Plasma Isoflavones and Fibrocystic Breast Conditions and Breast Cancer Among Women in Shanghai, China

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Abstract

Background: Proliferative benign breast conditions are associated with elevated risk of breast cancer, whereas nonproliferative conditions are not strongly associated with risk. Factors acting before onset of hyperplasia might be associated with both benign conditions and breast cancer, whereas those on the proliferative disease-to-cancer pathway would be associated only with cancer. Soy isoflavone exposure may influence breast cancer risk, but little is known of its association with benign conditions. Materials and Methods: We examined possible relationships between plasma genistein and daidzein concentrations and risk of breast disease in women, in a breast self-examination trial in Shanghai, China, diagnosed with breast cancer (n = 196) or a benign breast condition (n = 304), and 1,002 age-matched controls with no known breast disease. Benign conditions were classified as nonproliferative (n = 131) or proliferative with or without atypia (n = 173).

Results: Isoflavone concentrations were inversely associated with risk of nonproliferative and proliferative benign fibrocystic conditions, as well as with breast cancer, both with and without concomitant proliferative changes in ipsilateral noncancerous mammary epithelium (P trend < 0.01 for all comparisons with controls). Women in the highest quartile of plasma genistein (>76.95 ng/mL) were less likely to have breast cancer (odds ratio, 0.26; 95% confidence interval, 0.13-0.50) or benign conditions (odds ratio, 0.40; 95% confidence interval, 0.23-0.70) compared with women in the lowest quartile (<9.42 ng/mL). Observed risks for breast cancer with and without surrounding proliferative changes were not different, respectively, from observed risks for benign proliferative and nonproliferative conditions alone.

Conclusion: Isoflavone exposure was inversely associated with fibrocystic breast conditions and breast cancer, and the results suggest that effects on cancer risk occur early in carcinogenesis. (Cancer Epidemiol Biomarkers Prev 2007;16(12):2579–86)

Introduction

Proliferative fibrocystic breast conditions without and with atypia have been associated with a 1.5-fold to 2-fold and 3.5-fold to 5-fold increased risk of breast cancer, respectively (1, 2). Nonproliferative fibrocystic breast conditions do not seem to be associated with an increased risk of neoplasia unless a strong family history is present (2); however, they are common, place a burden on health care resources, and cause psychological stress to the patient (3).

Exposures and reproductive lifestyle factors that modulate steroid hormones are associated with both breast cancer and fibrocystic breast conditions (4). Dietary isoflavones, found almost exclusively in soy foods, are estrogenic phytochemicals that bind to estrogen receptors α and β (5). Because of their ability to compete with endogenous estrogens for estrogen receptor binding, these compounds have received considerable attention for their potential chemopreventive effects in relation to breast cancer (6). Epidemiologic studies of the relationship between intake of soy foods and breast cancer have yielded inconsistent results (7). One study found a significant inverse association between soy food intake and risk of proliferative fibrocystic breast conditions, but not of nonproliferative fibrocystic conditions, or of breast cancer with or without concomitant proliferative fibrocystic conditions in extratumoral tissue (4). For the most part, these studies have relied on estimating soy and isoflavone intake using food frequency questionnaires or other dietary report instruments. However, isoflavones undergo significant intestinal bacterial metabolism, such that urinary recovery of the parent isoflavones, genistein and daidzein, and their metabolites is typically 7% to 18% and 42% to 62%, respectively (8). Consequently, use of biomarkers that integrate dietary intake and metabolic handling and

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absorption of isoflavones may be more informative than measuring dietary intake alone.

Only a few studies have examined the relationship between breast cancer risk and biomarkers of isoflavone exposure. Generally, among Asian populations, urinary or circulating isoflavone levels are associated with lower breast cancer risk (9), whereas among Western populations wherein soy food intake is typically low, associations have been positive (10), inverse (11-13), or null (14, 15). The relationship between plasma isoflavone concentrations and fibrocystic breast conditions has not been evaluated.

Our objective was to investigate possible associations between soy isoflavone exposure, as measured by plasma genistein and daidzein concentrations, and risk of proliferative fibrocystic changes in the mammary epithelium in women with and without concomitant breast cancer. For comparison, we also assessed these possible associations with nonproliferative fibrocystic changes alone and in the extratumoral tissue of women with concomitant breast cancer.

Materials and Methods

Study Population. The participants in this study were selected from women who participated in a randomized trial of breast self-examination in Shanghai, China (16). Briefly, 266,064 women born between January 1, 1925 and December 31, 1958 (ages 30-64 years at enrollment) who were current or retired employees of the Shanghai Textile Industrial Bureau were enrolled in the trial between October 1989 and October 1991. The cases were identified through July 31, 2000, for development of benign and malignant breast diseases.

Case Selection. When a woman in the breast self-examination trial developed a breast lump or detected a breast lump, she was initially evaluated by a medical worker in the clinic in her factory and then, if indicated, was referred to one of three hospitals operated by Shanghai Textile Industrial Bureau or to other hospitals that had contracts with individual factories. The breast self-examination trial personnel identified all new cases of breast cancer and benign breast diseases by periodic review of the records of the factory medical clinics, visits to the three Shanghai Textile Industrial Bureau hospitals, and visits to other hospitals as needed. The tumor size, location within the breast, stage, and histologic classification were abstracted onto a standardized form, and histologic slides were obtained from each case for standardized review. Approximately, two thirds of the women in the cohort who had a breast biopsy were diagnosed and treated in one of the three hospitals operated by the Shanghai Textile Industrial Bureau. Women in the trial cohort, who received a breast biopsy in one of these hospitals and who were diagnosed with breast cancer or benign breast disease between September 1995 and July 2000, were eligible for the present study. A total of 622 women with fibrocystic conditions and 432 with breast cancer were detected during this study period. In-person interviews (described in ref. 4) were completed for 551 of the women with fibrocystic changes; 343 of these women had satisfactory slides (i.e., at least five scanning power fields) for pathologic review and a satisfactory blood sample. From these 343 women, we further excluded 23 women for whom the date of blood draw was >30 days before or 2 weeks after the date of diagnosis or >30 days from the date of interview and 16 women in whom neither daidzein or genistein could be measured. Thus, 304 women with fibrocystic changes were included in this study. In-person interviews were completed for 384 women with breast cancer, six of whom were excluded because of a history of previous breast cancer. Of the remaining 378 cases, 244 had both satisfactory noncancerous mammary epithelial tissue for histologic evaluation and a satisfactory blood draw. Forty of these women were excluded because their date of diagnosis was >2 weeks before or 30 days after the date of blood draw or the date of in-person interview was >30 days from the date of blood draw, and eight women in whom neither daidzein or genistein could be measured were also excluded, leaving 196 breast cancer cases in the analysis. Women in this study who were diagnosed with fibrocystic conditions (n = 135) or breast cancer (n = 93) between September 1995 and August 1997 were also enrolled in a concurrent study of cell proliferation. The remaining 269 women with fibrocystic conditions and 103 women with breast cancer were recruited only into the present study.

Control Selection. Controls were selected from unaffected women in the breast self-examination trial cohort. For the benign and malignant cases also enrolled in the cell proliferation study, 20 potential controls of the same age were randomly selected and listed. The women were contacted in the order listed until two women with the same age and menstrual status of the corresponding case were recruited. A total of 367 controls were recruited by this method (64% of the eligible women contacted). All were included in the present study, but individual matching of controls was not retained in these analyses. For the rest of the cases, controls were frequency matched by 5-year age group and hospital affiliation of their factory to the cases in this study and also to those in a concurrent study of fibroadenoma, so that there would be a 1:1 case-to-control ratio for the largest benign or malignant case group in each age stratum. In-person interviews were completed for 704 of 862 controls (82%) selected in this manner. One control woman with a presumed unreliable calculated daily energy intake of >4,000 kcal was excluded. Of the 1,070 remaining controls, blood samples were collected from 1,042 women. Five controls were excluded because their date of blood draw was >30 days from the date of in-person interview. We also excluded 35 control women for whom neither daidzein nor genistein could be measured, leaving 1,002 controls included in the analysis.

The Institutional Review Board of the Fred Hutchinson Cancer Research Center and the Station for Prevention and Treatment of Cancer in the Shanghai Textile Industry Bureau approved the study in accordance with the assurances of the Office for Human Research Protection of the U.S. Department of Health and Human Services. Informed consent was obtained from each woman before interview.

Diagnosis and Histologic Classification. A single study pathologist (M.L.) reviewed slides from the benign fibrocystic conditions and from the extratumoral tissue
from the cancer cases and classified them according to the scheme developed by Stalsberg. The following features were scored on a scale of 0 to 3 (normal/not present, mild, moderate, florid): adenosis, sclerosing adenosis, ductal hyperplasia, apocrine metaplasia, apocrine hyperplasia, cysts, fibrosis, calcification, duct ectasia, inflammatory reaction, and lactation change. For lobular atypia, ductal atypia, and apocrine atypia, another scoring system was applied: 0, none; 1, uncertain; 2, atypical hyperplasia.

A subset of samples of major types of benign breast conditions and extratumoral tissues of malignant cases was also read by Dr. Stalsberg. There was satisfactory concordance between readings by the two pathologists on assessing levels of proliferation and presence of atypia (weighted k coefficient, 0.4), but poor agreement on the detailed features of hyperplasia according to both scoring systems. Thus, we limited classification of benign breast conditions and the noncancerous breast tissue from the malignant cases to one of the following three categories for analyses: nonproliferative conditions (ductal hyperplasia and sclerosing adenosis with a score of 0 or 1), proliferative without atypia (ductal hyperplasia and sclerosing adenosis with a score of 2 or 3), and atypical hyperplasia (atypical ductal hyperplasia, atypical lobular hyperplasia, and atypical apocrine epithelium with a score of 2). The resultant classification is similar to that of Page. In all instances, the diagnosis of the study pathologist was used.

**Measurement of Plasma Daidzein and Genistein.** All plasma samples were frozen and stored at −70°C until assayed. Samples were batched with a similar distribution of cases and controls in each batch. The methods used to measure plasma daidzein and genistein concentrations have been described in detail previously. Two different methods were used. Initially, liquid chromatography (LC)–Coularray method was used. We later switched to a LC–mass spectrometry (MS) method to improve assay efficiency and precision of the sample measurements. Comparison of the two methods showed that mean serum daidzein concentrations were higher when run by LC-Coularray compared with LC-MS, but mean serum genistein concentrations were similar by analysis method. Samples from 124 women with fibrocystic changes (58 nonproliferative and 66 proliferative), 94 breast cancer cases (48 nonproliferative and 46 proliferative extratumoral tissue), and 261 controls were analyzed by the LC-Coularray method. Samples from 180 women with fibrocystic changes (73 nonproliferative and 107 proliferative), 102 breast cancer cases (54 nonproliferative and 48 proliferative extratumoral tissue), and 741 controls were analyzed by LC-MS. For both methods, daidzein and genistein concentrations of <1 ng/mL for genistein were considered below the limit of quantitation and were assigned the midpoint value of 0.5 ng/mL.

**Statistical Methods.** Plasma isoflavone variables were categorized into quartiles according to their distribution among the 1,002 controls. Odds ratios (OR) and 95% confidence intervals (95% CI) for the upper three quartiles compared with the lowest quartile were calculated using conditional logistic regression analysis. We computed the OR of fibrocystic conditions and breast cancer by comparing each case group to the control group, and we also directly compared the malignant and benign case groups to estimate risk of breast cancer relative to that of fibrocystic changes. All analyses were stratified by the proliferative status of the fibrocystic changes and of the extratumoral tissue of the cancer cases. Proliferative conditions with and without atypia were combined because the number of women with atypia was small. Age (5-year categories) and plasma isoflavone analysis method were included for adjustment in the multiple logistic models. These models were also stratified by year of blood draw (1995-1996, 1997, 1998-1999, 2000-2001), because dietary habits have changed rapidly over the past two decades in China and blood draws from controls tended to have been taken at a later date than those from the cases. We evaluated the possible confounding effects on the OR estimates of multiple factors, including age at first birth, the number of live births, total duration of lactation, years of oral contraceptive use, age at first menstrual period, menopausal status, prior breast lump, number of times of breast self-examination done per year, body mass index, and education. However, none of these factors changed the results appreciably (<10% change in the OR of the primary predictor variable) when added individually into multiple logistic models, and they were not included in the final models. Tests for trends were done by entering the categorical variables as continuous variables into the regression models. All statistical analyses were based on two-tailed probability and done using SAS version 8.2 (SAS Institute, Inc.).

**Results**

As shown in Table 1, the controls were older than the women with fibrocystic lesions and younger than the cases of breast cancer. More than 80% of women with fibrocystic breast conditions were younger than 50 years of age at diagnosis and over 40% of breast cancer cases were 50 years of age and older at diagnosis. General and reproductive risk factors for these conditions and soy intake in this population are also presented in Table 1. As reported previously, the risk of both proliferative fibrocystic breast conditions alone and with breast cancer were associated with low parity, a benign breast lump, and breast cancer in a first-degree relative. Similarly, nonproliferative fibrocystic conditions alone and with breast cancer were associated with the same risk factors as for proliferative conditions, although the association with parity was not as strong. Risk in relation to self-reported soy intake among women in this study was similar to that reported for the larger study. The OR for the highest quartile group of soy intake (compared with the lowest group) was 1.28 (95% CI, 0.64-2.58, \( P_{\text{trend}} = 0.47 \)) for nonproliferative fibrocystic conditions and 0.51 (95% CI, 0.26-1.0, \( P_{\text{trend}} = 0.02 \)) for proliferative fibrocystic conditions. The ORs were 1.02 (95% CI, 0.50-2.06; \( P_{\text{trend}} = 0.83 \)) for breast cancer with concurrent nonproliferative benign conditions and 0.71 (95% CI, 0.32-1.58; \( P_{\text{trend}} = 0.49 \)) for breast cancer with concurrent proliferative conditions.

Table 2 shows the OR for fibrocystic breast conditions and for breast cancer in relation to quartiles of plasma daidzein and genistein concentration. There was a significant inverse association of plasma concentrations...
of both daidzein and genistein with both nonproliferative and proliferative (including atypia) fibrocystic conditions, all fibrocystic conditions combined, and breast cancers with both nonproliferative and proliferative changes in the noncancerous breast tissue from the same breast, as well as all breast cancers combined. The associations with fibrocystic conditions and with breast cancer were of similar magnitude. Therefore, no significant trends in risk of breast cancer relative to risk of fibrocystic conditions in relation to either isoflavone were observed in either the women with or without benign epithelial proliferative changes.

When breast cancer cases with plasma isoflavone data, but without adequate materials for histologic

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review (57 cases for daidzein and 59 cases for genistein), were included in the analysis, the OR for total breast cancer did not change substantially; compared with lowest quartile, OR (95% CI) of second, third, and fourth quartiles were 0.82 (0.47-1.43), 0.43 (0.24-0.79), 0.25 (0.13-0.47) for daidzein ($P_{trend} < 0.0001$) and 0.54 (0.33-0.90), 0.63 (0.38-1.04), 0.30 (0.17-0.55) for genistein ($P_{trend} = 0.0003$), respectively.

**Discussion**

In this population-based case-control study, the risk of breast cancer and fibrocystic breast conditions, both with or without benign proliferative changes, decreased significantly with increasing plasma concentrations of genistein and daidzein. The highest quartiles of genistein and daidzein concentrations compared with the lowest were associated with approximately a 60% to 80% reduced risk of fibrocystic breast conditions and breast cancer, respectively.

Few studies have examined the relationship between circulating isoflavone concentrations and breast cancer risk, particularly among populations routinely consuming soy, and ours is the first also to evaluate the relationship between circulating isoflavone concentrations and fibrocystic breast conditions. Previously, Dai et al. (9) showed that, among 500 women in a case-control study in

### Table 2. OR and 95% CI of fibrocystic breast conditions and breast cancer in relation to quartiles of plasma daidzein and genistein concentrations

<table>
<thead>
<tr>
<th></th>
<th>Daidzein (ng/mL)</th>
<th>Genistein (ng/mL)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Nonproliferative</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Q1 (&lt;6.718)</td>
</tr>
<tr>
<td>956 (100)</td>
<td></td>
<td>239 (25.0)</td>
</tr>
<tr>
<td>FBCs versus controls</td>
<td>OR $^c$ (95% CI)</td>
<td>0.49 (0.26-0.94)</td>
</tr>
<tr>
<td>Cancer versus controls</td>
<td>OR $^c$ (95% CI)</td>
<td>0.56 (0.27-1.18)</td>
</tr>
<tr>
<td>Cancer versus FBCs</td>
<td>OR $^c$ (95% CI)</td>
<td>1.14 (0.52-2.48)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Q2 (6.718-18.515)</td>
</tr>
<tr>
<td>956 (100)</td>
<td></td>
<td>239 (25.0)</td>
</tr>
<tr>
<td>FBCs versus controls</td>
<td>OR $^c$ (95% CI)</td>
<td>0.42 (0.16-0.76)</td>
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<tr>
<td>Cancer versus controls</td>
<td>OR $^c$ (95% CI)</td>
<td>0.65 (0.31-1.32)</td>
</tr>
<tr>
<td>Cancer versus FBCs</td>
<td>OR $^c$ (95% CI)</td>
<td>1.39 (0.64-2.65)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Q3 (18.515-42.092)</td>
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<tr>
<td>956 (100)</td>
<td></td>
<td>239 (25.0)</td>
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<tr>
<td>FBCs versus controls</td>
<td>OR $^c$ (95% CI)</td>
<td>0.22 (0.10-0.50)</td>
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<tr>
<td>Cancer versus controls</td>
<td>OR $^c$ (95% CI)</td>
<td>0.20 (0.08-0.49)</td>
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<tr>
<td>Cancer versus FBCs</td>
<td>OR $^c$ (95% CI)</td>
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<td></td>
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<td>Q4 (≥42.092)</td>
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<tr>
<td>956 (100)</td>
<td></td>
<td>239 (25.0)</td>
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<tr>
<td>FBCs versus controls</td>
<td>OR $^c$ (95% CI)</td>
<td>0.42 (0.21-0.86)</td>
</tr>
<tr>
<td>Cancer versus controls</td>
<td>OR $^c$ (95% CI)</td>
<td>0.65 (0.34-1.23)</td>
</tr>
<tr>
<td>Cancer versus FBCs</td>
<td>OR $^c$ (95% CI)</td>
<td>1.39 (0.64-2.65)</td>
</tr>
</tbody>
</table>

Abbreviation: FBC, fibrocystic breast condition.

*Women with missing data were excluded from the analysis.

* Adjusted for age and isoflavone analysis method and stratified by year of blood draw.

To convert ng/mL to nmol/L, multiply by 3.94.

Futher adjusted for the status of proliferative changes.

To convert ng/mL to nmol/L, multiply by 3.70.
Shanghai, urinary excretion of isoflavonoids was significantly lower among breast cancer cases than controls. In Western populations, where exposure to low levels of isoflavonoids is mainly as food additives in baked products, urinary isoflavone concentrations have been shown to be inversely (11, 12) or not significantly (14) associated with breast cancer risk. Of the studies which measured circulating isoflavone concentrations, one reported a positive association with breast cancer risk among women in Norwich, England (10), whereas another showed no association in premenopausal women in Germany (15), and a recent study from the Netherlands, predominantly in postmenopausal women, reported an inverse association (13).

Differences in risk association between studies in Asian and Western populations have been attributed in part to differences in study design (i.e., prospective versus case-control; ref. 7); however, several other differences in isoflavone exposure between these populations should be considered. Dose, timing of exposure, and gut bacterial metabolism may also play a role. Soy intakes in Western, low-soy intake populations may not provide a sufficiently high-enough isoflavone dose to alter effectively the risk of breast cancer (22). Among Western women, median or geometric mean genistein concentrations have been reported in the range of 1 to 5 ng/mL (4-19 nmol/L; refs. 10, 13), whereas among the control subjects in our study, the geometric mean genistein concentration was 23 ng/mL (85 nmol/L; ref. 19). Isoflavone exposure also has been shown to be associated with lower breast cancer risk among women who were exposed to higher amounts of isoflavonoids prepubertally (23-25), and in rodents, early exposure to genistein enhances mammary gland differentiation and reduces tumor incidence and multiplicity (26). Therefore, Western women who adopt a diet higher in soy foods as adults may not derive the same benefit as Asian women who have been exposed continuously from earlier in life. Lastly, differences in gut microbial communities between populations may affect isoflavone bioavailability and microbial metabolism (27); for example, Asian populations tend to be more likely to convert the soy isoflavone daidzein to equol, a reaction dependent on harboring certain bacteria (28).

Overall, few dietary risk factors have been identified for fibrocystic breast conditions (20), and in general, dietary approaches to secondary prevention or treatment of fibrocystic breast conditions, which to date have not included soy, have not been effective (29). Because excessive estrogen or altered sensitivity to estrogen is a dominant hypothesis of fibrocystic breast etiology, dietary factors that may modulate endogenous steroid hormones and their metabolism have been proposed as target approaches. Previously, in this Shanghai population, higher soy intake (measured by food frequency questionnaire) was associated with lower risk of fibrocystic breast conditions with proliferative changes; however, soy intake was not associated with proliferative fibrocystic breast conditions with breast cancer or with nonproliferative fibrocystic breast conditions alone (4, 20). Further, soy intake also was not associated with overall risk of breast cancer (21). This discordance between observed relationships of breast disease risk and food frequency questionnaire–estimated soy intake compared with our present findings with plasma biomarkers may in part reflect the limitations of the food frequency questionnaire. Previously, we observed in this cohort a significant, but modest, linear trend for isoflavone concentrations with increasing quartiles of soy food consumption frequency and with increasing estimated isoflavone intake (19). Nonetheless, plasma isoflavone concentrations are an integration of dietary soy intake, as well as bioavailability, which is determined in part by bacterial metabolism of the isoflavones, as well as enteric recycling (i.e., in vivo conjugation and efflux; ref. 30). In humans, there is large interindividual variation in urinary recovery of genistein and daidzein and their metabolites, suggesting that bioavailability may be a critical aspect of exposure that is not captured with dietary report methods (8).

The etiology of fibrocystic breast conditions and the progression of proliferative fibrocystic breast conditions to breast cancer remain poorly characterized (31). Cyst formation is thought to occur through variation in normal lobular involution. Typically, lobular involution consists of orderly loss of both lobular epithelium and stroma (32), but early and uncoordinated stromal loss can result in remaining epithelial acini forming microcysts, thus setting the pattern for macrocyst development by obstruction of the efferent ductule (31). Breast cancer develops in part when a cell with an initiating genetic alteration is transformed into a cell with a proliferative advantage. For example, in women with benign breast disease, p53 protein accumulation and alterations in the p53 gene are associated with increased risk of progression to breast cancer (33). Subsequent cell proliferation expands the population of initiated cells, thereby enhancing the probability of further events leading to a malignant phenotype. Given the progression from fibrocystic breast conditions to breast cancer, risk factors for invasive breast cancer could act either before or after the development of hyperplasia. Thus, theoretically, factors acting before the onset of hyperplasia would be observed in both proliferative benign conditions and breast cancer, whereas those acting to increase the probability that proliferative disease progresses to breast cancer would be observed only in relation to breast cancer.

Our data showing an inverse association between plasma isoflavone concentrations and risk of both proliferative and nonproliferative fibrocystic breast conditions, with and without breast cancer, suggest that soy exposure before or during breast involution may be important. Isoflavone concentrations did not differ between the women with fibrocystic breast conditions and the women with breast cancer. Although the sample size for this test was small, the clear lack of a difference suggests that soy exposure may be a risk factor for both conditions. These findings are consistent with several other epidemiologic studies that suggest that earlier life exposures influence normal structural changes in the breast later in life. Baer et al. (34) reported that women who were heavier at young ages had lower incidence of proliferative benign breast disease. Byrne et al. (35) reported that higher alcohol intake among women ages 18 to 22 years was associated with later nonproliferative and proliferative benign breast disease, but not atypical hyperplasia. Our data are also consistent with those of...
other epidemiologic studies, suggesting that soy intake during childhood is important for a protective effect (23–25) and with those from animal studies, in which prepubertal exposure to genistein lowers tumor burden in adult animals (26).

This study has several strengths. It is a population-based study conducted in 1,502 women who typically consume soy foods, but with a wide range in frequency (19). We evaluated the effect of isoflavone exposure using a biomarker; plasma concentrations of daidzein and genistein are not dependent on recall of diet and take into account metabolism by colonic bacteria and bioavailability. Women had their blood drawn before diagnosis or treatment. Finally, all biopsies were histologically reviewed by one study pathologist.

Possible limitations influencing interpretation of these results are the case-control design of the study, and the use of circulating isoflavone concentrations as the biomarkers. Because women with breast conditions had their blood drawn at the time of biopsy, it is possible that the cases modified their diets as they approached the day of their hospital visit. However, the biopsy was considered a minor out-patient procedure in overall healthy women, women were not instructed to make any changes to their habitual activities or diet in preparation for the biopsy visit, and most samples were collected before diagnosis. Plasma isoflavone half-lives are short (6–8 h); therefore, circulating isoflavones are typically biomarkers of recent exposure. Nonetheless, in these women, we showed that plasma genistein and daidzein concentrations were significantly associated with frequency of soy food intake over most of their adult lives (19), and therefore, these biomarkers likely are a good measure of habitual exposure in this population.

A potential limitation related to the isoflavone analysis was the switch from use of LC-Coularray to LC-MS. Daidzein concentrations were slightly lower with LC-MS; however, there was no difference in genistein concentrations by method of analysis (19). Because similar significant effects on risk were observed for both plasma daidzein and genistein, we expect that the effect of method on our results is minimal; however, we did adjust for method in our statistical model. In addition, when we restricted the analysis to those samples run by LC-MS, the results and risk estimates were similar to those of the whole data set (data not shown). Finally, another possible limitation was misclassification on the basis of histologic classification used. Having only one study pathologist review the slides helped to minimize this. Furthermore, any misclassification is unlikely to be related to plasma isoflavone concentrations and would therefore only bias the results toward the null.

In conclusion, circulating daidzein and genistein concentrations were significantly higher in controls than in cases with fibrocystic breast conditions or breast cancer. There were no differences in isoflavone concentrations between the women with fibrocystic breast conditions and the women with breast cancer. These results suggest that isoflavone exposure may affect normal, age-related changes in breast structure (e.g., lobular involution) and therefore contribute to reduced risk of both fibrocystic breast conditions and breast cancer.

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