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Insulin-Like Growth Factor-I, Soy Protein Intake, and Breast Cancer Risk

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Abstract: *Previous studies have found that estrogen enhances the effect of insulin-like growth factor-I (IGF-I) levels on breast cancer cell growth. Participants in the Shanghai Breast Cancer Study (SBCS) consumed large amounts of soy that was high in isoflavones, which act as weak estrogens and as anti-estrogens. We assessed whether soy protein intake modified the effect of IGF-I levels on breast cancer risk. The SBCS is a population-based case-control study of breast cancer among women aged 25–64 conducted between 1996 and 1998 in urban Shanghai. In-person interviews were completed with 1,459 incident breast cancer cases ascertained through a population-based cancer registry and 1,556 controls randomly selected from the general population (with respective response rates of 91% and 90%). This analysis is restricted to the 397 cases and 397 matched controls for whom information on IGF-I levels was available. For premenopausal breast cancer, we found nearly significant interactions between soy protein intake and IGF-I levels (*P = *0.080) and insulin-like growth factor-binding protein-3 (IGFBP-3) levels (*P = *0.057). The direction of the interaction appeared to be negative for IGF-I levels but was positive for IGFBP-3 levels. No interaction was evident between soy protein intake and IGF-I or IGFBP-3 levels among postmenopausal women. Our results suggest that soy protein intake may negatively modulate the effect of IGF-I and may positively modulate the effect of IGFBP-3 levels on premenopausal breast cancer risk. Further studies are needed to confirm our finding and to understand the biological mechanisms of these potential interactions.*

Introduction

Insulin-like growth factor-I (IGF-I) is thought to play a role in the pathogenesis of breast cancer due to its mitogenic and anti-apoptotic effects on mammary cell lines (1). Insulin-like growth factor-binding protein-3 (IGFBP-3) regulates IGF-I bioactivity by binding to IGF-I (2). Of the nine human studies of IGF-I levels and premenopausal breast cancer (3–11), IGF-I was positively associated in four studies of Caucasian women (3–6) and in the Shanghai Breast Cancer Study (SBCS) of Asian women (7). Seven of these studies also investigated IGFBP-3, with four studies reporting positive associations (3,5–7) and three studies reporting no association (8,9,11). Only one human study, conducted among African-American women (12), of the 12 studies of IGF-I levels and postmenopausal breast cancer (4–7,9–11,13–16) found a positive relation. Similarly, only one study, using the SBCS (7), of the eight studies of IGFBP-3 and breast cancer (5,6,9,11,14–16) reported an elevated risk of postmenopausal breast cancer associated with increased IGFBP-3. In vitro studies have shown that estrogen enhances the effect of IGF-I on breast cancer cell growth (17,18), and thus the association of IGF-I with breast cancer risk may be modified by estrogens. One in vivo study, using the SBCS, investigated whether estrogen modified the effect of IGF-I on breast cancer risk (19). They reported synergistic effects between IGF-I levels and two estrogen-related hormones, estrone and testosterone, on breast cancer risk among women diagnosed premenopausally and postmenopausally.

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High consumption of soy during childhood and adulthood has been hypothesized to be protective against breast cancer. One of several mechanisms proposed for this effect is its richness in isoflavones, which may reduce estrogen activity in the breast by competing as weak estrogens for receptor sites (20). Isoflavones may also reduce estrogen synthesis (21) and increase sex hormone-binding globulin (22). Of the 12 human studies of adult soy intake on breast cancer risk (23–34) only 4 of those conducted among Asian or Asian-American populations, who consume large amounts of soy, have found statistically significant inverse associations (23–26). A recent Japanese cohort study identified significant inverse relations for isoflavones and breast cancer risk, especially among postmenopausal women, but not for soy in general (35). In one of the studies the reduction in breast cancer risk associated with high soy intake was seen among all women (23), whereas two studies were limited to premenopausal women (24,25). A previous report from the SBCS found that high adult soy intake was associated with a reduced risk of breast cancer for women with a higher body mass index (BMI) or with an estrogen receptor/progesterone receptor-positive breast cancer (26).

As indicated in the SBCS, high soy intake appeared to act as a weak estrogen or anti-estrogen only among women with a high BMI (26), IGF-I levels appeared to exert a mitogenic effect on premenopausal breast cancer (7), and estrogen-related hormones and IGF-I levels worked synergistically in the etiology of breast cancer among all women (19). Given that estrogen tends to enhance the action of IGF-I on mammary cell lines (17,19), we hypothesized that high soy protein intake and high IGF-I levels would act synergistically in increasing breast cancer risk. We also hypothesized that there would be a synergistic interaction between high soy protein intake and high IGFBP-3 on breast cancer because IGFBP-3 was positively associated with breast cancer in an earlier SBCS report (7). We collected information from an additional 97 case-control pairs to combine with the 300 case-control pairs from the previous SBCS report (7) to test these hypotheses.

Material and Methods

Detailed methods of this population-based case-control study appeared elsewhere (36). Briefly, all women aged 25–64 yr who were permanent residents of urban Shanghai at the time of diagnosis of first primary invasive breast cancer (August 1996 through March 1998) were eligible for the study. Two senior pathologists histologically confirmed all diagnoses. We used rapid case ascertainment supplemented by the Shanghai Cancer Registry to identify breast cancer cases who had no prior history of cancer. A total of 1,459 breast cancer cases (91.1% of eligible cases) completed a standardized in-person interview. Of potentially eligible cases, 109 refused (6.8%), 17 died prior to the interview (1.1%), and 17 were not located (1.1%).

The Shanghai Resident Registry, a listing of all permanent adult residents of urban Shanghai, was used to randomly select controls. Controls were frequency matched to cases on age (5-yr interval) based on the number of incident breast cancer cases by age group reported to the Shanghai Cancer Registry from 1990 through 1993. Women who did not reside at the registered address at the time of the study were ineligible. A total of 1,556 controls (90.4% of eligible controls) completed a standardized in-person interview. The remaining 166 potentially eligible controls (9.6%) refused participation. Two women died prior to the interview and were excluded.

The study was approved by relevant institutional review boards in Shanghai and the United States. Women were interviewed at hospitals (cases) or at home (cases and controls) by trained interviewers. The subject questionnaire collected information on demographic factors, reproductive and medical histories, family history of cancer, use of oral contraceptives and hormone replacement therapy, diet, physical activity, lifestyle factors, and body size. Adult soy consumption in the previous 5 yr was collected using a 76-item food-frequency questionnaire. Detailed methods of the calculation of soy protein equivalence appears elsewhere (18). Briefly, foods on the questionnaire used to calculate soy protein equivalence based on the Chinese Food Composition Table (37) were tofu, soy milk, fresh soybeans, dried soybeans, soybean sprouts, and other soy products. Weights were applied to these foods to account for the edible portion, the mixture of non-soyfoods, and seasonal variation. The soyfood items were then summed to estimate total soy protein.

After completing the interview, over 80% of women provided fasting blood samples (1,193 cases, 1,310 controls). Detailed methods of blood collection and testing appeared elsewhere (7). Briefly, plasma was separated from samples and stored at –70°C within 6 h of collection. Within the SBCS, a case-control substudy of quantitative biomarkers was conducted utilizing the 397 cases whose fasting blood samples were collected prior to therapy. A total of 397 controls were selected from the pool of controls who provided fasting blood samples. Cases and controls were individually matched on age (within 5 yr), date of blood collection (within 30 days), menopausal status, and, for premenopausal women, menstruation day (within the first 10 days of menstruation during follicular phase or within 3 days of the first 10 days during the follicular or luteal phases). Matched case-control pairs were analyzed in the same batch assay. Plasma IGF-I and IGFBP-3 concentrations were determined with enzyme-linked immunosorbent assay kits available from DSL, Inc. (Webster, TX). Previous studies of IGF-I and IGFBP-3 and cancer have used these methods with good reproducibility (4,38). The intra-assay and interassay precisions measured as coefficients of variation were 1.5–3.4% and 1.5–8.5% for IGF-I and 0.5–1.9% and 1.8–3.9% for IGFBP-3.

χ^2 tests for categorical variables and paired *t*-tests for continuous variables were used to assess differences in known breast cancer risk factors by case-control status. Spearman

correlation coefficients among controls were computed to evaluate whether levels of IGF-I and IGFBP-3 and soy protein intake were correlated. We used conditional logistic regression to estimate the relative risk of breast cancer associated with IGF-I levels, IGFBP-3 levels, and soy protein intake while controlling for confounders (39). Because these variables were skewed we used the decile distributions among controls and assigned the median of each decile a score for the continuous analysis. We used the tertile distribution among controls to categorize IGF-I levels, IGFBP-3 levels, and soy protein intake in the main effects analysis. The referent group for the main effects analysis was women whose IGF-I level, IGFBP-3 level, or soy protein intake was in the lowest tertile. Due to small numbers in some cells, the median distribution among controls was used to categorize IGF-I levels, IGFBP-3 levels, and soy protein intake in the joint effects analysis. In the joint effects analysis, the referent group was women whose IGF-I or IGFBP-3 levels were less than the median and who consumed less than the median of soy protein. Variables were categorized for all women combined and for premenopausal and postmenopausal women separately. Age, education, family history of breast cancer in a first-degree relative, history of fibroadenoma, leisure physical activity in past 10 yr, BMI, waist-to-hip ratio, parity, age at first live birth, age at menarche, oral contraceptive use, hormone replacement therapy use, and total energy intake were assessed as confounders of the associations between IGF-I levels, IGFBP-3 levels, and soy protein intake and breast cancer. Using a 10% change between unadjusted and adjusted odds ratios (ORs) as evidence of confounding, analyses were adjusted for leisure physical activity in the past 10 yr, parity, and age at first live birth.

Analyses are presented for all women and separately by menopausal status because the effect of some hormonal and growth factor exposures on breast cancer risk is thought to differ by menopausal status. In multiple logistic regression models, we assessed linear trend by treating categorical variables as continuous variables. Interaction terms between IGF-I or IGFBP-3 levels and soy protein intake were included in logistic regression models, and likelihood ratio tests were conducted to examine whether there was evidence of effect modification. We performed an ad hoc analysis that did not involve statistical testing to explore the direction of the effect modification. The OR for the group with high levels of IGF-I or IGFBP-3 and high soy protein intake was divided by the OR for the group with low levels of IGF-I or IGFBP-3 and high soy protein intake. This ratio of ORs was compared with the stratum-specific OR for high levels of IGF-I or IGFBP-3 levels and low soy protein intake.

Results

Table 1 compares known breast cancer risk factors of cases and controls. Compared with controls breast cancer cases were more likely to have a history of fibroadenoma, to have a higher BMI, and to have a later age at first birth and were less likely to have engaged in leisure physical activity in

Table 1. Comparison of Cases and Controls for Selected Risk Factors[a]

	Cases (n = 397)[b]	Controls (n = 397)[b]	P Value
Age	47.8 ± 7.8	47.6 ± 7.9	0.20
Education (%)			
No formal education + elementary school	12.6	14.6	
Middle school	44.3	43.1	
High school	30.5	31.5	
Profession, college, and above	12.6	10.8	0.74
Breast cancer in first-degree relatives (%)	3.0	1.5	0.15
Ever had breast fibroadenoma (%)	9.1	4.8	0.02
Leisure physical activity in past 10 yr (%)	20.9	29.7	<0.01
Body mass index	23.5 ± 3.3	22.9 ± 3.2	0.02
Waist-to-hip ratio	0.80 ± 0.1	0.80 ± 0.1	0.20
Nulliparous (%)	4.0	3.3	0.57
Age at first live birth (yr)[c]	26.9 ± 4.1	26.3 ± 3.9	<0.01
Menarcheal age (yr)	14.7 ± 1.7	14.9 ± 1.7	0.11
Oral contraceptive use (%)	21.9	25.4	0.24
Hormone replacement therapy use (%)	3.5	3.0	0.68
Postmenopausal (%)	36.9	36.4	0.88
Menopausal age (yr)[d]	48.5 ± 4.5	47.8 ± 4.5	0.12
Usual total energy intake (kcal/day)	1,905.7 ± 470.3	1,862.3 ± 481.9	0.16
Soy protein intake (g/day)	11.5 ± 10.8	12.0 ± 9.8	0.53
IGF-I level (ng/ml)[e]	150.6 (144.5–156.9)	138.5 (133.5–143.8)	<0.01
IGFBP-3 level (ng/ml)[e]	3,963.9 (3,813.6–4,119.5)	3,718.2 (3,586.5–3,854.8)	<0.01

a: Subjects with missing values were excluded from the analysis.
b: Unless otherwise specified, mean ± SD is presented.
c: Among women who had live births.
d: Among women with natural menopause.
e: Geometric mean and 95% confidence interval.

the past 10 yr. Mean soy protein intake did not differ significantly between cases and controls, but women with breast cancer had significantly higher levels of IGF-I and IGFBP-3 than did control women. In comparison with the larger study, cases in the substudy had a significantly smaller waist-to-hip ratio (0.80 vs. 0.81) and older age at menarche (14.7 vs. 14.3), whereas controls in the substudy were more likely to be physically active (29.7% vs. 23.7%) and had an older menarcheal age (14.9 vs. 14.7) (data not shown).

Table 2 shows the Spearman correlation coefficients for IGF-I and IGFBP-3 levels and soy protein intake. The correlation between IGF-I and IGFBP-3 levels in this study was not significant among all controls or by menopausal status nor was there a significant correlation between IGF-I levels and soy protein intake among any control subjects. Although the correlation between IGFBP-3 and soy protein intake was not correlated among all controls, there was a significant negative correlation among premenopausal (r = –0.1389; P = 0.03) and significant positive correlation among postmenopausal controls (r = 0.2123; P = 0.01).

Table 3 presents the ORs and 95% confidence intervals (CIs) for breast cancer associated with IGF-I levels, IGFBP-3 levels, and soy protein intake among all women and by menopausal status. There was an indication of significant associations in the continuous analyses of IGF-I or IGFBP-3 among all women. In addition, there were significant trends of increasing risk associated with increasing levels of IGF-I and IGFBP-3 among all women and by menopausal status. The highest tertile of IGF-I was associated with a twofold increase in breast cancer risk (OR = 2.2; 95% CI = 1.4–3.4) that was seen primarily among women who were diagnosed postmenopausally (OR = 2.2; 95% CI = 0.8–5.8). This pattern held for IGFBP-3 (all women OR = 2.6; 95% CI = 1.5–4.5; postmenopausal women OR = 8.1; 95% CI =

Table 2. Correlations Among IGF-I Levels, IGFBP-3 Levels, and Soy Protein Intake Among All Controls and by Menopausal Status

	Spearman Correlation Coefficient (P Value)		
	IGF-I	IGFBP-3	Soy Protein
All controls			
IGF-I	1.00	–0.0829 (0.10)	–0.0043 (0.93)
Soy protein		–0.0036 (0.94)	1.00
Premenopausal controls			
IGF-I	1.00	0.0240 (0.70)	0.0677 (0.28)
Soy protein		–0.1389 (0.03)	1.00
Postmenopausal controls			
IGF-I	1.00	0.0608 (0.47)	–0.0197 (0.82)
Soy protein		0.2123 (0.01)	1.00

Table 3. Odds Ratios of Breast Cancer Associated With Main Effects of IGF-I Levels, IGFBP-3 Levels, and Soy Protein Intake Among All Women and by Menopausal Status

	OR (95% CI)[a]		
	All Women (397 cases, 397 controls)	Premenopausal Women (250 cases, 252 controls)	Postmenopausal Women (147 cases, 145 controls)
IGF-I levels (ng/ml)			
Continuous	1.1 (1.1–1.2)	1.1 (1.0–1.2)	1.2 (1.0–1.3)
Categorical[b]			
Tertile 1	1.0 (referent)	1.0 (referent)	1.0 (referent)
Tertile 2	1.4 (0.9–2.1)	1.1 (0.6–2.0)	1.1 (0.5–2.6)
Tertile 3	2.2 (1.4–3.4)	1.7 (0.9–3.2)	2.2 (0.8–5.8)
P for trend	<0.001	0.003	0.017
IGFBP-3 levels (ng/ml)			
Continuous	1.2 (1.1–1.3)	1.2 (1.1–1.4)	1.2 (1.0–1.4)
Categorical[c]			
Tertile 1	1.0 (referent)	1.0 (referent)	1.0 (referent)
Tertile 2	1.3 (0.8–2.1)	1.8 (1.0–3.4)	0.5 (0.2–1.3)
Tertile 3	2.6 (1.5–4.5)	1.6 (0.9–2.9)	8.1 (2.5–26.0)
P for trend	<0.001	0.002	0.015
Soy protein (g/day)			
Continuous	1.0 (0.9–1.0)	1.0 (0.9–1.1)	1.0 (0.9–1.1)
Categorical[d]			
Tertile 1	1.0 (referent)	1.0 (referent)	1.0 (referent)
Tertile 2	1.0 (0.7–1.4)	0.9 (0.6–1.4)	1.2 (0.7–2.4)
Tertile 3	1.0 (0.7–1.5)	1.1 (0.7–1.6)	0.7 (0.4–1.5)
P for trend	0.455	0.8	0.485

a: Adjusted for leisure physical activity in past 10 yr, parity, and age at first live birth.

b: Tertiles 1–3 for IGF-I levels for all women were <117.7, 117.7–168.3, and ≥168.4; for premenopausal women were <135.9, 135.9–182.4, and ≥182.5; for postmenopausal women were <96.25, 96.25–130.1, and ≥130.2.

c: Tertiles 1–3 for IGFBP-3 levels for all women were <3,306, 3,306–4,190, and ≥4,191; for premenopausal women were <3,086, 3,086–4,003, and ≥4,004; for postmenopausal women were <3,698, 3,698–4,461, and ≥4,462.

d: Tertiles 1–3 for soy protein intake for all women were <6.96, 6.96–12.21, and ≥12.22; for premenopausal women were <6.89, 6.89–11.85, and ≥11.86; for postmenopausal women were <7.28, 7.28–13.18, and ≥13.19.

2.5–26.0). Soy protein intake was not associated with breast cancer risk. Additional adjustment of the IGF-I analysis for IGFBP-3 and the IGFBP-3 analysis for IGF-I weakened most of these associations (data not shown).

Table 4 shows the effect of increasing IGF-I or IGFBP-3 levels on breast cancer risk for women with low and high levels of soy protein intake among all women and by menopausal status. There were borderline significant associations for the continuous analysis of IGF-I and IGFBP-3 levels among all women regardless of level of soy protein intake. In the categorical analysis, high IGF-I level was associated with an increased risk of breast cancer among all women who consumed high levels of soy protein (OR = 1.7; 95% CI = 1.1–2.6). There were twofold elevations in risk associated

Table 4. Odds Ratios of Breast Cancer Associated With Joint Effects of IGF-I or IGFBP-3 Levels and Soy Protein Intake Among All Women and by Menopausal Status

	OR (95% CI)[a]	
	<9.5 g/day Soy Protein (median)	≥9.5 g/day Soy Protein (median)
All women (397 cases, 397 controls)		
IGF-I levels (ng/ml)		
Continuous	1.1 (1.0–1.2)	1.1 (1.0–1.3)
P for interaction	0.863	0.157
Categorical		
<141.0	1.0 (referent)	0.9 (0.6–1.4)
≥141.0	1.6 (1.0–2.5)	1.7 (1.1–2.6)
P for interaction		0.105
IGFBP-3 levels (ng/ml)		
Continuous	1.2 (1.0–1.4)	1.3 (1.0–4.5)
P for interaction	0.663	0.112
Categorical		
<3741.0	1.0 (referent)	0.9 (0.6–1.4)
<3741.0	2.2 (1.3–3.7)	2.3 (1.4–3.8)
P for interaction		0.265
	<9.1 g/day Soy Protein (median)	≥9.1 g/day Soy Protein (median)
Premenopausal women (250 cases, 252 controls)		
IGF-I levels (ng/ml)		
Continuous	1.1 (0.9–1.3)	1.1 (1.0–1.3)
P for interaction	0.393	0.517
Categorical		
<162.6	1.0 (referent)	1.1 (0.6–2.0)
≥162.6	1.6 (0.9–2.8)	1.7 (1.0–2.9)
P for interaction		0.080
IGFBP-3 levels (ng/ml)		
Continuous	1.4 (1.0–1.9)	1.2 (1.0–1.5)
P for interaction	0.297	0.292
Categorical		
<3,526.0	1.0 (referent)	1.1 (0.6–1.8)
≥3526.0	2.1 (1.0–4.3)	2.5 (1.3–5.0)
P for interaction		0.057
	<10.0 g/day Soy Protein (median)	≥10.0 g/day Soy Protein (median)
Postmenopausal women (147 cases, 145 controls)		
IGF-I levels (ng/ml)		
Continuous	1.1 (0.9–1.5)	1.3 (1.0–1.6)
P for interaction	0.689	0.111
Categorical		
<108.3	1.0 (referent)	0.7 (0.3–1.5)
≥108.3	1.5 (0.7–3.2)	1.5 (0.7–3.3)
P for interaction		0.823
IGFBP-3 levels (ng/ml)		
Continuous	1.2 (0.9–1.6)	1.5 (1.0–2.2)
P for interaction	0.689	0.110
Categorical		
<4,060.5	1.0 (referent)	0.9 (0.4–2.1)
≥4,060.5	2.0 (0.9–4.7)	1.4 (0.7–3.2)
P for interaction		0.176

a: Adjusted for leisure physical activity in past 10 yr, parity, and age at first live birth.

with high IGFBP-3 levels among all women and among premenopausal women regardless of amount of soy protein consumed. Although not significantly different, the OR for high IGFBP-3 levels was higher among premenopausal women with high soy protein intake than among premenopausal women with low soy protein intake, whereas the reverse was true for postmenopausal women. Most of these relations were weakened after additional adjustment for IGFBP-3 or IGF-I levels (data not shown).

The *P* values for interaction for IGF-I and IGFBP-3 levels and soy protein intake in the categorical analysis were nearly significant among premenopausal women (IGF-I $P = 0.08$; IGFBP-3 $P = 0.57$) but not among postmenopausal women. Among premenopausal women, the direction of the interaction for IGF-I levels was unclear (OR = 1.6; ratio of ORs = 1.6) but appeared to be positive for IGFBP-3 levels (OR = 2.1; ratio of ORs = 2.3). Although there was no evidence of statistical effect modification, the OR and ratio of ORs differed somewhat among postmenopausal women for IGF-I (OR=1.5; ratio of ORs = 2.1) and IGFBP-3 (OR = 2.0; ratio of ORs = 1.6).

Discussion

We found a nearly significant interaction between high soy protein intake and high IGF-I level and breast cancer risk among premenopausal women. The direction of this interaction was unclear, but the negative correlation between IGF-I level and soy protein intake among premenopausal controls would lead one to believe it was negative. This nonsignificant negative interaction was unexpected but could be due to soy inhibiting tumor cell growth stimulated by growth factors. Genistein, the most common isoflavone, has been shown to inhibit the proliferation of breast cancer cells stimulated by epidermal growth factor (40). In contrast, epidermal growth factor and IGF-I have been shown to act synergistically to stimulate breast cancer cell growth (41). Although not significant, the OR and ratio of ORs for IGF-I level and soy protein intake among postmenopausal women were strikingly different and appeared to be positive rather than negative. The mechanism of this potential positive interaction is unknown but could be related to soy's competition as a weak estrogen for receptor sites (20) or to soy acting as an anti-estrogen by reducing estrogen synthesis (21) and increasing sex hormone-binding globulin (22). A previous analysis of the SBCS identified synergistic effects between IGF-I levels and two estrogen-related hormones, estrone and testosterone, on breast cancer risk among women diagnosed premenopausally and postmenopausally (19).

For IGFBP-3 levels, there was a nearly significant positive interaction among premenopausal women. In contrast, the OR and ratio of ORs among postmenopausal women appeared to suggest a negative interaction. The SBCS is one of two studies that identified a stronger association for premenopausal breast cancer with IGFBP-3 levels than with IGF-I levels (6,7). Thus, the nearly significant positive interaction among premenopausal women was expected, but the nonsignificant negative interaction among postmenopausal women was not. The lack of significant correlations between these IGF-I and IGFBP-3 levels among premenopausal or postmenopausal controls suggests that the biological mechanisms may have differed by menopausal status.

An alternative explanation for soy enhancing the effect of IGF-I levels on breast cancer is that soy may indirectly affect IGF-I levels because estrogens regulate the expression of IGF-I (42), and selective estrogen receptor modulators such as tamoxifen reduce IGF-I levels (43). To determine whether soy was a confounder or intermediate of breast cancer risk, we assessed the correlation between soy protein intake and IGF-I or IGFBP-3 levels. Soy protein intake was not correlated with IGF-I among any control subjects, but IGFBP-3 was correlated among premenopausal and postmenopausal controls. Nagata et al. (44) did not find a significant correlation between soy and IGF-I or IGFBP-3 levels among premenopausal Japanese women; however, to our knowledge no other studies have assessed these correlations among postmenopausal women. In addition, we found no evidence of confounding after adjusting the IGF-I and IGFBP-3 main effect analyses for soy protein intake. This argues against soy being a confounder or in the causal pathway between IGF-I levels and breast cancer risk but does not rule out this possibility for IGFBP-3.

The nonsignificant positive interaction for IGF-I level and soy protein intake among postmenopausal women, indicating that soy protein may act as a weak estrogen or as an anti-estrogen, is in agreement with laboratory studies showing that estrogen enhanced the effect of IGF-I on breast cancer cell growth (17,18). The nearly significant negative interaction between IGF-I level and soy protein intake among premenopausal women could not be explained by the estrogen–IGF-I hypothesis. In our data, we found that soy protein intake was correlated with estrone sulfate ($r = 0.16$; $P = 0.04$) and sex hormone-binding globulin ($r = –0.14$; $P = 0.07$) levels among premenopausal controls and with testosterone ($r = 0.16$; $P = 0.08$) levels among postmenopausal controls. Soy protein intake was not correlated with any other hormones (dehydroepiandrosterone sulfate, estradiol, estrone, or progesterone), suggesting that the soy protein intake among the study population may not be high enough to alter the estrogen level. More studies are needed to better understand the combined effect of estrogen and growth factor on breast cancer.

This study was not without limitations. Data on IGF-I and IGFBP-3 levels were available for a subgroup of women, reducing statistical power to detect effect modification. IGF-I and IGFBP-3 levels among healthy women in our population were lower than those among Caucasian women in the Nurses' Health Study (4), somewhat limiting the generalizability of our results. A potential explanation for these lower levels is the smaller body size and increased physical activity of Asian women compared with American women. Although blood was collected from cases prior to therapy, there may have been an effect of the disease itself on IGF-I and IGFBP-3 levels. Re-

porting of soy intake is prone to misclassification. A recently completed dietary validation study showed that the correlation of soy protein intake derived from the food-frequency questionnaire that we used in the study and the mean of multiple 24-h dietary recalls was 0.49 (45). Misclassification in assessing soy intake may have compromised our ability to investigate the interactive effects of soy protein intake and IGF-I and IGFBP-3 levels. Change of dietary habits over time, particularly after cancer diagnosis, is another concern. A supplementary questionnaire completed by 295 of 397 controls in the present study indicated that soy consumption reported in the last week was highly correlated with soy consumption reported in the past 5 yr ($r = 0.28$; $P < 0.0001$). Main effects and joint effects analyses comparing women whose diets had not changed with all women were slightly more pronounced but fairly comparable.

Although in vitro (17,18) and in vivo (19) studies of breast cancer have investigated the interaction between estrogen and IGF-I levels, ours is the first in vivo study to investigate the interaction between soy protein, a weak estrogen and anti-estrogen, and IGF-I levels. The relatively high soy consumption among our population compared with the rest of the world made this analysis possible. Additional strengths of this study are its population-based nature and high response rates among subjects (cases: 91%; controls: 90%), which minimizes selection bias. We adjusted for known breast cancer risk factors and evaluated the IGF-I levels, IGFBP-3 levels, and soy protein intake and breast cancer associations in conjunction with menopausal status, a suspected effect modifier of these relations. We also assessed BMI, waist-to-hip ratio, and use of hormone replacement therapy as effect modifiers of the IGF-I–breast cancer association with no evidence of such (data not shown). With the exception of waist-to-hip ratio, age at menarche, and physical activity, we were successful in selecting women for this substudy who were comparable with women from the larger study.

In summary, our results suggest that soy protein intake may modify the effect of IGF-I and IGFPB-3 levels on premenopausal breast cancer risk. Further studies with larger sample sizes are needed to confirm our finding and to understand the biological mechanism of these potential interactions. Should these interactions persist in other studies, intervention studies using soy protein must account for womens' IGF-I and IGFBP-3 levels in their design.

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