were also reduced with increasing excretion rate of mammalian lignans, although no test for a linear association was statistically significant in stratified analyses. Findings from this study suggest that the potential protective association of phytoestrogens may be modified by BMI, WHR, and blood levels of SHBG, and steroid hormones.

Introduction

Phytoestrogens are plant-derived organic nonsteroidal molecules possessing a weak estrogenic, or antiestrogenic activity. Isoflavones and lignans are two principal groups of dietary phytoestrogens. Evidence from both animal and human studies suggests that high consumption of phytoestrogens may reduce the risk of breast cancer, and part of the protective effect may be through their role in regulating the synthesis, metabolism, and signal transduction of steroid hormones (1). After menopause, adipose tissue becomes the major site for estrogen synthesis. The ratio of breast tissue:plasma E_2[3] changes from 1:1 in premenopausal to 10–50:1 in postmenopausal women. E_2 level in breast tissue primarily regulated by three enzymes: aromatase, estrone sulfatase, and 17β-HSD1. Several *in vitro* studies have found that isoflavonoids or their metabolites inhibited 17β-HSD1 (2, 3) and estrone sulfatase (4). On the other hand, lignans, but not isoflavonids, were found to moderately inhibit aromatase (1) in human preadipocytes (5). In addition, among postmenopausal women, high intake of lignans or soy increased the ratio of urinary 2:16-α-hydroxyestrone (6, 7), and soy intake has also been shown to significantly increase SHBG levels (8). Therefore, it appears that some beneficial effects of phyestrogens may be dependent on the endogenous levels of steroid hormones and SHBG.

We reported recently in a case-control study conducted in Shanghai that regular soyfood intake was related to a reduced risk of breast cancer, particularly for cancers positive for estrogen and progesterone receptors (9). This inverse association was more pronounced among women with a high BMI [weight (kg)/height (m)^2] or WHR (9). We also showed in the study that increasing excretion of urinary isoflavonoids and lignans was associated with a reduced risk of breast cancer (10, 11). In this report, we evaluated whether the association between urinary excretion of phytoestrogens and the risk of breast cancer may

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[3] The abbreviations used are: E_2, estradiol; 17β-HSD1, 17β-hydroxysteroid dehydrogenase type 1; SHBG, sex hormone-binding globulin; BMI, body mass index; WHR, waist:hip ratio; *O*-DMA, *o*-desmethylangolensin; CV, coefficient of variation; E_1, estrone; E_1-S, estrone sulfate; DHEA-S, dehydroepiandrosterone sulfate; OR, odds ratio; CI, confidence interval.

be modified by body weight and fat distribution, and circulating SHBG and sex hormone levels.

Materials and Methods

Study Design and Data Collection. Data and biological samples from this project came from the Shanghai Breast Cancer Study, a population-based case-control study conducted among Chinese women in urban Shanghai during 1996–1998. The detailed study methods have been reported elsewhere (9–15). Through a rapid case-ascertainment system, supplemented by the population-based Shanghai Tumor Registry, 1602 eligible breast cancer cases aged between 25 and 64 years were identified during the study period. Of them, 1459 patients (91.1%) completed an in-person interview. Controls were randomly selected from the general population using the files kept at the population-based Shanghai Resident Registry. Cases and controls were frequency-matched to cases by age (5-year interval). Of the 1724 eligible controls identified, in-person interviews were completed for 1556 (90.3%). A structured questionnaire was used in the in-person interview to elicit detailed information on demographic factors, dietary habits, and known and suspected lifestyle risk factors for breast cancer. After completing the interview, body weight and circumferences of waist and hip were measured. Among those who completed the interviews and anthropometrics, 83.1% of cases and 83.8% of controls donated a fasting blood sample, and 99.2% of cases and 99.5% of controls donated a spot urine sample. Ascorbic acid (125 mg/100 ml urine sample) was added to prevent degradation of labile compounds. Immediately after collection, samples were placed in a portable insulated case with ice pads (0–4°C) and transported to the central laboratory for processing. All of the specimens were collected in the morning before breakfast. Within 6 h after collection, samples were aliquoted and stored at −70°C. For nearly 50% of the cases, biospecimen collections and in-person interviews were completed before any cancer therapy (10, 11). To increase the comparability between cases and controls for studying quantitative biomarkers, we individually matched a control to each of the cases whose blood and urine samples were collected before cancer treatment on age (±3 years), menopausal status (yes or no), and date of sample collection (±30 days). Successful match was accomplished for 300 case-control pairs for the study of endogenous estrogens. Of them, 250 pairs were selected for the phytoestrogen study based on the availability of their urine samples. Included in the current study were 117 postmenopausal case-control pairs who were included in both estrogen and phytoestrogen studies. To eliminate the effect of between-assay variability on study results, samples for each case-control pair were assayed in the same batch. Technicians who performed the assays were unaware of any information on the study subjects (*e.g.*, disease status).

Laboratory Assays of Urinary Phytoestrogens and Creatinine. Urinary isoflavonoid and lignan analysis was performed as described previously (11, 16–18). In brief, urine was mixed with triethylamine acetate (pH 7.0), β-glucuronidase, and arylsulfatase, and then incubated for 1 h at 37°C. This mixture was extracted three times with 2 ml of ethyl ether. The combined organic phases were dried under nitrogen, redissolved in methanol/acetate buffer, and analyzed immediately by injecting 20 μl into the liquid chromatography system or stored at −20°C until analyzed. Liquid chromatography photodiode array mass spectrometry analyses were performed by using a HydroBond PS 18 reversed-phase guard and analytical column (18). Included in the study were daidzein, dihydrodaidzein, glycitein, genistein, dihydrogenistein, *O*-DMA, enterodiol, and enterolactone. Final phytoestrogen excretion rates in urine were expressed in nmol/mg creatinine by adjusting for urinary creatinine concentrations, which were determined by a test kit based on the Jaffe reaction (Sigma, St. Louis, MO; Ref. 10). The mean CVs for intra-assay variations of phytoestrogens was 10.4%. With the exception of *O*-DMA and enterodiol, all of the other intra-assay CVs were <15%. The interassay variability was larger than intra-assay variability, particularly for *O*-DMA. For major isoflavonoids and lignans, the interassay CVs were 7.2% for daidzein, 11.8% for genistein, 8.5% for glycitein, 11.4% for dihydrodaidzein, 8.2% for enterodiol, and 8.9% for enterolactone. However, for isoflavonoid metabolites, interassay CVs were 14.8% for dihydrodaidzein, 18.9% for dihydrogenistein, and 60.0% for *O*-DMA. Because the samples for each case-control pair were assayed in the same batch, the potential influence of interassay variation on results was minimized.

Laboratory Assays of Plasma Steroid Hormones and SHBG. All of the assays for steroid hormones and SHBG were conducted in the Diagnostic Systems Laboratory, Inc. (Webster, TX), a reference laboratory certified by Clinical Laboratory Improvement Amendments and the International Standard ISO 9002. Each sample was tested in duplicate, and two internal quality control samples were included in each run of the assay. Commercial RIA from Diagnostic Systems Laboratories Inc. were used for measuring steroid hormones, and an immunoradiometric assay from Diagnostic Laboratory Systems, Inc. was used for measuring SHBG. Plasma levels of E_2, E_1, E_1-S, testosterone, and DHEA-S were determined directly without any extraction procedures (19). The CVs for the intra- and interassay variations of these assays ranged from 1.1 to 11.5%, with the majority of CVs <10%.

Data Analyses. For case-control comparisons of selected demographic and risk factors, McNemar's test was used for categorical variables and the paired Student *t* test was conducted for continuous variables. Because the data for most biomarkers were skewed to the right, the Wilcoxon signed rank test was used for comparisons of the median difference between cases and controls. ORs and 95% CIs were estimated to measure the strength of the association using conditional logistic regression. Cases and controls were categorized according to the control tertile distribution of urinary excretion rates of phytoestrogens, and ORs were estimated for the upper two tertile groups, the referent being the lowest tertile group (20). Stratum-specific ORs were also derived from conditional regression with the inclusion of terms for main effect along with two interaction terms, the cross-products of the stratum variable with two dummy variables for the upper two tertile levels of phytoestrogens. By adding these interaction terms, case-control pairs were not broken, and all of the subjects were included in the model building. Multivariate analyses were performed to adjust for potential confounding variables. Tests for trend across tertiles were performed in conditional logistic regressions by assigning the score j to the j^{th} level of the variable selected. Multiplicative interaction tests were evaluated for log-transformed continuous variables of exposure (phytoestrogens) and potential modifiers (steroid hormones, BMI, and WHR). All of the statistical analyses were based on two-tailed probability.

Results

The comparisons of cases and controls on demographic factors and known lifestyle risk factors of breast cancer, including dietary factors, are shown in Table 1. Cases and controls were comparable

Table 1 Comparison of cases and controls by selected demographic and risk factors, the Shanghai Breast Cancer Study, 1996–1998

Characteristic[a]	Cases (n = 117)	Controls (n = 117)	P^b
Demographic factors			
Age (years)	56.8 ± 4.3	56.9 ± 4.1	0.63
Education elementary and under (%)	31.6	34.2	0.63
Nondietary risk factors			
First degree relative with breast cancer (%)	5.1	2.6	0.32
Ever diagnosed with fibroadenoma (%)	7.8	2.6	0.06
BMI[a]	24.6 ± 3.8	23.9 ± 3.7	0.15
WHR[a]	0.81 ± 0.06	0.81 ± 0.06	0.80
Ever exercise (%)	35.0	47.9	0.06
Age at menarche[a]	14.9 ± 1.8	15.1 ± 1.9	0.50
Age at menopause (years)	48.8 ± 4.3	48.2 ± 4.4	0.22
Nulliparity	3.4	4.3	0.74
Number of live birth[c]	2.2 ± 1.0	2.2 ± 0.9	0.36
Age at first live birth (years)[c]	25.6 ± 4.8	24.2 ± 3.8	0.01
Nutritional factors			
Total energy intake (kcal/day)	1895.2 ± 408.1	1866.1 ± 462.6	0.62
Total fat intake (g/day)[d]	33.8 ± 1.02	36.5 ± 1.02	0.07
Total fruit and vegetable intake (g/day)[d]	482.0 ± 24.3	481.4 ± 24.3	0.99
Soy protein intake (g/day)[d]	10.8 ± 0.8	13.2 ± 0.8	0.03
Total meat (g/day)[d]	83.0 ± 5.0	76.7 ± 5.0	0.37

[a] Unless otherwise specified, mean ± SD are presented.
[b] *P*s were from the McNemar's test (for categorical variables) or paired Student t test (for continuous variables).
[c] Among ever pregnant women.
[d] Total energy adjusted means were presented.

Table 2 Comparison of urinary excretion rates of isoflavonoids and lignans, and blood levels of steroid hormones and SHBG between cases and controls, the Shanghai Breast Cancer Study, 1996–98

	Median (25^{th}, 75^{th} percentile)		Percentage[a] difference	P from paired Wilcoxon rank test
	Cases (n = 117)	Controls (n = 117)		
Total isoflavonoids[b,c]	18.38 (5.36, 44.91)	26.41 (8.31, 62.32)	−30.4	0.04
Daidzein[b]	5.10 (1.97, 21.30)	11.57 (4.02, 26.19)	−55.9	0.03
Genistein[b]	2.42 (0.50, 9.37)	5.80 (0.96, 13.23)	−58.3	0.12
Glycitein[b]	0.56 (0.14, 2.35)	1.31 (0.30, 3.37)	−57.3	0.01
Total mammary lignans[b,d]	1.72 (0.32, 4.34)	2.64 (0.43, 7.01)	−34.8	0.06
Enterolactone[b]	0.20 (0.04, 0.44)	0.29 (0.11, 0.68)	−31.0	0.02
Enterodiol[b]	1.22 (0.14, 3.89)	2.11 (0.23, 6.57)	−42.2	0.04
E_2 (pg/ml)	5.73 (3.82, 13.65)	5.73 (3.82, 14.96)	0.0	0.59
E_1 (pg/ml)	19.10 (9.57, 24.20)	17.85 (9.05, 20.65)	7.0	<0.01
E_1-S (ng/ml)	1.14 (0.51, 6.68)	0.96 (0.48, 6.68)	18.8	0.40
Testotesterone	0.20 (0.13, 0.28)	0.16 (0.12, 0.22)	25.0	<0.01
DHEA-S (ng/ml)	671.8 (485.5, 988.9)	589.8 (404.4, 811.7)	13.9	0.05
SHBG (nmol/liter)	74.90 (55.65, 113.95)	81.40 (56.40, 121.80)	−8.0	0.81

[a] Expressed as $(\text{median}_{\text{cases}} - \text{median}_{\text{controls}})/\text{median}_{\text{controls}}$.
[b] Expressed as nmoles per mg creatinine.
[c] Including daizein, genistein, glycitein, *O*-DMA, dihydro-daidzein, and dihydro-genistein.
[d] Including mammary enterolactone and enterodiol.

in age. Compared with controls, cases had an earlier age at menarche, an older age at menopause or at the first live birth, a higher BMI or WHR, and were less likely to exercise. Cases also appeared to have a higher educational achievement, and a higher frequency of prior history of breast fibroadenoma or family history of breast cancer among first-degree relatives. Although not statistically significant, cases tend to have a higher energy intake than controls. After adjusted for total energy intake, cases had a lower intake mean level of soy protein and fat, but higher mean intake of total meat or fruits and vegetables. These results are consistent with those from analyses using all subjects of the Shanghai Breast Cancer Study (12), although some of the outcomes in the current analysis were not statistically significant because of a small sample size. As with our previous report (11), we adjusted for the following variables in the analyses presented in this report: age at first live birth (continuous in year), ever diagnosed with fibroadenoma (yes/no), total meat intake (continuous in g/per day), ever physically active (yes/no), and BMI [continuous in weight(kg)/height $(m)^2$].

Presented in Table 2 are comparisons of median levels of plasma E_2, E_1, E_1-S, testosterone, DHEA-S, SHBG, and urinary excretion rates of isoflavonoids and lignans between cases and controls. Cases excreted substantially lower rates of urinary isoflavonoids and lignans than controls. These results are similar to those in our previous report for both pre- and postmenopausal women (11). The results for the association with specific isoflavonoids and lignans are not presented in Tables 3 and 4, because the pattern of the association was very similar across specific phytoestrogens, and each major specific phytoestrogen was highly correlated with total isoflavonoids or lignans (cor-

Table 3 OR and 95% CIs for urinary phytoestrogen excretion rates in relation to breast cancer risk, stratified by levels of BMI and WHR, the Shanghai Breast Cancer Study, 1996–1998

BMI or WHR (by median)		Urinary excretion rate of isoflavonoids by tertile[a]			
		T1 (low)	T2	T3	*P* for trend
Total isoflavonoids					
Cases/controls		53/38	37/39	27/40	
All subjects combined		1.00	0.79 (0.42–1.49)	0.46 (0.22–0.95)	0.04
BMI[b]	<25	1.00	1.68 (0.70–4.06)	0.54 (0.21–1.41)	0.34
	≥25	1.00	0.30 (0.10–0.87)	0.38 (0.13–1.17)	0.06
			P for interaction, 0.23		
WHR[c]	<0.84	1.00	0.80 (0.35–1.84)	0.66 (0.28–1.54)	0.33
	≥0.84	1.00	0.80 (0.27–2.33)	0.18 (0.05–0.68)	0.02
			P for interaction, 0.36		
Total lignans					
Cases/controls		43/39	51/38	23/40	
All subjects combined		1.00	1.06 (0.54–2.08)	0.50 (0.23–1.10)	0.09
BMI[b]	<25	1.00	1.53 (0.61–3.86)	0.70 (0.26–1.88)	0.37
	≥25	1.00	0.72 (0.27–1.87)	0.27 (0.07–1.10)	0.08
			P for interaction, 0.71		
WHR[c]	<0.84	1.00	1.04 (0.45–2.41)	0.86 (0.32–2.29)	0.67
	≥0.84	1.00	1.33 (0.42–4.22)	0.17 (0.04–0.71)	0.02
			P for interaction, 0.38		

[a] Cases and controls were categorized according to the tertile level of controls.
[b] Adjusted for age at first live birth, ever diagnosed with fibroadenoma, total meat intake, and ever physically active.
[c] Additionally adjusted for BMI.

Table 4 ORs and 95% CIs for urinary phytoestrogens in relation to breast cancer risk, stratified by blood levels of steroid hormones and SHBG, the Shanghai Breast Cancer Study, 1996–1998

Steroid hormones (by median)		Urinary excretion rate of isoflavonoids by tertile[a,b]				Urinary excretion rate of lignans by tertile[a,b]			
		T1 (low)	T2	T3	*P* for trend	T1 (low)	T2	T3	*P* for trend
E_2 (pg/ml)	≤5.73	1.00	1.04 (0.43–2.51)	0.76 (0.26–2.18)	0.65	1.00	0.68 (0.24–1.87)	0.37 (0.12–1.17)	0.08
	>5.73	1.00	0.60 (0.21–1.74)	0.22 (0.07–0.68)	0.01	1.00	1.45 (0.51–4.09)	0.61 (0.20–1.89)	0.41
		P for interaction, 0.18				*P* for interaction, 0.40			
E_1 (pg/ml)	≤17.85	1.00	0.67 (0.25–1.76)	0.34 (0.11–1.04)	0.07	1.00	0.68 (0.25–1.87)	0.46 (0.17–1.30)	0.13
	>17.85	1.00	0.95 (0.37–2.46)	0.47 (0.16–1.41)	0.19	1.00	1.26 (0.50–3.15)	0.49 (0.16–1.51)	0.23
		P for interaction, 0.07				*P* for interaction, 0.58			
E_1-S (ng/ml)	≤0.96	1.00	0.66 (0.23–1.85)	0.20 (0.06–0.64)	0.01	1.00	1.46 (0.50–4.29)	0.62 (0.19–2.02)	0.35
	>0.96	1.00	1.05 (0.42–2.61)	0.78 (0.27–2.27)	0.67	1.00	0.75 (0.30–1.85)	0.38 (0.11–1.35)	0.15
		P for interaction, 0.74				*P* for interaction, 0.71			
Testosterone (ng/ml)	≤0.16	1.00	0.63 (0.20–1.99)	0.47 (0.14–1.61)	0.26	1.00	1.11 (0.33–3.76)	0.58 (0.16–2.11)	0.30
	>0.16	1.00	1.05 (0.44–2.53)	0.37 (0.13–1.07)	0.09	1.00	0.98 (0.43–2.24)	0.41 (0.15–1.17)	0.12
		P for interaction, 0.30				*P* for interaction, 0.94			
DHEA-S (ng/ml)	≤589.8	1.00	0.63 (0.21–1.91)	0.49 (0.16–1.49)	0.24	1.00	0.82 (0.29–2.31)	0.38 (0.11–1.32)	0.14
	>589.8	1.00	0.90 (0.36–2.22)	0.38 (0.14–1.05)	0.06	1.00	1.17 (0.43–3.15)	0.53 (0.19–1.53)	0.23
		P for interaction, 0.74				*P* for interaction, 0.91			
SHBG (nmol/liter)	≤81.4	1.00	0.52 (0.22–1.22)	0.36 (0.13–0.97)	0.03	1.00	1.35 (0.58–3.10)	0.64 (0.23–1.82)	0.60
	>81.4	1.00	1.32 (0.48–3.68)	0.67 (0.24–1.85)	0.47	1.00	0.67 (0.20–2.27)	0.33 (0.09–1.24)	0.06
		P for interaction, 0.46				*P* for interaction, 0.40			

[a] Cases and controls were categorized according to the tertile level of controls.
[b] Adjusted for age at first live birth, ever diagnosed with fibroadenoma, total meat intake, ever physically active, BMI, and blood SHBG level.

relation coefficient: 0.42–0.99 with only one below 0.56; Ref. 11). The medians of urinary creatinine (mg/liter) were 648.8 for cases and 516.8 for controls. On the other hand, cases had a higher median level of plasma E_1, testosterone, or DHEA-S and a lower median level of SHBG than controls. There was no difference in plasma E_2 level between cases and controls.

As shown in Table 3, inverse associations of isoflavonoids and lignans with breast cancer risk were observed regardless of BMI and WHR strata. However, the inverse associations were only statistically significant or of borderline significance among women with a high BMI or WHR. Similar stratified analyses were performed by medians of blood E_2, E_1, E_1-S, testosterone, DHEA-S, and SHBG (Table 4). Again, inverse associations were observed in all of the strata for both isoflavonoids and lignans. For urinary isoflavonoids, the inverse association was statistically significant only among women with a high E_2 (trend test; $P = 0.01$), low E_1-S ($P = 0.01$), and low SHBG ($P = 0.03$). No statistically significant inverse association was observed for urinary lignans. Data for crude ORs were not shown, but the pattern of the associations is similar between crude and adjusted estimates. None of the tests for a multiplicative interaction were statistically significant. However, the statistical power to evaluate multiplicative interaction was low. For example, the minimal detectable size of

interaction is >10 for a study with 117 case-control pairs to evaluate an interaction of two categorical variables that are independently associated with a 2-fold risk of disease.

Discussion

Our findings for a stronger inverse association of urinary isoflavonoids and lignans with breast cancer risk among women with a high BMI or WHR were consistent with the results from our previous report based on dietary assessment of usual soyfood intake (9). As with studies conducted elsewhere, BMI and WHR were associated positively with breast cancer risk among Chinese women in Shanghai (21). BMI is related positively to blood level of estrogens among postmenopausal women (22), although the association of WHR with estrogens is less clear. Both BMI and WHR have also been linked to an elevated level of blood insulin (23), and a reduced level of SHBG (24) or 2-hydroxyestrone:16α-hydroxylation ratio (25). All of these factors may be affected by phytoestrogens. *In vitro* and *in vivo* studies found that phytoestrogens may compete with estrogens to bind to estrogen receptors (1). *In vitro* studies showed that lignans moderately inhibit aromatase in human preadipocytes (1, 5), whereas intake of isoflavonoids were shown to increase SHBG level in intervention studies (8). Human intervention studies also suggest that both soy and lignan intakes increase the urinary 2-hydroxyestrone:16α-hydroxyestrone ratio (6, 7). Several animal studies have shown recently that soy intake improves glucose tolerance and insulin sensitivity (26). In an intervention study, Duncan *et al.* (27) found that a high-soy diet appeared to result in a lower level of blood insulin. Therefore, it is possible that women with a high BMI or WHR may have a high level of estrogens and insulin, and a low level of SHBG and 2-hydroxyestrone:16α-hydroxylation ratio and may particularly benefit from high intake of phytoestrogens.

Consistent with the results on potential modifying effect of BMI and WHR on urinary phytoestrogen excretion and breast cancer risk, we found that the inverse association between urinary excretion of isoflavonoids and breast cancer risk was more pronounced among women with a higher level of E_2, or a lower level of SHBG than those with a lower level of E_2 or a higher level of SHBG. It is unclear why the inverse association of urinary phytoestrogen and breast cancer was stronger among women with a higher E_2 but not among those with a higher E_1 in our study. Although after menopause, E_1 substitutes E_2 and becomes the major circulating estrogen, E_2 level in breast tissue remains high (28), and the tissue:plasma ratio of E_2 increases from 1:1 in premenopausal women to 10–50:1 in postmenopausal women. *In vitro* studies found that isoflavonoids modestly inhibit 17β-HSD1, a key enzyme in catalyzing E_1 to the biologically more active E_2 (1, 2). Most of E_1 is in the form of the sulfate conjugate (E_1-S), which cannot cross breast cell membrane (28). Estrogen sulfotransferase is the major enzyme catalyzing the conversion of estrogens to their inactive sulfated forms, and estrone sulfatases catalyze the conversion of sulfated estrogens back to E_1 or E_2. A recent study showed that sulfated isoflavonoids were potent inhibitors of sulfatases (4). Therefore, it is possible that women with a high sulfatase activity (and, thus, low E_1-S) may particularly benefit from intake of sulfatase inhibitors, isoflavonoids. Our observation for a stronger inverse association of isoflavonoids among women with a lower E_1-S appears consistent with this hypothesis.

In our study, we did not find any statistically significant association for urinary lignans with breast cancer risk after stratifying by steroid hormones or SHBG. It is possible that the mechanisms for isoflavonoids and lignans differ. However, the level of urinary lignans was 10 times lower than that of isoflavonoids, and effect of lignans may be masked by isoflavonoids. Indeed, in our previous report, we found that the inverse association between lignans and breast cancer risk was primarily among women who excreted low rate of isoflavonoids (11). Because of a small sample size, we were unable to evaluate the association of urinary lignans after stratifying by both isoflavonoids and steroid hormones at the same time.

Blood and urine samples in this study were collected at the same time. It is possible that measured levels of plasma steroid hormones or SHBG may have been affected by current intake of phytoestrogens, and, thus, some of the modifying effect may have already been reflected in the levels of blood steroid hormones and SHBG. This limitation is likely to attenuate the association, which is unavoidable even in any cohort studies. However, BMI and WHR are unlikely to be influenced by urinary phytoestrogen excretion rates measured in the study, which may explain the more consistent findings observed in this study for these anthropometrics than blood steroid hormones.

Other limitations of the study include the use of postdiagnostic samples for the study. However, urine samples of all of the cases included in this study were collected before any cancer therapy, minimizing influence of breast cancer and its sequelae on the levels of urinary analytes. Although bias could still occur, if the breast cancer patients had substantially changed their dietary habits after being diagnosed, there is no reason to speculate that cases would have decreased their intake of soyfoods, fruits, and vegetables, particularly because these foods are traditionally regarded in Shanghai as well-balanced foods that are suitable for cancer patients. Although it cannot eliminate the possibility that urinary isoflavonoids are merely surrogate biomarkers of other anticarcinogenic compounds, such as trypsin inhibitors, saponins, and phytic acid, no evidence suggests that urinary isoflavonoids are highly correlated with the bioavailability of these heat-labile compounds (29). Finally, the sample size for this study was small, and statistical power for evaluating interaction was limited.

In summary, we found that the association of phytoestrogens with breast cancer may be modified by BMI, WHR, and blood level of sex hormones and SHBG. These results are consistent with findings from *in vitro* and *in vivo* studies. Our findings, if confirmed in future larger studies, could have significant public health implications, as women with a high risk of breast cancer could be specifically targeted for increasing phytoestrogen intake.

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