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Plasma Isoflavone Level and Subsequent Risk of Breast Cancer Among Japanese Women: A Nested Case-Control Study From the Japan Public Health Center-Based Prospective Study Group

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ABSTRACT

Purpose
Because they have large variations in consumption, Asian countries are suitable settings for studies of the effect of relatively high-dose isoflavone intake on breast cancer risk. Nevertheless, no prospective study from Asia has assessed blood or urine levels as biomarkers of isoflavone intake.

Patients and Methods
A total of 24,226 women ages 40 to 69 years in the Japan Public Health Center–based prospective study who responded to the baseline questionnaire and provided blood in 1990 to 1995 were observed to December 2002. During a mean 10.6 years of follow-up, 144 patients newly diagnosed with breast cancer were identified. Two matched controls for each patient were selected from the cohort. Isoflavone levels were assessed by plasma level and food frequency questionnaire, and the odds ratio of breast cancer according to isoflavone level was estimated using a conditional logistic regression model.

Results
We found a statistically significant inverse association between plasma genistein and risk of breast cancer, but no association for plasma daidzein. Adjusted odds ratios for the highest versus lowest quartile of plasma level were 0.34 for genistein (95% CI, 0.16 to 0.74; *P* for trend, .02) and 0.71 for daidzein (95% CI, 0.35 to 1.44; *P* for trend, .54). Median plasma genistein values in the control group were 31.9 ng/mL for the lowest and 353.9 ng/mL for the highest quartile groups. Regarding dietary intake of isoflavones, nonsignificant inverse associations were observed for both genistein and daidzein.

Conclusion
This nested case-control study found an inverse association between plasma genistein and the risk of breast cancer in Japan.

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INTRODUCTION

Soy foods, a traditional staple dish in Asian countries, are a primary source of isoflavones, such as genistein and daidzein, which are classified as phytoestrogens. Because breast cancer risk is substantially lower in Asian than Western countries,[1] the contribution of a high isoflavone intake to low breast cancer risk has been hypothesized.[2] This hypothesis has been supported by in vitro studies at high genistein concentrations and in the majority of animal studies, which together have demonstrated various anticancer effects of isoflavones acting via both estrogen-dependent and -independent mechanisms.[3,4] Estrogen-dependent mechanisms arise through the mediation of estrogen receptor α and β, owing to the similar chemical structure of isoflavones to the human estrogen hormone and their binding affinity to estrogen receptors.[4,5] For this reason, they have been hypothesized to behave like selective estrogen receptor modulators. In contradiction to potential protective effects, however, genistein exhibits estrogenic properties at low concentrations, which could theoretically enhance breast cancer risk.[3,4] In fact, some animal studies have reported that genistein stimulates tumor development and growth.[6,7] Although a recent meta-analysis found that soy intake was associated with a

small reduction in breast cancer risk, the authors concluded that in view of these risk-enhancing effects, recommendations for high-dose isoflavone supplementation to prevent breast cancer or its recurrence were premature.[8] Phytoestrogen supplements, however, are commercially marketed for use by postmenopausal women as natural and safe alternatives to hormone replacement therapy. The effect of relatively high-dose isoflavone on breast cancer risk is now of concern.

Because they have large variations in consumption among individuals, Asian countries serve as suitable venues for studies of the effect of relatively high-dose isoflavone intake on breast cancer risk. Despite this advantage, only a few epidemiological studies on soy or isoflavone intake and breast cancer risk from Asia have been reported.[9] In particular, no prospective study on isoflavone levels in blood or urine samples has been reported, notwithstanding that, because they are partly determined by individual differences in absorption and metabolism, blood or urine levels might better reflect interperson differences than dietary assessment. The three nested case-control studies which have investigated this association in Western populations have been inconsistent, with one reporting an inverse association with plasma genistein in the Netherlands,[10] the second showing no association with urinary genistein in the Netherlands,[11] and the third finding a positive association with urine and serum phytoestrogens in the United Kingdom.[12] This inconsistency might be in part explained by the apparently small variation in isoflavone levels in Western countries. For example, studies in the Netherlands, which has a high incidence of breast cancer (age-standardized rate per 100,000 world population, 86.7 in 2002),[13] reported a median genistein intake of 0.14 mg/d in women ages 49 to 70 years,[14] and a median plasma genistein level of 4.89 ng/mL in the control group of a nested-case control study.[10] In contrast, a study in Japan, where the incidence of breast cancer is low (age-standardized rate per 100,000 world population, 32.7 in 2002),[13] reported a median genistein intake of 22.3 mg/d and median serum level of 90.2 ng/mL.[15] This substantial variation in isoflavone levels suggests that the Japanese population represents an ideal setting for determining whether an association exists at relatively high levels achievable from dietary intake only.

Herein, to clarify the effect of relatively high-dose isoflavone exposure on breast cancer risk, we conducted a nested case-control study within a large-scale population-based prospective study in Japan.

PATIENTS AND METHODS

Study Population

The Japan Public Health Center–based prospective study, which began in 1990 for cohort I and in 1993 for cohort II, included 140,420 subjects (68,722 men and 71,698 women) living in the municipalities supervised by 11 public health centers (PHC). Details of the study design have been described elsewhere.[16] The study protocol was approved by the institutional review board of the National Cancer Center, Tokyo, Japan.

The study population comprised registered Japanese inhabitants living in each PHC area, ages 40 to 59 years in cohort I and 40 to 69 years in cohort II. In this analysis, one PHC area was excluded since data on cancer incidence were not available. We thus defined a population-based cohort of 67,426 women (27,389 in cohort I and 40,037 in cohort II) after the exclusion of ineligible subjects (n = 95).

Questionnaire Survey

A baseline survey was conducted from 1990 to 1994. A total of 55,891 women (83%) returned the questionnaire, which contained questions concerning demographic characteristics, medical history, menstrual and reproductive history, anthropometric factors, physical activity, smoking and drinking habits, and diet.

Blood Collection

Subjects voluntarily provided 10 mL of blood during health check-ups from 1990 to 1995. Blood samples were divided into plasma and buffy layers and stored at −80°C until analysis. Among respondents to the baseline questionnaire, a total of 24,996 women (45%) donated blood.

Follow-Up

All registered subjects were observed from the start of the study period to December 31, 2002. Data on residential relocation were obtained from residential registries. Among study subjects (n = 24,996), 1,289 subjects (5.2%) moved out of the study area and 5 (0.02%) were lost to follow-up within the study at-risk period.

Selection of Patients and Controls

Incidence data on breast cancer were collected for the Japan Public Health Center cancer registry through two data sources—major local hospitals and population-based cancer registries. Death certificates were used to supplement information on cancer incidence. Site of origin and histologic type were coded by members of our study group (Appendix A1, online only) using the International Classification of Diseases for Oncology, third edition, code C500-509. Up to the end of the study period, 144 new breast cancer cases (97 in cohort I and 47 in cohort II) were identified among the 24,226 women (9,689 in cohort I and 14,537 in cohort II) who had returned the baseline questionnaire, reported no history of breast cancer or ovarian cystoma, and provided blood samples. Diagnosis was microscopically verified in 98% of patients, and based on death certificates only in 0.7%. The mortality/incidence ratio was 0.14.

For each patient, two controls were selected using incidence density sampling from subjects who were not diagnosed with breast cancer during the follow-up period when the patient was diagnosed. Control selection was done without reference to incidence of other cancer sites. Controls were matched with each patient for age (within 3 years), PHC area, area (city or town and village), date of blood collection (within 90 days), time of day of blood collection (within 3 hours), fasting time at blood collection (within 3 hours), and baseline menopausal status.

Assessment of Dietary Intake

Dietary intakes of genistein and daidzein were assessed by a food frequency questionnaire of 44 items for cohort I and 52 for cohort II. Isoflavone intake was defined for this study as the sum of genistein and daidzein intake. We documented the questionnaire assessment of isoflavone intake to be reasonably valid (details in Appendix A1).[15,17]

Laboratory Assay

Plasma levels of isoflavone were analyzed using high-performance liquid chromatography with a coulometric array detector in accordance with the modified methods of Gamache and Acworth.[18] Concentrations of genistein and daidzein were determined by linear regression of the peak height for each standard, and adjusted according to the recovery rate of the internal plasma standard. The regression coefficient of peak height and concentration calculated for isoflavones revealed a linearity range of 0 to 0.75 μg/mL, with correlation coefficient values higher than 0.938. Voltametric response for the standard solution displayed coefficients of variation of 8% for intra- and 11% for interday variation. Recovery rates of isoflavones in plasma samples ranged between approximately 73% and 98%. Detection limits were 2.2 ng/mL for genistein and 2.7 ng/mL for daidzein. Laboratory personnel were blinded to case-control status when performing the analyses.

Statistical Analysis

Comparison of baseline characteristics, as well as plasma levels and dietary intake of isoflavones, between cases and controls was evaluated by the Mantel-Haenszel test using matched-set strata. Spearman's correlation coefficients were calculated among plasma levels and dietary intakes of isoflavone

among control subjects. Using a conditional logistic regression model, we calculated odds ratios (ORs) and 95% CIs of breast cancer for plasma levels and dietary intake of isoflavone divided into quartiles based on control distribution. The ORs were adjusted for number of births and age at first birth as potential confounders. The adjusted ORs were calculated based on a total of 405 subjects with complete information for covariates. Linear trends for ORs were tested in the conditional logistic regression model using the exposure categories as ordinal variables. All P values reported are two sided, and significance level was set at $P < .05$. All statistical analyses were performed with SAS software, version 9.1 (SAS Institute Inc, Cary, NC).

RESULTS

Case subjects and controls had significantly different distribution for number of births (Table 1). Other characteristics, such as age at menarche, age at first birth, body mass index (BMI), alcohol consumption, or dietary intake did not substantially differ between the two groups.

Plasma genistein was significantly lower among cases than controls whereas plasma daidzein values were similar (Table 2). No significant differences between the groups were seen for dietary genistein, daidzein, or isoflavone intake. Median isoflavone intake in the control group was 34.8 mg/d (36.1 in cohort I and 29.9 mg/d in cohort II). Genistein and daidzein were highly correlated for both plasma level ($r = 0.72$) and dietary intake ($r = 0.99$). Correlation coefficients between plasma and dietary levels were relatively low for both genistein ($r = 0.23$) and daidzein ($r = 0.31$).

We found a statistically significant inverse association between plasma genistein and the risk of breast cancer (P for trend, .02), but no statistically significant association for plasma daidzein (P for trend, .54; Table 3). Adjusted ORs for the highest versus lowest quartile of plasma level were 0.34 for genistein (95% CI, 0.16 to 0.74; $P \leq .01$) and 0.71 for daidzein (95% CI, 0.35 to 1.44; $P = .34$). Moreover, the results did not change substantially after adjustment for dietary intake of isoflavone or other potential confounders such as age at menarche, menopausal status at baseline, age at menopause, height, BMI, and alcohol consumption. Further, exclusion of cases diagnosed before the first 3 years of follow-up did not substantially change the results, nor did the exclusion of subjects who used vitamin supplements or who provided a nonfasting blood sample (ie, within 6 hours after a meal). Regarding dietary intake, we observed inverse associations for both genistein and daidzein but neither was statistically significant (Table 3). In addition, adjusted ORs by isoflavone intake were closely similar to those by genistein intake (data not shown).

A stratified analysis according to baseline menopausal status showed no remarkable difference between two strata for either genistein and daidzein, regardless of whether the values were assessed by plasma or questionnaire, although the inverse association between plasma genistein and risk of breast cancer tended to be more stable in postmenopausal than premenopausal women (Table 4).

Table 1. Characteristics of Patients and Matched Control Subjects at Baseline

Characteristic	Patients (n = 144) No.	Patients (n = 144) %	Controls (n = 288) No.	Controls (n = 288) %	P*
Mean age, years	51.7		51.8		
Standard deviation	7.1		7.1		—
Family history of breast cancer	2	1.4	2	0.7	.48
Premenopausal women	59	42	118	42	—
Postmenopausal women					
Natural menopause	70	50	140	50	—
Surgical menopause	10	7.2	20	7.2	—
Mean age at menopause, years	50.0		49.8		.76
SE†	0.38		0.27		
Mean age at menarche, years	14.6		14.8		.33
SE†	0.15		0.10		
Mean No. of births	2.3		2.8		.01
SE†	0.12		0.09		
Mean age at first birth, years	25.7		25.0		.22
SE†	0.30		0.21		
Use of exogenous female hormones (current use)	4	3.0	2	0.8	.10
Mean height, cm	151.7		151.4		.70
SE†	0.46		0.33		
Mean body mass index, kg/m^2	23.4		23.5		.49
SE†	0.25		0.18		
Smoking (current smoker)	5	3.5	17	5.9	.23
Alcohol drinking (regular drinker)	18	13	26	9.1	.28
Leisure-time physical activity (≥ once per week)	30	21	57	20	.42
Vitamin supplement user	33	24	61	23	.65
Green tea intake (≥ five cups per day)	36	25	71	25	.42
Mean total energy intake, kcal/d	1,269.4		1,271.0		.41
SE‡	26.5		19.2		
Mean fish and shellfish intake, g/d	45.4		45.7		.75
SE‡	2.5		1.8		
Mean meat intake, g/d	30.5		28.5		.15
SE‡	1.7		1.2		
Mean vegetable intake, g/d	121.2		115.9		.20
SE‡	5.7		4.1		
Mean fruit intake, g/d	104.8		99.4		.79
SE‡	5.9		4.3		

*P for Mantel-Haenszel test with matched-set strata.
†Adjusted for age.
‡Adjusted for age and cohort.

DISCUSSION

In this study, we found a statistically significant inverse association between plasma genistein and the risk of breast cancer, but no association for plasma daidzein. This finding suggests that genistein may

Table 2. Plasma Levels and Dietary Intake of Isoflavone in Patients and Matched Controls

Parameter	Patients (n = 144) Median	Patients (n = 144) Interquartile Range	Controls (n = 288) Median	Controls (n = 288) Interquartile Range	P*
Plasma level					
Genistein, ng/mL	131.8	67.9-202.6	144.5	78.8-255.6	.046
Daidzein, ng/mL	16.7	7.0-34.0	17.9	5.5-40.8	.45
Dietary intake					
Genistein, mg/d	19.9	16.6-24.0	21.7	16.8-26.1	.37
Daidzein, mg/d	12.5	10.1-14.8	13.3	10.3-16.3	.36
Isoflavone, mg/d†	32.5	26.8-38.7	34.8	27.0-42.4	.36

*P for Mantel-Haenszel test with matched-set strata.
†Isoflavone intake = sum of genistein and daidzein intake.

Table 3. ORs and 95% CIs of Breast Cancer According to Plasma Level and Dietary Intake of Isoflavone

Parameter	Quartile 1	2	3	4	*P* for trend
Plasma level					
Median genistein, ng/mL	31.9	108.1	190.8	353.9	
No. of patients	41	37	45	21	
No. of controls	72	72	72	72	
OR	1.00	0.84	1.04	0.46	.07
95% CI	Reference	0.47 to 1.51	0.57 to 1.91	0.23 to 0.91	
Adjusted OR*	1.00	0.69	0.87	0.34	.02
95% CI	Reference	0.36 to 1.32	0.45 to 1.67	0.16 to 0.74	
Median daidzein, ng/mL	0	12.0	27.0	53.7	
No. of patients	30	45	44	25	
No. of controls	72	72	72	72	
OR	1.00	1.50	1.44	0.79	.59
95% CI	Reference	0.85 to 2.64	0.80 to 2.61	0.41 to 1.54	
Adjusted OR*	1.00	1.30	1.51	0.71	.54
95% CI	Reference	0.70 to 2.42	0.80 to 2.86	0.35 to 1.44	
Dietary intake					
Median genistein, mg/d	15.7	18.5	22.9	27.3	
No. of patients	42	36	37	29	
No. of controls	69	75	71	73	
OR	1.00	0.78	0.83	0.58	.15
95% CI	Reference	0.46 to 1.35	0.47 to 1.48	0.30 to 1.12	
Adjusted OR*	1.00	0.81	0.92	0.58	.21
95% CI	Reference	0.46 to 1.45	0.50 to 1.70	0.29 to 1.18	
Median daidzein, mg/d	9.4	11.4	14.1	17.1	
No. of patients	40	39	35	30	
No. of controls	70	74	72	72	
OR	1.00	0.91	0.82	0.65	.21
95% CI	Reference	0.52 to 1.58	0.46 to 1.47	0.33 to 1.27	
Adjusted OR*	1.00	0.96	0.94	0.67	.34
95% CI	Reference	0.54 to 1.74	0.50 to 1.74	0.33 to 1.39	

Abbreviation: OR, odds ratio.
*Adjusted for number of births (0, 1, 2, 3, 4, 5+) and age at first birth (−21, 22-25, 26-29, 30+, nulliparous). Adjusted ORs were calculated based on a total of 405 subjects with complete information of covariates.

play a more important role in the etiology of breast cancer than daidzein. Our findings are in general agreement with those of a recent nested case-control study in the Netherlands,[10] albeit that our inverse association occurred at substantially higher plasma concentrations. For example, median plasma genistein values in the control group of the Netherlands study were 3.75 ng/mL for premenopausal and 4.89 ng/mL for postmenopausal women.[10] In contrast, the median value in our control group was 144.5 ng/mL, and only 3.2% of control subjects was under 5 ng/mL. This apparently high level is not surprising considering that the median value of 353.9 ng/mL in our highest plasma genistein quartile group, which had a significantly lower risk of breast cancer than the lowest group, corresponded to a median dietary intake of 28.5 mg/d for genistein and 46.5 mg/d for isoflavone, as estimated by the validation study data. Although some in vivo and in vitro studies have shown risk-enhancing effects of genistein, our study suggests that relatively high-dose isoflavones exposure achievable from dietary intake alone is associated with a decreased rather than increased risk.

We observed an approximately 65% reduction in breast cancer risk in the highest plasma genistein quartile group but no decrease in the other quartiles, indicating that only the highest group benefited from risk reduction. The apparent lack of a dose-response relationship might imply the presence of a threshold level of effect. Interestingly, this idea contradicts findings in Western populations, in whom inverse associations are seen despite materially low levels of isoflavones. Given the differences in hormonal milieu between the two populations, the potential protective effect of isoflavones in breast cancer might act differently between Western and Asian populations: sex hormone levels are higher in Western than Asian women,[19] for example, as is the prevalence of obesity.[20,21] In this regard, a case-control study in Shanghai found that the inverse association between urinary isoflavone level and breast cancer risk was stronger among women in the high BMI, waist-hip ratio, and estradiol level groups and in the low sex hormone-binding globulin level group than in the respectively converse low and high groups.[22] Alternatively, the apparent lack of a dose-response relationship might merely reflect uncontrolled confounding by other dietary characteristics or risk-lowering behaviors.

The reason for a role for genistein but not daidzein in the etiology of breast cancer is unclear, but several possibilities can be speculated. Genistein possesses stronger binding affinity for estrogen receptor than daidzein.[5] Further, a pharmacokinetic study showed higher plasma levels and a 1.5-fold longer half-life for genistein than daidzein

Table 4. ORs and 95% CIs of Breast Cancer According to Plasma Level and Dietary Intake of Isoflavone By Baseline Menopausal Status

Parameter	Quartile 1	2	3	4	*P* for trend
Premenopausal women					
Plasma genistein, ng/mL					
No. of patients	24	14	19	2	
No. of controls	41	28	25	24	
Adjusted OR*	1.00	0.76	1.75	0.14	.20
95% CI	Reference	0.31 to 1.86	0.68 to 4.50	0.03 to 0.69	
Plasma daidzein, ng/mL					
No. of patients	17	21	15	6	
No. of controls	27	45	23	23	
Adjusted OR*	1.00	0.80	1.27	0.49	.48
95% CI	Reference	0.34 to 1.88	0.48 to 3.38	0.15 to 1.57	
Dietary genistein intake, mg/d					
No. of patients	21	16	14	8	
No. of controls	35	31	32	20	
Adjusted OR*	1.00	0.92	0.86	0.62	.43
95% CI	Reference	0.41 to 2.05	0.34 to 2.18	0.21 to 1.84	
Dietary daidzein intake, mg/d					
No. of patients	20	17	14	8	
No. of controls	36	30	32	20	
Adjusted OR*	1.00	1.07	0.93	0.67	.53
95% CI	Reference	0.46 to 2.51	0.37 to 2.34	0.22 to 2.03	
Postmenopausal women					
Plasma genistein, ng/mL					
No. of patients	17	23	25	15	
No. of controls	28	41	46	45	
Adjusted OR*	1.00	0.54	0.57	0.36	.10
95% CI	Reference	0.18 to 1.62	0.20 to 1.65	0.12 to 1.12	
Plasma daidzein, ng/mL					
No. of patients	13	23	27	17	
No. of controls	40	27	47	46	
Adjusted OR*	1.00	2.86	2.06	1.16	.95
95% CI	Reference	1.03 to 7.98	0.82 to 5.17	0.43 to 3.15	
Dietary genistein intake, mg/d					
No. of patients	20	20	22	18	
No. of controls	33	42	35	50	
Adjusted OR*	1.00	0.73	0.93	0.52	.31
95% CI	Reference	0.30 to 1.77	0.38 to 2.27	0.19 to 1.42	
Dietary daidzein intake, mg/d					
No. of patients	19	22	20	19	
No. of controls	33	42	36	49	
Adjusted OR*	1.00	0.89	0.93	0.64	.43
95% CI	Reference	0.38 to 2.10	0.38 to 2.29	0.23 to 1.72	

Abbreviation: OR, odds ratio.
*Adjusted for number of births (0, 1, 2, 3, 4, 5+) and age at first birth (−21, 22-25, 26-29, 30+, nulliparous).

after ingestion of baked soybean powder containing closely similar amounts of the two.[23] Moreover, the absence of an association for plasma daidzein might be attributable to misclassification arising from the metabolization of this compound. Daidzein can be metabolized by intestinal bacteria to equol and O-desmethylangolites; because approximately only 30% to 50% of individuals are capable of equol production, probably due to differences in gut microflora, daidzein-to-equol metabolizers may have lower plasma daidzein levels than nonmetabolizers.[24] Equol has been suggested to have greater biologic activity than daidzein,[24] and an inverse association between equol level and breast cancer risk has been reported.[25] Here, the lowest plasma daidzein quartile group might conversely have had a lower breast cancer risk than the higher groups due to its inclusion of equol metabolizers, and such misclassification, if present, would lead to a null result.

Our study has several methodological advantages over previous studies of isoflavones and the risk of breast cancer. First, the direct measurement of plasma isoflavone levels provides not only an index of intake but also of the absorption and metabolism of isoflavone, an understanding of which is important to elucidating the mechanisms by which isoflavones might influence breast cancer development. Indirect measurement by dietary intake of genistein is likely a major reason for the present smaller and nonsignificant risk reduction of breast cancer than by plasma genistein. Exposure assessment using

blood samples is therefore likely a more sophisticated means of detecting an association. Second, two case-control studies in Australia and China showed an inverse association between urinary isoflavones and breast cancer risk.[25,26] In view of the retrospective design of these studies, however, blood or urine levels of isoflavones in breast cancer cases might have been influenced by metabolic changes after the breast cancer was detected or by altered eating habits among case subjects. In our nested case-control study within a prospective cohort, in contrast, blood samples were collected before cancer diagnosis, obviating any potential bias due to the presence of cancer. Third, cases and controls were selected from the same cohort, thereby avoiding the selection bias inherent to case-control studies.

Several limitations of this study warrant mention. First, we measured plasma isoflavones only once for each individual. The consumption of soy foods is a personal dietary preference, and intake levels of most individuals are assumed to be relatively stable over time in Japan, as suggested by our validation study, which showed high reproducibility of repeated measurements of genistein intake by food frequency questionnaire (correlation coefficient = 0.72 for 1-year interval and 0.61 for 5-year interval).[15,17] By comparison, plasma isoflavone levels may reflect short-term rather than long-term intake: isoflavones have short half-lives in blood (eg, 6 to 8 hours),[23,27] and plasma levels are particularly affected by time elapsed since the last meal. To minimize the attenuation of risk estimates derived from random measurement errors, we matched fasting time between cases and controls. Second, despite a reasonably large cohort population (24,226 women) and long follow-up period (average, 10.6 years), the number of breast cancer cases was relatively small, reflecting the low incidence rate in Japan (age-standardized rate per 100,000 world population, 32.7 in 2002).[13] The interpretability of our results might therefore be limited, particularly in stratified analyses. Third, although our cohort subjects were selected from the general population, subjects were restricted to the 24,226 women respondents (43%) to the baseline questionnaire who provided blood samples. Although health check-up examinees in our previous report had a different socioeconomic status than nonexaminees and a more favorable lifestyle profile,[28] no apparent difference in isoflavone intake and breast cancer risk factors was found between subjects in the subcohort for this study and the original cohort; median isoflavone intake, for example, was 32.5 and 32.1 mg/d, respectively, and the average number of births was 2.8 and 2.7, respectively.[29] Nevertheless, any extrapolation of the results to the general population should be done cautiously, particularly in view of a previous report showing the difficulty of extrapolating relative risk estimates for a subcohort to an entire cohort. This difficulty might in fact be inherent to prospective studies in general.[30]

Allowing for these methodological issues, we found an inverse association between plasma genistein and the risk of breast cancer in a nested case-control study in Japan. This finding suggests a risk-reducing rather than a risk-enhancing effect of isoflavones on breast cancer, even at relatively high concentrations within the range achievable from dietary intake alone.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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Appendix

Assessment of Dietary Intake. The cohort I questionnaire asked about average consumption during the previous month of 44 food items, including three items contributing to isoflavone intake, namely, (1) miso soup, (2) soybeans, tofu, deep-fried tofu, and natto, and (3) vegetables other than yellow and green vegetables, such as Chinese cabbage, radish, tomato, cucumber, and so on—whose items includes soybean sprouts. The cohort II questionnaire asked about average consumption during the previous month of 52 food items, including three items contributing to isoflavone intake: miso soup, tofu, and natto. The cohort I questionnaire had four frequency categories: rarely, 1 to 2 days/week, 3 to 4 days/week, and almost everyday, while the cohort II questionnaire had five frequency categories of never, less than 1 day/week, 1 to 2 days/week, 3 to 4 days/week, and almost everyday. Portion size and isoflavone content were estimated from a validation study (Yamamoto S, Sobue T, Sasaki S, et al: J Nutr 131:2741-2747, 2001; Tsubono Y, Kobayashi M, Sasaki S, et al: J Epidemiol 13:S125-133, 2003), in which 14- to 28-day dietary records and blood samples were collected from subsamples among the Japan Public Health Center cohort members, comprising 107 women in cohort I and 178 women in cohort II. Spearman's correlation coefficients between genistein and daidzein intake estimated from the questionnaire and from dietary records were 0.54 for genistein and 0.55 for daidzein in cohort I, respectively (Tsubono Y, Kobayashi M, Sasaki S, et al: J Epidemiol 13:S125-133, 2003), and 0.53 for genistein and 0.50 for daidzein in cohort II, respectively (unpublished data). To include intakes calculated from the two different questionnaires in the analysis, we estimated genistein and daidzein intake for each subject by cohort from the questionnaires based on a regression function derived from the validation study data.

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