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

The Appendix is included in the full-text version of this article, available online at www.jco.org. It is not included in the PDF version (via Adobe® Reader®).

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EPIDEMIOLOGY

Dietary isoflavone intake and breast cancer risk in case–control studies in Japanese, Japanese Brazilians, and non-Japanese Brazilians

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Abstract Although epidemiologic studies have shown an inverse association between isoflavones and breast cancer risk, little evidence for a dose–response relation is available. We conducted hospital-based case–control studies of patients aged 20–74 years with primary, incident, histologically confirmed invasive breast cancer, and matched controls from medical checkup examinees in Nagano, Japan and from cancer-free patients in São Paulo, Brazil. A total of 850 pairs (390 Japanese, 81 Japanese Brazilians and 379 non-Japanese Brazilians) completed validated food frequency questionnaires. The odds ratio of breast cancer according to isoflavone intake was estimated using a conditional logistic regression model. We found a statistically significant inverse association between isoflavone intake and the risk of breast cancer for Japanese Brazilians and non-Japanese Brazilians. For Japanese, a non-significant inverse association was limited to postmenopausal women. In the three populations combined, breast cancer risk linearly decreased from 'no' to 'moderate' isoflavone intake and thereafter leveled off. Compared to non-consumers, adjusted odds ratios (95% confidence interval) for consumers in increasing quintile intake categories (median intake in each category: 8.7, 23.1, 33.8, 45.7, and 71.3 mg/day) were 0.69 (0.44–1.09), 0.54 (0.31–0.94), 0.45 (0.26–0.77), 0.34 (0.19–0.62), and 0.43 (0.24–0.76), respectively. Overall, we found an inverse association between dietary isoflavone intake and risk of breast cancer. Our finding suggests a risk-reducing rather than risk-enhancing effect of isoflavones on breast cancer within the range achievable from dietary intake alone. In addition, women may benefit

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from risk reduction if they consume at least moderate amounts of isoflavones.

Keywords Breast cancer · Dietary isoflavones · Case–control study · Immigrants

Abbreviations

CI	Confidence interval
ER	Estrogen receptor
FFQ	Food-frequency questionnaire
OR	Odds ratio
PR	Progesterone receptor

Introduction

Soy foods, which are rich in isoflavones, are habitually consumed by Asian populations in large amounts. Isoflavones, of which genistein and daidzein are major examples, are classified as phytoestrogens, which are plant-derived non-steroidal compounds with estrogen-like biological properties. A high intake of isoflavones has therefore been hypothesized to contribute to the lower incidence of breast cancer in Asia than Western countries [1]. This hypothesis is supported by not only in vitro studies at high genistein concentrations and the majority of animal studies [2, 3] but also epidemiological studies [4–10]. In particular, a recent meta-analysis showed a small decrease in risk of breast cancer with higher soy intake [11] while a more recent meta-analysis indicated that risk reduction was limited to Asian populations [12]. In apparent contradiction to potential protective effects, however, genistein exhibits estrogenic properties at low concentrations, which could theoretically enhance breast cancer risk [2, 3], and some animal studies have in fact reported that genistein stimulates tumor development and growth [13, 14].

Although research remains insufficient for any comprehensive determination of whether isoflavones are protective or harmful for breast cancer, interest in soy foods and isoflavones is nevertheless increasing. This increase may reflect an expectation of potential benefits in a wide variety of medical conditions, including cancer of the endometrium and prostate as well as breast, cardiovascular diseases, osteoporosis, and menopausal symptoms. In fact, consumption of soy foods in the United States has increased over the past ten years, against fairly constant intake in Japan over the past four decades [15]. Moreover, phytoestrogen supplements are commercially marketed for use by postmenopausal women as natural and safe alternatives to hormone replacement therapy. A dose–response pattern, in particular the effect of relatively high-dose isoflavones on breast cancer risk, is thus now of concern. Nevertheless, little evidence of any dose–response relationship is available—indeed, we do not know the answer to 'how much isoflavones is needed?' This is partly because few studies have estimated isoflavone intake using a validated food-frequency questionnaire (FFQ) [4–6, 16, 17], and also because most studies in Western countries have involved only a small variation in isoflavone intake [6, 7, 16–20].

Here, to evaluate the dose–response relationship between isoflavone intake and the risk of breast cancer, ranging from zero to the relatively high levels achievable from dietary intake only, we conducted hospital-based case–control studies in Nagano, Japan and São Paulo, Brazil, areas with a low and middle incidence of breast cancer, respectively (age-standardized rate per 100,000 world population, 32.7 and 46.0 in 2002, respectively) [21], using validated FFQs with relatively high validity in three populations: Japanese living in Japan, Japanese Brazilians living in São Paulo, and non-Japanese Brazilians living in São Paulo. The mortality of breast cancer among these three populations has increased over the last 20 years, with that in Japanese Brazilians intermediate between that in Japanese and Brazilians [22]. In addition, because amounts and variations in isoflavone intake are expected to be high and large for Japanese, intermediate and relatively large for Japanese Brazilians, and low and small for non-Japanese Brazilians, respectively, these populations serve as suitable venues for studies of the effect of dose–response relations.

Materials and methods

Study subjects

These multicenter, hospital-based case–control studies of breast cancer were designed to determine lifestyle factors and genetic susceptibility to the risk of breast cancer and to compare potential risk factors among Japanese living in Nagano, Japan, and Japanese Brazilians and non-Japanese Brazilians living in São Paulo, Brazil. Eligible cases were a consecutive series of female patients aged 20–74 years with newly diagnosed and histologically confirmed invasive breast cancer. Cases were recruited between 2001 and 2005 at four hospitals in Nagano, and between 2001 and 2006 at eight hospitals in São Paulo. A total of 405 cases (98%) participated in Nagano, and 83 Japanese Brazilians (91%) and 389 non-Japanese Brazilians (99%) in São Paulo. In the study in Nagano, eligible controls were selected from medical checkup examinees in two of the four hospitals and confirmed not to have cancer. One control was matched for each case by age (within 3 years) and residential area during the study period. Among potential controls, one examinee refused to participate and two refused to provide blood samples. Consequently, we

obtained written informed consent from 405 matched pairs. In the study in São Paulo, eligible controls were preferentially selected from cancer-free patients who visited the same hospital as the index cases. One control was matched for each case by age (within 5 years) and ethnicity during the study period. Among potential controls, 22 patients refused to participate (participation rate = 96%). Consequently, we obtained written informed consent from 472 matched pairs (83 for Japanese Brazilians and 389 for non-Japanese Brazilians). The study protocol was approved by CONEP (Comissão Nacional de Ética em Pesquisa), Brasília, Brazil and by the institutional review board of the National Cancer Center, Tokyo, Japan.

Data collection

Participants in Nagano were asked to complete a self-administered questionnaire, while in-person interviews were conducted by trained interviewers using a structured questionnaire in São Paulo. The two questionnaires contained closely similar questions concerning demographic characteristics, medical history, family history of cancer, menstrual and reproductive history, anthropometric factors, physical activity, and smoking habits. For dietary habits, we used a semi-quantitative FFQ (136 items for the Japanese version and 118 items for the Brazilian version) which was developed and validated in each population [23, 24]. Information on estrogen receptor (ER) and progesterone receptor (PR) status was obtained from medial records. Hormone receptor status was determined by either enzyme-linked immunoassay or immunohistochemical assay. Hormone receptor positivity values were determined either as specified by the laboratory that performed the assay, or in accordance with the laboratory's written interpretation thereof, or both.

Dietary assessment

In the FFQ, participants were questioned on how often they consumed the individual food items (frequency of consumption), as well as relative sizes compared to standard portions. Response choices for frequency were never or less than once/month, 1–3 times/month, 1–2 times/week, 3–4 times/week, 5–6 times/week, once/day, 2–3 times/day, 4–6 times/day, and 7 times/day or more, and relative sizes to a standard portion were small (50% smaller than standard), medium (same as standard), and large (50% larger). For the Japanese version, white rice intake was determined in terms of the relative size of the rice bowl used and the frequency of intake, with the nine choices of less than 1–10 bowls per day. Frequency for miso soup intake was given in the six choices of almost never, 1–3 times/month, 1–2 times/week, 3–4 times/week, 5–6 times/week, or daily, while amount was given in nine categories ranging from less than 1–10 bowls per day, without reference to the relative size of the bowl used. Daily food intake was calculated by multiplying frequency by standard portion and relative size for each food item in the FFQ. Daily intakes of genistein and daidzein were calculated using a food composition table of isoflavones developed previously [25, 26]. Isoflavone intake was defined for this study as the sum of genistein and daidzein intake. Other nutrients were calculated using the Japanese Standard Tables of Food Composition, 5th ed. for the Japanese version [27] and the United States Department of Agriculture (USDA) food composition tables for the Brazilian version [28]. For some Japanese-specific foods in the Brazilian version, the Japanese Standard Tables of Food Composition, 5th ed. was used.

The validity of isoflavone intake estimated from the Japanese version of the FFQ was evaluated in a subsample of the Japan Public Health Center-based Prospective Study, which includes Nagano as one of the study areas. The estimated intake according to the FFQ was compared to that in four consecutive 7-day dietary records, one conducted in each the four seasons. Spearman's correlation coefficients between energy-adjusted genistein and daidzein intake estimated from the FFQ and from dietary records were 0.59 for genistein and 0.60 for daidzein [24]. For the Brazilian version, the validity of isoflavone intake estimated from the FFQ was evaluated in a subsample of the control group in this case–control study by comparing the estimated intake according to the FFQ to that in two consecutive 4-day dietary records, one each in two seasons. Spearman's correlation coefficients between energy-adjusted genistein and daidzein intake estimated from the FFQ and from dietary records were 0.76 for genistein and 0.76 for daidzein (unpublished data).

Statistical analysis

We excluded subjects who reported extremely low or high total energy intake (<500 or ≥ 4000 Kcal), leaving 390 pairs of Japanese, 81 pairs of Japanese Brazilians and 379 pairs of non-Japanese Brazilians for use in the present analyses. Comparison of baseline characteristics between cases and controls was evaluated by the Mantel-Haenszel test using matched-pair strata in each population. Dietary intake of isoflavones was adjusted for total energy intake by the residual method and divided into median or tertile categories based on control distribution for Japanese and Japanese Brazilians, respectively. Because of the small proportion of consumers, non-Japanese Brazilians were categorized into non-consumers and consumers of isoflavones. Using a conditional logistic regression model, we calculated odds ratios (ORs) and 95% confidence intervals

(CIs) of breast cancer for isoflavone intake. An unconditional logistic regression model was used for stratified analyses according to menopausal status. Associations between isoflavone intake and hormone receptor-defined breast cancer were assessed by an unconditional polytomous logistic regression model. Linear trends for ORs were tested in the logistic regression model using the exposure categories as ordinal variables. The following variables, which were mainly selected based on comparison of baseline characteristics between cases and controls, were adjusted for as potential confounders: menopausal status, number of births, family history of breast cancer, smoking status, moderate physical activity in the past 5 years, and vitamin supplement use. We did not include a history of benign breast disease as a covariate since we regarded it as an intermediate variable in the causal pathway between isoflavone intake and breast cancer. All p values reported are two-sided, and significance level was set at $P < 0.05$. All statistical analyses were performed with SAS software version 9.1 (SAS Institute, Inc., Cary, NC).

Results

Characteristics of cases and controls and isoflavone intake (Table 1)

For Japanese, the proportion of premenopausal women, current smokers, and vitamin supplement users was higher in cases than in controls, and cases tended to have a family history of breast cancer and history of benign breast disease. Cases were less likely than controls to breast-feed, be physically active, and eat vegetables. For Japanese Brazilians, cases were less likely than controls to give birth and be physically active and more likely to eat vegetables and fruits. For non-Japanese Brazilians, the proportion of premenopausal women and current smokers was higher in cases than controls while the proportion of physically active women and vitamin supplement users was lower. Isoflavone intake substantially varied among populations, with mean intakes (mg/day) in control subjects of 46.1 for Japanese, 24.9 for Japanese Brazilians, and 4.4 for non-Japanese Brazilians. Because genistein and daidzein intakes were highly correlated, with a Spearman's correlation coefficient for the three populations of 0.99, only isoflavone intake was used for the following analyses.

ORs in the three populations (Table 2)

We found a statistically significant inverse association between isoflavone intake and the risk of breast cancer for Japanese Brazilians and non-Japanese Brazilians but not for Japanese. Adjusted OR for the highest versus lowest tertile of isoflavone intake was 0.25 (95% CI 0.09–0.68; P for trend <0.01) for Japanese Brazilians. For non-Japanese Brazilians, adjusted OR for consumers versus non-consumers of isoflavones was 0.56 (95% CI 0.35–0.90). No substantial change was seen after further adjustment for other potential confounders, such as age at menarche, age at menopause, age at first birth, history of breast feeding, body mass index, alcohol drinking, or vegetable and fruit intake.

A stratified analysis according to menopausal status revealed that an inverse association was limited to postmenopausal women in Japan although it was not statistically significant. Adjusted OR for the highest versus lowest tertile of isoflavone intake was 0.62 (95% CI 0.38–1.01; P for trend = 0.06) for postmenopausal women, but 1.35 (95% CI 0.72–2.54; P for trend = 0.41) for premenopausal women. The inverse association was stronger in premenopausal than postmenopausal women for Japanese Brazilians but no remarkable difference between the two strata was seen for non-Japanese Brazilians.

ORs of hormone receptor-defined breast cancer (Table 3)

Information on the combined ER and PR status of the breast tumor was available for 387 (99%) Japanese, 61 (75%) Japanese Brazilians, and 264 (70%) non-Japanese Brazilians cases. The following subtypes were used for modeling in an unconditional polytomous logistic regression model: positive for both receptors (ER+/PR+), ER-positive and PR-negative (ER+/PR−), and negative for both receptors (ER−/PR−) for Japanese, and ER+/PR+, ER+/PR−, ER−/PR−, and unknown for Japanese Brazilians and non-Japanese Brazilians. Overall, we found no remarkable difference in risk by hormone receptor-defined subtype.

Dose–response pattern (Table 4; Fig. 1)

To evaluate dose–response relations using a wide range of isoflavone intake, we combined individual study data from three populations and categorized the subjects into six groups, namely non-consumers and quintiles among isoflavone consumers based on the combined control distribution. Compared to non-consumers, adjusted ORs (95% CI) for consumers in increasing quintile categories (median intake in each category: 8.7, 23.1, 33.8, 45.7, and 71.3 mg/day) based on a conditional logistic regression model were 0.69 (0.44–1.09), 0.54 (0.31–0.94), 0.45 (0.26–0.77), 0.34 (0.19–0.62), and 0.43 (0.24–0.76), respectively. A stratified analysis according to menopausal status based on an unconditional logistic regression model revealed that this inverse association was more prominent in postmenopausal

Table 1 Characteristics of case and matched control subjects

	Japanese living in Nagano, Japan			Japanese Brazilians living in São Paulo, Brazil			Non-Japanese Brazilians living in São Paulo, Brazil		
	Case ($n = 390$)	Control ($n = 390$)	P^a	Case ($n = 81$)	Control ($n = 81$)	P^a	Case ($n = 379$)	Control ($n = 379$)	P^a
Age (years), mean	53.8	54.0	–	56.6	56.5	–	52.4	52.5	–
Premenopausal women, %	46	35	<0.01	31	30	0.80	42	38	0.04
Age at menopause (years), mean[b]	49.0	49.4	0.15	49.9	50.6	0.73	49.1	48.4	0.13
Age at menarche (years), mean[b]	13.4	13.2	0.42	12.9	12.9	0.20	13.2	13.1	0.96
Nulliparous women, %	13	14	0.66	23	16	0.24	11	10	0.91
Number of births (≥4 births), %	2	3	0.16	7	20	0.02	29	35	0.10
Age at first birth (years), mean[b, c]	26.9	26.4	0.42	28.6	27.5	0.25	23.2	22.5	0.24
Breast feeding (yes), %[c]	91	96	0.03	92	91	0.56	88	91	0.67
Oral contraceptives user, %	3	3	1.00	29	36	0.30	63	65	0.62
Family history of breast cancer, %	11	6	0.02	15	12	0.65	6	6	0.88
History of benign breast disease, %	12	7	0.03	12	6	0.17	7	7	1.00
Height (cm), mean[b]	155.3	155.5	0.50	154.0	153.9	0.91	158.2	158.4	0.96
Body mass index (kg/m^2), mean[b]	22.7	23.0	0.07	24.3	24.5	0.43	26.6	26.1	0.11
Smoking (current smoker), %	8	5	<0.01	11	2	0.07	17	11	0.04
Alcohol drinking (regular drinker), %	26	29	0.25	2	6	0.26	6	6	0.65
Moderate physical activity past 5 years (yes), %	32	40	0.02	19	32	0.03	9	14	0.03
Vitamin supplement user, %	18	12	0.03	19	26	0.27	3	9	<0.01
Total energy intake (kcal/day), mean[b]	1881.6	1949.3	0.27	1662.0	1587.7	0.44	1847.0	1752.8	0.09
Fish and shellfish intake (g/day), mean[b]	87.6	94.4	0.11	27.4	30.5	0.56	13.7	16.6	0.24
Meat or red meat intake (g/day), mean[b, d]	58.1	57.6	0.36	54.3	53.3	0.44	72.1	64.2	0.14
Vegetable intake (g/day), mean[b]	257.6	310.5	<0.01	146.7	93.0	<0.01	77.7	86.4	0.96
Fruit intake (g/day), mean[b]	288.6	287.7	0.69	364.0	311.0	0.02	260.2	250.9	0.35
Isoflavone intake (mg/day), mean[b]	43.5	46.1	<0.01	16.5	24.9	0.15	1.1	4.4	0.01
Genistein intake (mg/day), mean[b]	27.0	28.6	<0.01	10.2	15.8	0.15	0.73	3.1	0.01
Daidzein intake (mg/day), mean[b]	16.5	17.5	<0.01	6.3	9.1	0.15	0.33	1.4	0.01

[a] P for Mantel-Haenszel test with matched-pair strata

[b] Adjusted for age

[c] Among parous women

[d] Meat intake for Japanease and red meat intake for Japanese Brazilians and non-Japanese Brazilians

than premenopausal women. To clarify the effect of high isoflavone intake in detail, subjects were further categorized into 11 groups, namely non-consumers and deciles of isoflavone consumers. We found a linear decrease in breast cancer risk from zero to moderate intake (20–30 mg/day) and a leveling-off thereafter based on a conditional logistic regression model (Fig. 1). No increasing trend was found for relatively high intake.

Discussion

In these case–control studies of Japanese, Japanese Brazilians, and non-Japanese Brazilians, overall, we found an inverse association between dietary isoflavone intake and the risk of breast cancer. Our finding is in general agreement with those of a recent meta-analysis [11] and in five of the ten previous studies examining the association between isoflavone intake as estimated by FFQ and breast cancer risk [4–8]. It is noteworthy that, although several experimental studies have suggested adverse effects from soy constituents [2, 3, 13, 14], no epidemiological study estimating isoflavone intake by FFQ has reported an increased risk of breast cancer. Our study also suggests a risk-reducing rather than risk-enhancing effect of isoflavones on breast cancer within the range achievable from dietary intake alone. It remains unclear, however, whether isoflavone exposure other than dietary intake is associated with the risk of breast cancer.

We found a linear decrease in breast cancer risk from zero to moderate intake (20–30 mg/day) and thereafter a leveling-off. This dose–responses pattern might imply the

Table 2 Odds ratios (ORs) and 95% confidence intervals (CIs) of breast cancer according to dietary isoflavone intakes

	Median isoflavone intake[a]	All subjects						Premenopausal women				Postmenopausal women			
		No.		OR[b]	95% CI	OR[c]	95% CI	No.		OR[d]	95% CI	No.		OR[d]	95% CI
		Case	Control					Case	Control			Case	Control		
Japanese living in Nagano, Japan															
Tertile 1	22.1	152	129	1.00		1.00		80	67	1.00		72	62	1.00	
Tertile 2	39.6	118	131	0.75	(0.53–1.07)	0.86	(0.59–1.27)	52	44	0.99	(0.58–1.71)	66	87	0.79	(0.48–1.29)
Tertile 3	69.1	120	130	0.75	(0.52–1.10)	0.83	(0.54–1.28)	46	26	1.35	(0.72–2.54)	74	104	0.62	(0.38–1.01)
P for trend				0.12		0.39				0.41				0.06	
Japanese Brazilians living in São Paulo, Brazil															
Tertile 1	4.7	41	26	1.00		1.00									
Tertile 2	13.4	25	28	0.51	(0.23–1.15)	0.48	(0.20–1.16)								
Tertile 3	42.8	15	27	**0.35**	**(0.15–0.80)**	**0.25**	**(0.09–0.68)**								
P for trend				**0.01**		**<0.01**									
Median 1	8.0	48	40	1.00		1.00		16	10	1.00		32	30	1.00	
Median 2	35.0	33	41	0.68	(0.37–1.26)	0.52	(0.26–1.06)	9	14	**0.17**	**(0.03–0.84)**	24	27	0.84	(0.37–1.92)
Non-Japanese Brazilians living in São Paulo, Brazil															
Non-consumers	0	343	318	1.00		1.00		147	124	1.00		196	194	1.00	
Consumers	15.0	36	61	**0.54**	**(0.34–0.84)**	**0.56**	**(0.35–0.90)**	14	21	0.54	(0.26–1.13)	22	40	0.58	(0.33–1.03)

[a] Crude intake (mg/day)

[b] Crude OR

[c] Conditional model adjusting for menopausal status (premenopausal women, postmenopausal women), number of births (0, 1, 2, 3, 4, 5+), family history of breast cancer (yes, no), smoking status (never, past, current smokers), moderate physical activity in the past 5 years (no, less than 3 days/month, 1–4 days/week, more than 5 days/week), and vitamin supplement use (yes, no)

[d] Unconditional model adjusting for matching factors (age and area for Japanese; age and hospital for Japanese Brazilians; age and ethnicity for non-Japanese Brazilians), number of births (0, 1, 2, 3, 4, 5+), family history of breast cancer (yes, no), smoking status (never, past, current smokers), moderate physical activity in the past 5 years (no, less than 3 days/month, 1–4 days/week, more than 5 days/week), and vitamin supplement use (yes, no)

Bold characters indicates statistically significant values

Table 3 Odds ratios (ORs) and 95% confidence intervals (CIs) of hormone receptor-defined breast cancer according to dietary isoflavone intakes

	No. of controls	ER+/PR+			ER+/PR−			ER−/PR−			Unknown		
		No. of cases	OR[a]	95% CI	No. of cases	OR[a]	95% CI	No. of cases	OR[a]	95% CI	No. of cases	OR[a]	95% CI
Japanese living in Nagano, Japan, all subjects													
Tertile 1	129	82	1.00		23	1.00		38	1.00				
Tertile 2	131	70	0.98	(0.64–1.51)	24	1.10	(0.58–2.08)	21	0.58	(0.32–1.07)			
Tertile 3	130	67	0.97	(0.62–1.51)	22	0.71	(0.36–1.43)	28	0.71	(0.40–1.28)			
P for trend			0.89			0.35			0.23				
Japanese living in Nagano, Japan, premenopausal women													
Tertile 1	67	46	1.00		8	1.00		18	1.00				
Tertile 2	44	40	1.35	(0.74–2.46)	4	0.80	(0.22–2.89)	6	0.47	(0.17–1.32)			
Tertile 3	26	27	1.51	(0.74–3.07)	7	1.64	(0.48–5.58)	10	0.94	(0.34–2.56)			
P for trend			0.22			0.52			0.65				
Japanese living in Nagano, Japan, postmenopausal women													
Tertile 1	62	36	1.00		15	1.00		20	1.00				
Tertile 2	87	30	0.68	(0.37–1.25)	20	1.25	(0.57–2.73)	15	0.65	(0.30–1.44)			
Tertile 3	104	40	0.68	(0.38–1.22)	15	0.53	(0.22–1.26)	18	0.57	(0.27–1.22)			
P for trend			0.21			0.14			0.15				
Japanese Brazilians living in São Paulo, Brazil, all subjects													
Median 1	40	24	1.00		7	1.00		7	1.00		9	1.00	
Median 2	41	16	0.63	(0.27–1.45)	2	0.22	(0.04–1.36)	4	0.34	(0.08–1.49)	11	1.24	(0.38–4.03)
Non-Japanese Brazilians living in São Paulo, Brazil, all subjects													
Non-consumers	318	97	1.00		41	1.00		76	1.00		108	1.00	
Consumers	61	8	0.46	(0.21–1.004)	9	1.10	(0.50–2.41)	10	0.67	(0.33–1.40)	7	**0.35**	**(0.16–0.80)**

[a] Unconditional model adjusting for matching factors (age and area for Japanese; age and hospital for Japanese Brazilians; age and ethnicity for non-Japanese Brazilians), menopausal status (premenopausal women, postmenopausal women), number of births (0, 1, 2, 3, 4, 5+), family history of breast cancer (yes, no), smoking status (never, past, current smokers), moderate physical activity in past 5 years (no, less than 3 days/month, 1–4 days/week, more than 5 days/week), and vitamin supplement use (yes, no)

Bold characters indicates statistically significant values

Table 4 Odds ratios (ORs) and 95% confidence intervals (CIs) of breast cancer according to dietary isoflavone intake based on combined individual study data from three populations

	Non-consumers and quintile category among consumers						*P* for trend
	0	1	2	3	4	5	
Median isoflavone intake (mg/day)[a]	0	8.7	23.1	33.8	45.7	71.3	
Japanese living in Nagano, Japan							
No. of cases/No. of controls	0/0	49/31	93/90	89/85	72/96	87/88	
Japanese Brazilians living in São Paulo, Brazil							
No. of cases/No. of controls	9/5	46/41	16/12	3/8	1/6	6/9	
Non-Japanese Brazilians living in São Paulo, Brazil							
No. of cases/No. of controls	343/318	27/33	5/3	2/13	2/3	0/9	
All subjects in three populations							
No. of cases/No. of controls	352/323	122/105	114/105	94/106	75/105	93/106	
OR	1.00	0.69	**0.54**	**0.45**	**0.34**	**0.43**	**<0.01**
(95% CI)[b]		(0.44–1.09)	**(0.31–0.94)**	**(0.26–0.77)**	**(0.19–0.62)**	**(0.24–0.76)**	
Premenopausal women in three populations							
No. of cases/No. of controls	150/127	48/37	58/52	49/37	23/30	36/23	
OR	1.00	0.68	0.44	0.54	**0.27**	0.62	0.27
(95% CI)[c]		(0.33–1.39)	(0.19–1.01)	(0.24–1.24)	**(0.10–0.69)**	(0.25–1.54)	
Postmenopausal women in three populations							
No. of cases/No. of controls	202/196	74/68	56/53	45/69	52/75	57/83	
OR	1.00	0.70	0.52	**0.31**	**0.34**	**0.33**	**<0.01**
(95% CI)[c]		(0.40–1.24)	(0.26–1.04)	**(0.15–0.64)**	**(0.17–0.71)**	**(0.16–0.66)**	

[a] Energy adjusted by residual method

[b] Conditional model adjusting for menopausal status (premenopausal women, postmenopausal women), number of births (0, 1, 2, 3, 4, 5+), family history of breast cancer (yes, no), smoking status (never, past, current smokers), moderate physical activity in the past 5 years (no, less than 3 days/month, 1–4 days/week, more than 5 days/week), and vitamin supplement use (yes, no)

[c] Unconditional model adjusting for age (continuous), study population (Japanese living in Nagano, Japan; Japanese Brazilians living in São Paulo, Brazil; non-Japanese Brazilians living in São Paulo, Brazil), number of births (0, 1, 2, 3, 4, 5+), family history of breast cancer (yes, no), smoking status (never, past, current smokers), moderate physical activity in the past 5 years (no, less than 3 days/month, 1–4 days/week, more than 5 days/week), and vitamin supplement use (yes, no)

Bold characters indicates statistically significant values

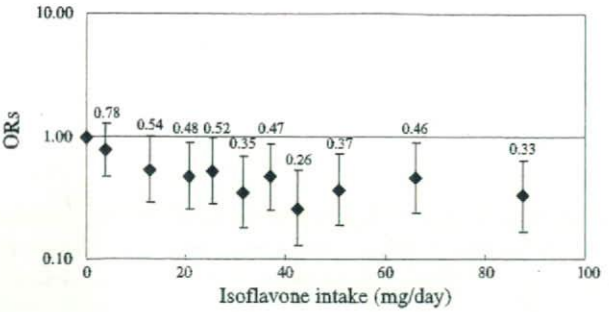

Fig. 1 Odds ratios (ORs) and 95% confidence intervals of breast cancer according to dietary isoflavone intake based on combined individual data from three populations. Subjects were categorized into 11 groups: non-consumers and deciles of isoflavone consumers based on the control distribution. ORs were estimated using matching pairs with adjustment for menopausal status (premenopausal women, postmenopausal women), number of births (0, 1, 2, 3, 4, 5+), family history of breast cancer (yes, no), smoking status (never, past, current smokers), moderate physical activity in the past 5 years (no, less than 3 days/month, 1–4 days/week, more than 5 days/week), and vitamin supplement use (yes, no)

presence of a ceiling effect and suggests that women may benefit from risk reduction if they consume at least a moderate amount of isoflavones. Alternatively, it might merely reflect differences in measurement errors due to the use of different FFQs, selection bias, and residual confounding among the three populations, notwithstanding that it clearly reflected the results of separate analyses. Specifically, consumers had lower risk than non-consumers in non-Japanese Brazilians, whose average intake of isoflavone was 4.4 mg/day among the control group; the risk of breast cancer decreased with increasing intake of isoflavone in Japanese Brazilians, whose average intake of isoflavone was 24.9 mg/day among the control group; while higher intake of isoflavone was not associated with further risk reduction in Japanese, whose average intake of isoflavone was 46.1 mg/day among the control group. Confirmation of this pattern would require further prospective cohort studies using blood or urine samples as an exposure assessment, because these could minimize the measurement errors and selection bias mentioned above.

Our stratified analysis by menopausal status using data from the three populations combined showed that an inverse association was more prominent among postmenopausal than premenopausal women. In addition, our separate analyses showed somewhat different patterns in the three populations: the inverse association was limited to postmenopausal women in Japanese; it was stronger in premenopausal than postmenopausal women in Japanese Brazilians; and no remarkable difference was found in non-Japanese Brazilians. These findings are inconsistent with a recent meta-analysis showing an inverse association regardless of menopausal status [11]. Moreover, findings to date on the association of isoflavone intake and the risk of breast cancer stratified by menopausal status have been inconsistent, with one prospective cohort study in Japan [4] and one case–control study in the United States [8] reporting that an inverse association was limited to postmenopausal women; one case–control study in Japan [5] showing it was limited to premenopausal women; and one prospective cohort study in the United States [16] and three case–control studies [6, 17, 18] finding no difference between the two strata.

Several mechanisms by which isoflavones may reduce the risk of breast cancer have been proposed [2, 3]. The most prominent and thoroughly investigated mechanisms are mediated via estrogen receptors, arising due to the similar chemical structure of isoflavones to the human estrogen hormone and their binding affinity to estrogen receptors [3, 29]. Given that the action of estrogen on breast cell proliferation appears to be mediated by estrogen receptors, therefore, any association between isoflavone intake and breast cancer risk might differ by hormone receptor-defined subtype. The present study did not support this hypothesis, however, showing no apparent difference in risk by subtype. Moreover, results for the few studies to date have been inconsistent [7, 16, 18, 19]. Although our findings might merely be explained by a lack of statistical power, they suggest that the anti-cancer effects of isoflavones might be evoked not only by mechanisms mediated by estrogen receptors but also by other mechanisms, such as the modulation of endogenous hormones via inhibition of the key enzyme involved in estrogen biosynthesis and metabolism; the arrest of cell cycle progression; induction of apoptosis; inhibition of tyrosine kinase activity, topoisomerase II activity, and angiogenesis; and antioxidant activity [2, 3].

Our study has several methodological advantages over previous studies of isoflavones and the risk of breast cancer. First, isoflavone intake differed considerably among the three populations, with median levels (interquartile rage) in the control group (mg/day) of 40.6 (25.9–61.2) among Japanese, 13.4 (8.1–35.0) among Japanese Brazilians, and 0 (0–0) among non-Japanese Brazilians. This range allowed the detailed evaluation of dose–response relations, ranging from zero to a relatively high level achievable from dietary intake only, and is unique to the present study. Second, the overall consistency of findings in the three populations allowed for the greater generalizability of results as compared to those from a single population.

Several limitations of this study warrant mention. First, dietary intake of isoflavone was assessed after the diagnosis of breast cancer and is therefore sensitive to recall bias. Second, although the substantially high participation rates among both eligible cases and controls minimized potential biases related to control selection, the use of controls from

medical checkup examinees and cancer-free patients, whose dietary habits may differ from the general population due to health consciousness or disease, might have lead to selection bias. Third, stratified analyses were performed based on a relatively small number of cases. The interpretability of our results might therefore be limited.

Allowing for these methodological issues, we found an inverse association between dietary isoflavone intake and the risk of breast cancer in case–control studies of Japanese, Japanese Brazilians, and non-Japanese Brazilians. Our findings suggest a risk-reducing rather than risk-enhancing effect of isoflavones on breast cancer within the range achievable from dietary intake alone. In addition, women may benefit from risk reduction if they consume at least moderate amounts of isoflavones.

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Current Organ Topics:

Breast and Endocrine Tumor
乳腺・内分泌 腫瘍

Ⅲ．乳がんのリスクファクター 世界のエビデンスと日本のエビデンス

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1．乳がんの動向

乳がんは世界的にみても女性の最も多いがんであり，International Agency for Research on Cancer（IARC，国際がん研究機関）の推計によると，2002年に新たに115万人が罹患している。また，女性のがんによる死亡の第1位となっており，2002年における女性乳がん死亡者数は41万人であった[1]。

欧米諸国のなかには，罹患率の増加に歯止めがかかり，死亡率の減少がみられる国もあるが，日本ではいまだに罹患率（粗罹患率，年齢調整罹患率），死亡率（粗死亡率，年齢調整死亡率）ともに増加している。地域がん登録による推計では，2002年における全国の乳がん粗罹患率は人口10万対64.4人と大腸がんに次いで2番目に高く（大腸がんを結腸がんと直腸がんに分けると乳がんが最も高い），年齢調整罹患率では，人口10万対52.2人と，乳がんが女性のがんにおいて最も高くなっている[2]。乳がんは他のがんに比べ比較的予後のよいがんであるが，人口動態統計によると，2006年の女性の乳がん死亡者数は11,177人であり，粗死亡率は人口10万対17.3人と，大腸がん，胃がん，肺がん，肝臓がんに次いで高い。また，年齢調整死亡率では人口10万対11.7人と，大腸がん，胃がん，肺がんに次いで高くなっている[3]。

年齢調整罹患率でみると，日本人の乳がんは増加傾向にあるものの，日本を含む東アジアの人々の乳がんは国際的には依然として少なく，米国白人やヨーロッパ人などに比べ罹患率は低い。また，アメリカに移住した日系人の移民の罹患率は，移住国の罹患率に近くなり，日本に住む日本人よりも高くなっている[4]。このことからも，乳がん罹患率における国際的な違いは，生活習慣など環境要因が強く影響していることが示唆される。そのため，乳がん予防において，食事や栄養，身体活動などの生活習慣が注目されてきた。そこで本稿では，乳がんのリスクファクターとしてこれら生活習慣に焦点を当て，世界と日本のエビデンスレビューを紹介する。

2．世界におけるエビデンス

世界におけるエビデンスについては，World Cancer Research Fund（WCRF，世界がん研究基金）/American Institute for Cancer Research（AICR，米国がん研究財団）が食事，栄養，身体活動に関してレビューを行っている。本稿では，2007年11月に出版された報告書“Food, Nutrition, Physical Activity and the Prevention of Cancer: a Global Perspective”[5]の内容を紹介したい。

この報告書の前に発行されたFirst reportである“Food, Nutrition and the Prevention of Cancer: a Global Perspective”はがんと食事に関する疫学研究を中心に論文のレビューを行い，がんを予防するための食事の勧告を全世界に向けて策定し，発信した。このなかで特に野菜や果物ががん予防にはきわめて重要であることが示されている。First reportは1997年に発行されて以来10年の間，がんと食事に関する最も権威と影響力のある報告とされてきた。また，政府関係者や医療従事者，研究者などの標準的なテキストとして，広く世界中で用いられてきた。

この分野における論文が劇的に増加したことや，エビデンスの分析や評価における新たな方法が開発されたことにともない，Second reportであるこの報告書が作成された。この報告書では，食事や栄養に加えて，前回には取り上げられなかった体格や身体活動についても検討が行われ，過体重や肥満，身体活動量の少なさががんのリスクファクターとなることが強調されている。

報告書の作成にあたっては，評価の客観性と透明性を最大限にするために，エビデンスの収集と，評価および判定とを分けて行った。具体的なプロセスは下記のとおりである。1）専門家委員会により，膨大な科学論文のシステマティックレビューを行う方法を作成，2）作成された方法論に基づき，リサーチチームが文献の収集とレビューを行う，3）専門家パネルがエビデンスと推奨の評価と判定を行う。

構成は，まず要約（Summary）に全体像が述べられており，Part 1の背景（Background）では，がんに関する統計や国際比較，エビデンスの判定などについて書かれている。Part 2のエビデンスと判定（Evidence and

表 1 WCRF/AICR による食事，栄養，身体活動と乳がんとの関連（閉経前）

	リスクを減少させるもの	リスクを上昇させるもの
Convincing（確実）	授乳	アルコール摂取
Probable（ほぼ確実）	体脂肪（肥満）	成人期の身長[a] 出生時体重の大きさ
Limited-suggestive（可能性あり）	身体活動[b]	
Limited-no conclusion（証拠不十分）	穀類と穀類製品，食物繊維，芋類，野菜，果物，マメ科の植物（マメ類），大豆と大豆製品，肉，鶏肉，魚，卵，牛乳と乳製品，脂質，総食物脂肪，植物性脂肪，脂肪酸組成，トランス脂肪酸，コレステロール，砂糖（スクロース），その他の糖類，糖類を含む食品と飲み物，コーヒー，紅茶，炭水化物，でんぷん，グリセミックインデックス（GI），プロテイン，ビタミン A，リボフラビン，ビタミン B6，葉酸，ビタミン B12，ビタミン C，ビタミン D，ビタミン E，カルシウム，鉄分，セレン，カロテノイド，イソフラボン，ジクロロジフェニルジクロロエチレン（DDE），ジヒドロジフェニルトリクロロエタン（DDT），ディルドリン，ヘキサクロロベンゼン，ヘキサクロロシクロヘキサン，トランス-ノナクロル，ポリ塩化ビフェニール類（PCB），食事パターン，文化的に規定される食事，成人後の体重の増加，エネルギー摂取，母乳で育てられること	
Substantial effects on risk unlikely（大きな関連なし）	特定されるものはない	

a）成人期の身長はがんのリスクに直接影響するものではおそらくない。成人期の身長は，受胎前から成人までの成長期間における，成長に影響する遺伝的，環境的，ホルモン的，栄養的な要因のマーカーである。
b）すべての身体活動: 仕事，家事，移動，余暇

出典: World Cancer Research Fund/American Institute for Cancer Research. Food, Nutrition, Physical Activity and the Prevention of Cancer: a Global Perspective. http://www.dietandcancerreport.org/（Accessed October 12, 2008）

Judgments）では，食事，飲酒，運動，体型，肥満などのリスクやがんの部位別のリスク，がんサバイバーの予後などについて述べられている。Part 3 の推奨（Recommendations）では，公衆衛生上の目標や推奨について述べられている。

がんサバイバーについても，研究が行われ始めているが，エビデンスの判定に十分な結果は得られていない。しかし，定期的な身体活動などにより体重を維持することは，少なくとも乳がんにとっては再発を防ぐ可能性もあり，また一般的な健康にもよいと記載されている。

報告書では最後に，レビュー結果に基づき，がん予防に有用な 10 の推奨事項が示されている。

推奨 1 体脂肪: 適正体重の範囲内で，体重をできるだけ少なめに維持する

推奨 2 身体活動: 毎日の生活の中で身体活動を活発に行う

推奨 3 体重増加を促進する食品と飲料: エネルギー密度の高い食品の摂取を制限し，砂糖入り飲料を避ける

推奨 4 植物性食品: 植物性食品を多く摂取する

推奨 5 動物性食品: 赤身肉の摂取を制限し，加工肉製品は避ける

推奨 6 アルコール飲料: アルコール飲料を制限する

推奨 7 保存，加工，調理: 食塩の摂取を減らす，カビの生えた穀類や豆類は避ける

推奨 8 サプリメント: 必要な栄養素は食事のみから摂取する

推奨 9 授乳: 母親が授乳を行うことも，子どもが母乳で育てられることも重要である

推奨 10 がんサバイバー: がんサバイバーはがん予防のための推奨に従う

以下では，報告書のなかで乳がんのリスクファクターについて具体的に取り上げている部分を紹介する。

乳がんはホルモン関連がんであり，閉経前に診断される場合と閉経後に診断される場合（閉経後に診断される方が多い）で，リスクファクターは同じではない。そのため，リスクファクターに関しては閉経前乳がん，閉経後乳がんに分けられている。

パネルの判定結果のまとめを，閉経前乳がんについては表 1，閉経後乳がんについては表 2 に示す。

1）ホルモン関連

授乳が乳がんリスクを低減することは，閉経前後を問

表2　WCRF/AICRによる食事，栄養，身体活動と乳がん（閉経後）

	リスクを減少させるもの	リスクを上昇させるもの
Convincing （確実）	授乳	アルコール摂取 体脂肪（肥満） 成人期の身長[a]
Probable （ほぼ確実）	身体活動[b]	腹部の脂肪 成人期の体重の増加
Limited-suggestive （可能性あり）		総食物脂肪
Limited-no conclusion （証拠不十分）	穀類と穀類製品，食物繊維，芋類，野菜，果物，マメ科の食物（マメ類），大豆と大豆製品，肉，鶏肉，魚，卵，牛乳と乳製品，脂質，植物性脂肪，脂肪酸組成，コレステロール，砂糖（スクロース），糖類を含む食品と飲み物，コーヒー，紅茶，炭水化物，でんぷん，グリセミックインデックス（GI），プロテイン，ビタミンA，リボフラビン，ビタミンB6，葉酸，ビタミンB12，ビタミンC，ビタミンD，ビタミンE，カルシウム，鉄分，セレン，カロテノイド，イソフラボン，ジクロロジフェニルジクロロエチレン（DDE），ジヒドロジフェニルトリクロロエタン（DDT），ディルドリン，ヘキサクロロベンゼン，ヘキサクロロシクロヘキサン，トランス-ノナクロル，ポリ塩化ビフェニール類（PCB），食事パターン，文化的に規定される食事，出生時体重，出生時身長，エネルギー摂取，母乳で育てられること	
Substantial effects on risk unlikely （大きな関連なし）	特定されるものはない	

a）成人期の身長はがんのリスクに直接影響するものではおそらくない。成人期の身長は，受胎前から成人までの成長期間における，成長に影響する遺伝的，環境的，ホルモン的，栄養的な要因のマーカーである。
b）すべての身体活動: 仕事，家事，移動，余暇

出典: World Cancer Research Fund/American Institute for Cancer Research.
Food, Nutrition, Physical Activity and the Prevention of Cancer: a Global Perspective.
http://www.dietandcancerreport.org/（Accessed October 12, 2008）

わず，すべての年齢において「確実（Convincing）」である。

2）食事・栄養

食事に関して確立されたリスクファクターは，現在のところアルコール摂取のみである。アルコール摂取が乳がんのリスクファクターであることは，閉経前後を問わずすべての年齢において「確実」である。穀類や食物繊維，野菜と果物，大豆，乳製品，脂質，糖類，ビタミン，イソフラボンなどが注目され，乳がんとの関連が検討されてきたが，これらに関しては「証拠不十分（Limited-no conclusion）」である。総食物脂肪については，閉経後乳がんのリスクであることが「可能性あり」とされている。

3）体　　格

成人期の身長の高さが乳がんのリスクになることは，閉経後乳がんにおいては「確実」，閉経前乳がんにおいても「ほぼ確実（Probable）」である。

出生時の高体重が，閉経前乳がんのリスクであることは「ほぼ確実」である。

体脂肪（肥満）が閉経後乳がんのリスクであることは「確実」であり，腹部の脂肪も「ほぼ確実」である。一方で，体脂肪（肥満）が，閉経前乳がんにおいては予防的な効果をもつことも「ほぼ確実」である。

4）身体活動

身体活動量が多いと乳がんリスクが低減することは，閉経後乳がんに対しては「ほぼ確実」であり，閉経前乳がんに対しては「可能性あり（Limited-suggestive）」である。身体活動については，月経・排卵周期や内因性ホルモンレベルへの影響を介したメカニズムも考えられているが，身体活動を行うことによって体脂肪の増加や体重の増加，閉経後の肥満が予防され，結果として乳がんのリスク低下に関連していると考えられている。

5）そ の 他

報告書では，食事，栄養，身体活動が中心となっているため，エビデンスレベルの判定が行われていないリスクファクターが存在する。それらについては，表として示されていないが，本文中には，その他の確立されたリスクファクターとして紹介されている。これらについては，閉経前乳がんと閉経後乳がんは分けられていない。

①遺伝的素因

乳がんの4～9%は遺伝性であり，BRCA1またはBRCA2遺伝子の生殖細胞性変異によって生じている。