Effect of Dietary Intake of Phytoestrogens on Estrogen Receptor Status in Premenopausal Women With Breast Cancer

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Abstract: Although many dietary studies have focused on breast cancer risk, few have examined dietary influence on tumor characteristics such as estrogen receptor (ER) status. Because phytoestrogens may modulate hormone levels and ER expression, we analyzed ER status and phytoestrogen intake in a case-case study of 124 premenopausal breast cancer patients. We assessed intake with a food-frequency questionnaire and obtained ER status from medical records. Rather than focusing on risk, we evaluated whether low intakes were more strongly associated with ER-negative tumors than with ER-positive disease. In logistic regression adjusting for potential confounders, threefold greater risks of ER-negative tumors relative to ER-positive tumors were associated with low intake of the isoflavones genistein (odds ratio, OR = 3.50; 95% confidence interval, CI = 1.43–8.58) and daidzein (OR = 3.10; 95% CI = 1.31–7.30). Low intake of the flavonoid kaempferol (OR = 0.36; 95% CI = 0.16–0.83), the trace element boron (OR = 0.33; 95% CI = 0.13–0.83), and the phytosterol β-sitosterol (OR = 0.42; 95% CI = 0.18–0.98) were associated with decreased risk of ER-negative tumors relative to ER-positive disease. Other phytoestrogens were not significantly associated with ER status. Thus, in premenopausal patients, some phytoestrogens may affect breast carcinogenesis by influencing ER status. Such findings suggest new directions for mechanistic research on dietary factors in breast carcinogenesis that may have relevance for prevention and clinical treatment.

Introduction

Although a large number of studies have focused on identifying dietary risk factors for breast cancer, proportionately less research has been conducted on the potential influence of diet on tumor characteristics, such as estrogen receptor (ER) status. ERs are high-affinity steroid hormone receptors whose activation by the binding of estrogens is considered to play a major role in both the growth and differentiation of normal breast tissues and the promotion and progression of breast tumors (1). Tumors with high ER concentrations (that is, ER-positive tumors) are thought to be more responsive to the proliferative action of estrogens than are ER-negative tumors (2). Moreover, the concentration of ERs in breast tumors has been shown to be important in the progression and clinical prognosis. ER-positive tumors tend to be more differentiated and less aggressive, to respond more favorably to endocrine therapy, and to have a better prognosis (2). Currently, little is known about the mechanisms that determine the levels of ERs in breast tumors, although 11 breast cancer studies of diet suggest that dietary factors may have some influence on ER status (3–13).

Much evidence for a protective effect against the development of breast cancer exists in general for diets rich in vegetables and, to a lesser extent, fruits (14,15). Phytoestrogens are plant compounds that can bind to ERs and have weak agonist or antagonist estrogenic effects. Phytoestrogens are found in high concentrations in soybeans, other legumes, whole-grain foods, nuts, and various seeds (16). The three main classes of phytoestrogens are the isoflavones (found in soy-based foods), the lignans (found in fruits, vegetables, and grains), and the coumestans (including essential coumestrol, which is found in alfalfa sprouts). Other potential estrogen modulators include flavonoids (that is, flavonols and flavones), phytosterols, and the trace element boron.

Phytoestrogens have become a focus of breast cancer research because they have a variety of anticarcinogenic effects (including antioxidative and antiproliferative effects) demonstrated in vitro and they modulate the production and metabolism of estrogens in humans (17,18). Isoflavones are hypothesized to exert anticarcinogenic effects in premenopausal women by lowering the level of circulating estrogen, consequently reducing the exposure of breast cells to...
endogenous estrogens (19). However, the biological mechanisms at play are complex. For example, proliferative effects of phytoestrogens have been demonstrated in healthy breast cells of premenopausal women in feeding studies (20,21). In contrast, in vitro experiments have shown DNA damage associated with phytoestrogens in hormone-sensitive breast cancer cell lines (22). Issues related to metabolism and dose of phytoestrogens also complicate understanding of these complex relationships because phytoestrogens can have either agonist or antagonist effects depending on concentration (21). Notwithstanding these observations and in light of ecological and migrant studies of breast cancer risk and diet, phytoestrogens continue to receive considerable attention as dietary factors that may contribute to the large international and geographical differences in breast cancer incidence (23).

Since the first epidemiological study on soy foods and breast cancer risk in 1991, more than 20 epidemiological studies have evaluated the association between breast cancer risk and phytoestrogens, captured as dietary intake or urinary excretion (reviewed in Ref. 24) (25–31). In the absence of consistent results, the positive studies have suggested that high consumption of soy-based foods (especially early in life) (32) and lignans might be associated with decreased risk of breast cancer (24,26). Generally, such studies have had narrow ranges or low levels of phytoestrogen consumption, which has raised questions about whether levels of intake common in Western diets are sufficient to generate biological effects because, in Asian diets, phytoestrogens are typically consumed in higher quantities than in Western diets (33). One recent study by Horn-Ross et al. reported strong protective effects for dietary intake of isoflavones and lignans against another hormonally responsive cancer, endometrial cancer, among a sample of African-American, Latina, and white women living in the United States with modest average intake for Western populations (34). This finding suggests that consumption at such levels may exert some influence in hormonal carcinogenesis, as low estrogen exposure can (35). Although the study by Horn-Ross et al. and other in vitro and in vivo studies support the plausibility of phytoestrogenic influence on hormonally responsive cancers, the relationship between dietary phytoestrogen intake and ER status in breast cancer has yet to be fully characterized.

We determined the relationship between the ER status of breast tumors and the dietary intake of phytoestrogens in a case-case study of 124 premenopausal breast cancer patients. Because higher levels of isoflavones may reduce the effects of estrogens in premenopausal women and elevated levels of endogenous estrogens down-regulate ER expression, it is biologically plausible that increased levels of these phytoestrogens may help up-regulate ER expression. For consistency in our analysis, we adopted a uniform hypothesis that women having lower levels of phytoestrogen intake before their diagnosis of breast cancer would be more likely to have ER-negative than ER-positive tumors, even though all phytoestrogens do not exert similar effects. In our analysis, we considered the potential effects of 18 individual and 5 classes of phytoestrogens to identify those that were more strongly associated with the development of ER-negative disease than ER-positive disease in premenopausal women with breast cancer.

Subjects and Methods

Study Population

The study population and data collection procedures have already been described in our previous publication (12). In brief, from 1998 to 2000, we enrolled premenopausal women diagnosed with nonmetastatic (that is, stage I–III) breast cancer within the previous 6 mo into two hospital-based studies conducted at The University of Texas M. D. Anderson Cancer Center, Houston. At recruitment, each study subject was required to read and sign an informed consent form, to undergo a personal interview, and to complete and return by mail a food-frequency questionnaire.

Collection of Demographic and Clinical Data

During the personal interview at recruitment, each study participant provided information about her age, education, ethnicity, and annual household income. We obtained height and weight from the anthropometric section of the diet questionnaire. We calculated body mass index [BMI, weight in kg/(height in m)²] and classified the women as normal weight (BMI < 25 kg/m²), overweight (BMI = 25–29.9 kg/m²), or obese (BMI ≥ 30 kg/m²) (36). We abstracted other information from the clinical and pathological reports in the patients’ medical records, including ER levels in the tumor, stage of breast cancer, and use of oral contraceptives at the time of diagnosis. Tumors were staged according to the American Joint Commission on Cancer system (37). Tumor ER levels obtained in the pathology reports referred to the α subtype of the receptor determined at diagnosis by immunohistochemistry in the Department of Pathology, M. D. Anderson Cancer Center. We defined tumors with fewer than 10% ER-positive cells as ER negative and tumors with 10% or more ER-positive cells as ER positive.

Diet Questionnaire

Diets during the 1-yr period preceding the diagnosis of breast cancer were assessed using a self-administered food-frequency questionnaire modified from the 98 food-item dietary section of the National Cancer Institute’s Health Habits and History Questionnaire, which has been validated in several populations (38–40). Briefly, this standardized questionnaire had been modified and validated (data not published) to capture the usual intake of a broad range of dietary phytoestrogens from foods commonly consumed in the southwest United States, using lists of foods known to be high in phytoestrogen content and an open-ended section specifically to report additional soy-based foods (16). Using the adapted 207 food-item questionnaire, we calculated the average daily
dietary intake of four isoflavones (genistein, daidzein, formononetin, and biochanin A), five flavonoids other than isoflavones (quercetin, kaempferol, luteolin, apigenin, and myricetin), three phytoestrogens (β-sitosterol, campesterol, and stigmasterol), two mammalian lignans (enterolactone and enterodiol), two lignan precursors (secoisolariciresinol and matairesinol), a coumestan (coumestrol), and boron. Total intake of each of the five classes of phytoestrogens (isoflavones, flavonoids, phytoestrogens, lignans, and lignan precursors) was defined as the sum of the individual phytoestrogen intakes. We also evaluated the daily intake of certain food groups, including soy-based foods, all vegetables, and grouped teas and spices. The participants were divided into high- and low-consumption groups based on the median intake level for each individual phytoestrogen class, and food group among all the study participants. “Low intake” was defined as less than or equal to the median intake of all participants and “high intake” was defined as greater than the median.

Dietary Assessment

As part of study enrollment, each participant completed and returned the self-administered food-frequency questionnaire by mail (response rate = 78%). The questionnaires were checked for completeness, accuracy, unusual responses, and major errors. When necessary, the women were contacted by mail or phone to verify demographic and dietary information. The Dietary Analysis Personal Computer System (DietSys, version 4.0; National Cancer Institute, Bethesda, MD) was used to enter and double-key questionnaire data, to identify outliers and keying errors, and to calculate the nutrient consumption for each woman.

Data Analysis

Of the 164 women who completed the diet questionnaire, we excluded 30 participants diagnosed with in situ breast cancer because the ER status of these tumors in situ had not been determined. We also excluded eight breast cancer patients whose ER status was not recorded in their medical records. Two other women were excluded because they reported consumption of more than 5,000 kcal/day, which was considered improbable. The final data set used for analysis included responses from 124 women.

Differences in the distributions of the two ER case groups were tested for statistical significance by using nonparametric tests (the two-sided Pearson χ² test for nominal data and the two-tailed Mann-Whitney U-test for ordinal variables and for continuous variables not normally distributed). To measure the strength of the associations between ER status and dietary variables, we calculated crude odds ratios (ORs) and 95% confidence intervals (CIs) as estimates of the relative risks. We performed multivariate unconditional logistic regression analyses, adjusting for the confounding factors age, ethnicity, BMI, and caloric intake. Because specific nutrient or food intakes are correlated with total energy intake and because assessing diet by using a food-frequency questionnaire can be inaccurate due to recall bias and methodological errors in reporting food intake, we used the standard multivariate method of adjustment in logistic regression to adjust daily intake of phytoestrogens and food groups by total daily energy intake (41). We then estimated the odds of being diagnosed with ER-negative breast cancer compared with the odds of diagnosis with ER-positive breast cancer to generate an OR estimating the relative risk of ER-negative disease versus ER-positive disease for women consuming low levels of selected phytoestrogens and food groups compared with women with higher consumption.

Results

General Demographics

In our sample, 36 (29%) premenopausal breast cancer patients had ER-negative tumors and 88 (71%) premenopausal breast cancer patients had ER-positive tumors. At the time of recruitment, the mean age was 41 yr (SD = 5.5), ranging from 28 to 52 yr. Most patients (81%) were non-Hispanic whites, and eight were Hispanic, eight were African American, four were Asian or Pacific Islander, two were American Indian, and one was Middle Eastern. The participants were highly educated, with about two-thirds having completed a bachelor’s or higher degree, and household incomes were high, with about half being greater than $75,000 per year. Although most of the women (57%) were of normal body size, 33 (27%) were overweight and 20 (16%) were obese. Mean height was 65 in (SD = 3). Approximately one-third of the women were taking oral contraceptives at the time of their diagnosis. The ER-negative and ER-positive groups were similar in all selected characteristics except stage at diagnosis (Table 1). As expected, a higher proportion of women with ER-positive tumors than women with ER-negative tumors was diagnosed with stage I breast cancer (P = 0.04).

Dietary Patterns

In general, the women in the ER-negative and ER-positive case groups had diets similar in total energy and macronutrients intake (Table 2). Compared with the ER-positive group, women in the ER-negative group consumed fewer soy-based foods, albeit not significantly fewer. Women with ER-negative disease also consumed a nonsignificantly higher quantity of vegetables and teas and spices (Table 2), which are the major sources of phytoestrogens other than the isoflavones (16).

After adjustment for age, ethnicity, BMI, and caloric intake, multivariate logistic regression analyses revealed that women who had low vegetable intake had a borderline significant decreased risk of ER-negative breast cancer relative to ER-positive disease (Table 2). Low consumption of teas and spices did not significantly affect the risk of being diagnosed with ER-negative breast cancer compared with ER-positive disease, whereas low consumption of soy-based foods (<21
g/wk) doubled the relative risk, although the CI included 1.0 and was not statistically significant.

Phytoestrogens

The patterns of associations with ER status observed for food groups were reflected in those for individual phytoestrogens. Women with ER-negative disease had significantly lower intake of two isoflavones, genistein and daidzein, than did women with ER-positive disease, whereas the consumption of all isoflavones grouped together was not significantly different between the two ER case groups (Table 3). Univariate and adjusted analyses revealed that women who consumed low amounts of genistein and daidzein had more than threefold greater risks of being diagnosed with ER-negative than ER-positive disease (adjusted ORgenistein = 3.50, 95% CI = 1.43–8.58; adjusted ORdaidzein = 3.10, 95% CI = 1.31–7.30).

The intakes of total flavonoids and total phytosterols were each similar between the two ER case groups. However, for the individual compounds including boron, the flavonoid kaempferol, and the phytosterol \( \beta \)-sitosterol, women with ER-positive breast cancer reported a lower dietary intake than did women with ER-negative tumors, the difference being statistically significant for boron only. Low intakes of boron, kaempferol, and \( \beta \)-sitosterol were significantly associated with 58–67% reductions in risk of ER-negative tumors relative to ER-positive tumors (adjusted ORboron = 0.33, 95% CI = 0.13–0.83; adjusted ORkaempferol = 0.36, 95% CI = 0.16–0.83; adjusted OR\( \beta \)-sitosterol = 0.42, 95% CI = 0.18–0.98).

In general, the results for the other individual and classes of phytoestrogens were not statistically significant but showed some suggestive trends. The women with ER-negative disease tended to have consumed more total and individual phytosterols, the most abundant phytoestrogens in foods, than did the women with ER-positive disease.
### Table 2. Daily Intake of Macronutrients and Selected Foods by ER Status of 124 Premenopausal Women During the Year Before Their Diagnosis of Breast Cancer and Estimates of Risk for Diagnosis With ER-Negative Breast Cancer in Association With Low Dietary Intake of Selected Food Groups

<table>
<thead>
<tr>
<th>Dietary Factor</th>
<th>ER Positive (n = 88)</th>
<th>ER Negative (n = 36)</th>
<th>P Valuea</th>
<th>Adjusted ORb,c</th>
<th>95% CI</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Energy from fat</td>
<td>35 (30, 41)</td>
<td>36 (31, 40)</td>
<td>0.95</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Energy from carbohydrates</td>
<td>47 (42, 54)</td>
<td>47 (42, 53)</td>
<td>0.69</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Energy from proteins</td>
<td>15 (14, 17)</td>
<td>15 (13, 16)</td>
<td>0.21</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total energy, kcal/day</td>
<td>1,838 (1,475, 2,298)</td>
<td>2,044 (1,460, 2,387)</td>
<td>0.27</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat, g/day</td>
<td>73 (51, 97)</td>
<td>80 (57, 102)</td>
<td>0.39</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbohydrates, g/day</td>
<td>224 (161, 283)</td>
<td>234 (171, 281)</td>
<td>0.56</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proteins, g/day</td>
<td>68 (52, 91)</td>
<td>72 (51, 92)</td>
<td>0.80</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total vegetables, g/day</td>
<td>178 (123, 243)</td>
<td>206 (137, 278)</td>
<td>0.16</td>
<td>0.44</td>
<td>0.19–1.02</td>
<td></td>
</tr>
<tr>
<td>Total soy-based foods, g/day</td>
<td>4 (1, 20)</td>
<td>2 (0, 15)</td>
<td>0.11</td>
<td>2.12</td>
<td>0.93–4.83</td>
<td></td>
</tr>
<tr>
<td>Total teas and spices, g/day</td>
<td>28 (3, 196)</td>
<td>61 (8, 178)</td>
<td>0.40</td>
<td>0.71</td>
<td>0.32–1.57</td>
<td></td>
</tr>
</tbody>
</table>

*a: From the two-tailed Mann-Whitney U-test comparing median values of ER-negative and ER-positive groups.

*b: The odds ratio (OR) compares risk of diagnosis with ER-negative breast cancer with risk of diagnosis of ER-positive breast cancer. The OR is calculated in association with “low dietary intake” (defined as less than or equal to the median intake of all participants) compared with “high dietary intake” (defined as greater than the median intake of all participants) as the reference group.

*c: Adjusted for age, ethnicity, BMI, and caloric intake.

### Table 3. Daily Intake of Selected Phytoestrogens by ER Status of 124 Premenopausal Women During the Year Before Their Diagnosis of Breast Cancer and Estimates of Risk for Diagnosis With ER-Negative Breast Cancer in Association With Low Dietary Intake of Phytoestrogens

<table>
<thead>
<tr>
<th>Phytoestrogen</th>
<th>Median (25th, 75th)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoflavones (µg)</td>
<td>425 (181, 1,190)</td>
</tr>
<tr>
<td>Genistein (µg)</td>
<td>220 (65, 689)</td>
</tr>
<tr>
<td>Daidzein (µg)</td>
<td>130 (44, 458)</td>
</tr>
<tr>
<td>Formononetin (µg)</td>
<td>1 (0, 3)</td>
</tr>
<tr>
<td>Biochanin A (µg)</td>
<td>55 (23, 126)</td>
</tr>
<tr>
<td>Flavonoids (µg)</td>
<td>13,118 (6,199, 22,840)</td>
</tr>
<tr>
<td>Quercetin (µg)</td>
<td>9,330 (4,743, 15,671)</td>
</tr>
<tr>
<td>Kaempferol (µg)</td>
<td>1,021 (481, 2,915)</td>
</tr>
<tr>
<td>Luteolin (µg)</td>
<td>9 (2, 32)</td>
</tr>
<tr>
<td>Apigenin (µg)</td>
<td>45 (8, 164)</td>
</tr>
<tr>
<td>Myricetin (µg)</td>
<td>1,209 (289, 3,745)</td>
</tr>
<tr>
<td>Boron (µg)</td>
<td>800 (622, 1,148)</td>
</tr>
<tr>
<td>Lignans (µg)</td>
<td>224 (142, 332)</td>
</tr>
<tr>
<td>Enterolactone (µg)</td>
<td>128 (79, 206)</td>
</tr>
<tr>
<td>Enterodiol (µg)</td>
<td>92 (63, 130)</td>
</tr>
<tr>
<td>Lignan precursors (µg)</td>
<td>3,159 (1,284, 5,301)</td>
</tr>
<tr>
<td>Secoisolariciresinol (µg)</td>
<td>3,121 (1,255, 5,100)</td>
</tr>
<tr>
<td>Matairesinol (µg)</td>
<td>28 (12, 172)</td>
</tr>
<tr>
<td>Coumestrol (µg)</td>
<td>70 (28, 144)</td>
</tr>
<tr>
<td>Phytoesters (mg)</td>
<td>173 (95, 432)</td>
</tr>
<tr>
<td>β-Sitosterol (mg)</td>
<td>125 (69, 400)</td>
</tr>
<tr>
<td>Campesterol (mg)</td>
<td>17 (11, 25)</td>
</tr>
<tr>
<td>Stigmasterol (mg)</td>
<td>13 (9, 17)</td>
</tr>
</tbody>
</table>

*a: Two-tailed Mann-Whitney U-test comparing median values of ER-negative and ER-positive groups.

*b: OR compares risk of diagnosis with ER-negative breast cancer with risk of diagnosis of ER-positive breast cancer. OR is calculated in association with “low dietary intake” (defined as less than or equal to the median intake of all participants) compared with “high dietary intake” (defined as greater than the median intake of all participants) as the reference group.

*c: Adjusted for age, ethnicity, BMI, and caloric intake.
Western diets are typically lower than Asian diets in soy-based foods and vegetables. For isoflavones, the usual daily consumption in Asia typically ranges from 20 to 100 mg/day, whereas usual consumption in the United States and Europe is less than 1 mg/day (42,43). Similarly, the usual intake of phytosterols is higher in vegetarian (345 mg/day) and Japanese (400 mg/day) diets than in the typical Western diet (80 mg/day) (44). In our study, the median intake of isoflavones was less than 1 mg/day and that of phytosterols was less than 200 mg/day, both of which are in the range of the typical Western diet. Moreover, the ranges of isoflavone and lignan intakes in our analysis were comparable with those observed in other studies reporting phytoestrogen intake in Western populations (26,28,45). The criteria we used to define high and low consumers of each phytoestrogen were above or below the median intake among all study participants, respectively. Although the range of phytoestrogen intake in our study may not have been sufficiently wide given our sample size for detecting associations modest in strength (especially if a threshold effect exists), it was sufficient for detecting strong associations with isoflavones, the most potent phytoestrogens, and phytosterols, the most prevalent phytoestrogens.

Our analyses revealed strong associations between ER status and the most potent isoflavones genistein and daidzein. Experimental studies provide evidence that, similar to selective ER modulators, genistein and daidzein can have either estrogenic or antiestrogenic effects on breast tissue depending on the endogenous hormonal environment (17). Dietary genistein and daidzein have been shown to decrease circulating endogenous sex hormone levels in humans (46), which may be clinically relevant because lowered endogenous estrogen levels up-regulate ER expression. Our results on genistein and daidzein are consistent with these previous findings and support our hypothesis that high intakes of genistein and daidzein may help up-regulate ER expression in breast cancer cells of premenopausal women. The other isoflavones evaluated, formononetin and biochanin A, although efficiently metabolized into genistein and daidzein, differ from these compounds by one methoxy group (47). This difference could alter the bioavailability of formononetin and biochanin A or modify their biological activity, possibly explaining why their consumption was not associated with ER status in our analysis.

Bound to glycosides in plant foods, flavonoids have been shown to have limited bioavailability in experimental feeding studies (48). This is consistent with the general lack of strong associations between flavonoid intake and ER status in our analysis, except for kaempferol, which has different biological properties from the other flavonoids and similar structure to genistein and daidzein (48). The strong, opposite direction of kaempferol’s association with ER status compared with those for genistein and daidzein suggests that the structural difference of one hydroxyl group in kaempferol and the other flavonoids may be important for their specific biological actions.

Considerable epidemiological evidence in general suggests a protective effect of high consumption of green vegetables and legumes and, to a lesser extent, fruits against breast cancer (14,15); for ER status specifically, other studies have revealed positive associations between ER-positive breast tumors and greater consumption of green and yellow vegetables as well as higher intake of vitamins A and B6, grain cereals, and fiber (3,4). Although phytoestrogens have not been directly evaluated, these findings are interesting because fruits and vegetables are high in phytosterol content (16). We found a consistent pattern of associations with ER status for phytosterols, reflecting the association found for the vegetable group. Only the association for β-sitosterol was statistically significant after adjustment. The interpretation of this borderline association is limited by homogenous intakes and modest statistical power but suggests possible biological effects of phytoestrogens on ER regulation. Although β-sitosterol may be as potent as the isoflavone genistein to bind to ERs, β-sitosterol was shown to have a higher binding affinity to ERα relatively to genistein (49). Because the predominant ER form in breast tissue cells is ERα and we determined ER status based on the α subtype only, the association of β-sitosterol intake with ER status that we observed may be more strongly related to ER-mediated mechanisms than the association with genistein intake. Phytoestrogens can also modify the fluidity of cholesterol-rich cell membranes (without altering their integrity) and inhibit membrane-bound molecules (50), thus indirectly affecting cellular processes such as estrogen metabolism, ER function, and ER expression. The consistency of the inverse associations between lower intake and ER-negative status in our analysis suggests that further assessment of the influence of phytoestrogens on breast carcinogenesis is warranted, particularly if such patterns are confirmed in studies with greater statistical power than ours.

Boron is a metalloid trace element found in borate complexes in many plant foods, particularly peanuts, avocados, and beans (51). Boron affects a number of biochemical functions, including hormonal and macromineral metabolism, and can alter endogenous estrogen levels in humans (52). Overall, participants in our analysis had low median boron intake; however, for many women, boron intake reached the level for which physiological actions have been demonstrated (1–13 mg/day) (53). Our findings showing that low boron intake was associated with lower risk of ER-negative disease suggest that low boron levels would favor ER expression in premenopausal breast cancer. As for phytoestrogens, the mechanisms by which boron may interfere with ER expression in breast cancer deserve more research.

In general, our study population was typical of a Western population of women with breast cancer with regard to their clinical tumor characteristics. However, compared with what would be observed in the U.S. population at large, the women in our study were slimmer and had higher education and income typical for patients treated at the M. D. Anderson Cancer Center. Our study lacked a healthy comparison group, which prohibited the direct estimation of risk of ER status. Still, our analysis provides information for understanding the
relationships between phytoestrogens and breast carcinogenesis in premenopausal women. For example, a major strength was our ability to assess individual phytoestrogen compounds as well as structural class groups. We found strong significant associations with the individual phytoestrogens genistein and daidzein but not for the overall class of isoflavones. Moreover, in several instances, the association for the phytoestrogen class differed in direction from that for an individual compound within the class. Such results might remain undetected in analyses that focused on foods, food groups, or phytoestrogen classes (that is, without considering individual compounds within classes) and may explain why some studies may not have detected associations with breast cancer risk for phytoestrogens (54,55).

As with all dietary studies that use food-frequency questionnaires, ours cannot address variation due to individual differences in digestion, gut microflora, and metabolism of foods. However, concern for differential recall bias by cases in our study was minimal because all participants were newly diagnosed breast cancer patients instructed to report their diets for the year before their diagnosis and were likely to do so without regard to their tumor ER status. A strength of our study was the modification of our food-frequency questionnaire to include, in addition to a variety of Mexican and Asian foods rich in phytoestrogens and consumed in the Southwest, an open-ended section specifically to report soy-based foods and other foods particularly rich in phytoestrogens. These modifications allowed us to evaluate the intake of phytoestrogens more accurately and to compare low and high phytoestrogen consumers, some of whom had intakes approximating modest consumption in some Asian countries. Although our results could be due to chance, we found significant associations for five compounds, the most potent phytoestrogens, which support our a priori hypothesis, and is more than the one or two that we would expect from analyzing 18 food compounds.

Our results provide epidemiological evidence that dietary intake of some phytoestrogens may influence the ER status of breast cancer tumors in premenopausal women. They suggest that isoflavones may exert antagonist effects on ER status by mostly modulating circulating hormonal levels and phytosterols may exert agonist effects mostly through ER-mediated mechanisms. Future studies should include more than just the isoflavones, as other phytoestrogens may also have important effects, as we found. Future studies should also consider evaluating postmenopausal women, as the effects of phytoestrogens may differ when the influence of ovarian hormones is diminished (17). Our findings, if confirmed by others, provide new directions for mechanistic research on dietary factors that may have important preventive and clinical relevance. Identifying factors that may mediate ER expression in breast cancer, particularly dietary factors whose consumption is modifiable, may ultimately be used to develop preventive strategies for women at high risk for ER-negative tumors. Although our knowledge of the effects of phytoestrogens on human health is limited, consuming high doses of phytoestrogen supplements should not be recommended until more is known about the metabolism of phytoestrogens and their long-term effects and risks of use. However, it is not premature to recommend a diet rich in fruits and vegetables as part of a healthy diet, in part because such diets have not been shown to be harmful but also because fruits and vegetables contain a number of micronutrients that are known to protect against cancer and other diseases.

Acknowledgments and Notes

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