

## Plasma Isoflavones and the Risk of Lung Cancer in Women: A Nested Case–Control Study in Japan

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### Abstract

**Background:** Although several epidemiologic studies have found that isoflavone intake assessed by questionnaire is associated with a decreased risk of lung cancer, no prospective study has investigated this association using blood concentrations of isoflavones.

**Methods:** We conducted a nested case–control study within a population-based prospective cohort study. A total of 24,127 women aged 40 to 69 years who returned the baseline questionnaire and provided blood samples were observed from 1990 through 2006. During a median follow-up period of 13.5 years, 126 newly diagnosed lung cancer cases were identified. For each case, we selected two controls matched for age, area, smoking status, and condition of blood draw. A conditional logistic regression model was used to estimate the odds ratios (ORs) and 95% CIs of lung cancer in relation to plasma concentrations of genistein, daidzein, glycitein, equol, and total isoflavones.

**Results:** After exclusion of 20 lung cancer cases diagnosed in the first 3 years after blood collection, an inverse association was found between plasma genistein concentration and lung cancer risk. The multivariate-adjusted OR (95% CI) of lung cancer in the highest quintile of plasma genistein concentration as compared with that in the lowest quintile was 0.31 (0.12, 0.86; *P* for trend = 0.085). Other isoflavones and total isoflavones were not associated with a significant decrease in the risk of lung cancer.

**Conclusion:** Plasma genistein concentration was inversely associated with lung cancer risk in Japanese women.

**Impact:** Our data support the previously observed association between isoflavone intake and lung cancer risk. *Cancer Epidemiol Biomarkers Prev*; 20(3); 419–27. ©2011 AACR.

### Introduction

Isoflavones, including genistein, daidzein, and glycitein, are found mainly in soy and soy products in Asian diets. They are similar in structure to the human female hormone 17- $\beta$  estradiol. They are also similar in function, as they have a high affinity for the beta-estrogen receptor (1) and act as estrogen agonists and antagonists

(2). Therefore, it has been hypothesized that isoflavones protect against the development of cancers related to sex hormones. Indeed, epidemiologic studies have shown an inverse association between isoflavones and the risks of breast (3–5) and prostate cancers (6–8).

In addition to these cancers, it has been suggested that estrogen has a role in lung carcinogenesis (9). Estrogen receptors are expressed in healthy lung tissue and in lung tumors (10), and estrogen induces proliferation of non-small-cell lung cancer (NSCLC) cells (11). Furthermore, randomized controlled trials have indicated that hormone replacement therapy which includes estrogens may increase lung cancer risk in women (12, 13). Thus, isoflavones may be related to the risk of lung cancer, in addition to other hormone-related cancers.

Although several *in vitro* and *in vivo* studies have shown a protective effect of genistein on lung carcinogenesis (14–16), epidemiologic studies have produced conflicting results regarding the association between lung cancer risk and isoflavone intake assessed by food frequency questionnaire (FFQ; ref. 17–20). Notably, 2 recent prospective studies in Asia observed an inverse association in never smokers (19, 20). Epidemiologic

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**Note:** Study group members are listed in the Appendix.

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studies using blood concentrations of isoflavones might clarify the association with lung cancer risk, because the concentration of isoflavone in blood reflects individual differences in absorption and metabolism, in which intestinal microflora have an important role (21). In particular, due most likely to differences in intestinal bacteria, only 30% to 50% of adults have the capacity to metabolize daidzein into equol, which is known to have stronger estrogenic activity than daidzein (22).

Here, in a nested case-control study within a large-scale, population-based, prospective study, we investigated the association between plasma isoflavone concentration and lung cancer risk among a population of Japanese women that varied substantially in isoflavone intake (3) and had a high prevalence of never smokers (23).

## Materials and Methods

### Study population

The Japan Public Health Center-based Prospective Study was launched in 1990–1994. The study population was defined as all Japanese inhabitants who had registered their address in administrative districts (city, town, or village) supervised by the 11 public health center (PHC) and were aged 40 to 69 years at the start of the baseline survey (24). Study participants were informed of the objectives and methods of the study in writing, and those who responded to the survey questionnaire and donated blood were regarded as having given informed consent to participate in the study. In addition, participants were notified that they could withdraw from the study at any time. Our study protocol follows the current ethical guidelines for epidemiological research in Japan (25). The study protocol was approved by the Institutional Review Board of the National Cancer Center, Japan.

For the present analysis we excluded one PHC area because data on cancer incidence were not available. After exclusion of ineligible participants ( $n = 144$ ), we identified 67,522 women as the cohort.

### Questionnaire survey

We distributed a baseline self-administered questionnaire survey on various health habits, including personal medical history, menstrual and reproductive history, anthropometric factors, smoking history, and other lifestyle factors in 1990 for Cohort I and in 1993 to 1994 for Cohort II. Women who reported first-degree relatives with lung cancer were considered to have a family history of lung cancer. Never smoking status was determined by answers to the question "Have you ever smoked cigarettes?" in Cohort I and by answers to the question "Are you currently smoking cigarettes?" and information on past history of smoking in Cohort II. Questions regarding age at initiation of smoking and average number of cigarettes smoked per day were also included. Information on passive smoking at the workplace was collected

using a question with 4 frequency categories: almost never, 1–3 days/month, 1–4 days/week, and almost daily. We defined women who drank alcoholic beverages less than 1 day/month as nondrinkers. Women who reported past or current use of female hormone drugs were classified as past or current exogenous female hormone users. We had no information on the type, duration, or dosage of such use.

The questionnaire survey also included validated FFQs that asked about average intake during the previous month of 44 food items (for Cohort I) or 52 food items (for Cohort II). The questionnaires had 6 frequency categories for beverages, ranging from 'rarely' to '5 glasses per day', and 4 (Cohort I) or 5 (Cohort II) categories for other items, ranging from 'never' or 'rarely' to 'almost daily'. The intakes of total energy, vegetables, fruit, and fish were calculated from these responses (26, 27), and portion sizes were estimated using data from a validation study (28).

Each questionnaire included 3 food items that contained genistein. In Cohort I, the percentages of women reporting 'almost daily' consumption of (i) miso soup, (ii) soybeans, tofu, deep-fried tofu, and natto (fermented soybeans), and (iii) vegetables other than yellow and green vegetables (e.g., Chinese cabbage, radish, tomato, and cucumber) were 79.4%, 52.2%, and 46.7%, respectively. In Cohort II, miso soup, tofu, and natto were consumed almost daily by 63.2%, 32.5%, and 9.5% of women, respectively.

A total of 55,842 women responded to the questionnaire, yielding a response rate of 83%. We then excluded 585 participants with incomplete information on smoking status and 1,525 participants who had received a diagnosis of cancer before the baseline questionnaire survey. Ultimately, a total of 53,732 women were eligible.

### Blood collection

Participants voluntarily provided 10 mL of blood during health checkups in 1990–1995. Blood samples were divided into plasma and buffy layers and preserved at  $-80^{\circ}\text{C}$  until analysis. Among the eligible participants, a total of 24,127 women (96.7% of participants in health checkups) donated blood.

### Follow-up

We followed study participants until December 31, 2006. Participants who died or moved to other municipalities were identified annually through residential registers in the respective PHC areas. Cause of death was confirmed using mortality data from the Ministry of Health, Labour and Welfare. Among the study participants ( $n = 24,127$ ), 1,160 (4.8%) died, 1,559 (6.5%) moved away, and 51 (0.2%) were lost to follow-up during the study period.

### Selection of cases and controls

We determined lung cancer incidence by using voluntary reports from local major hospitals in the study areas

and data linkage with population-based cancer registries, after obtaining permission. We used death certificate information as a supplementary information source. In our cancer registry system, the proportion of cases for which information was obtained only from death certificates was 5.1% during the study period. During the time from blood collection to the end of the study period, we identified 126 newly diagnosed lung cancer cases.

The site of origin and histologic type were coded using the International Classification of Diseases for Oncology, Third Edition (C34.0-C34.9; ref. 29). Diagnosis of lung cancer was confirmed by histologic or cytologic examination in 89% of cases ( $n = 112$ ), and was based on clinical findings or unspecified evidence in the remaining 11%. Histologic type was classified as adenocarcinoma ( $n = 94$ ; 75%), squamous cell carcinoma ( $n = 6$ ), large cell carcinoma ( $n = 4$ ), small cell carcinoma ( $n = 3$ ), or other histologic types ( $n = 5$ ), according to the World Health Organization histological classification of lung tumors (30).

For each case, 2 controls were selected at random from participants with no history of lung cancer when the case was diagnosed. Controls were matched for each case by age (within 3 years), PHC area, area (city, or town and village), date on which blood was collected (within 60 days), time of day of blood collection (within 3 hours), duration of fasting at blood collection (within 3 hours), and smoking status (never, past, and current).

### Laboratory assays

Plasma concentrations of isoflavones (i.e., genistein, daidzein, glycitein, and equol) were analyzed using triple-quadrupole tandem liquid chromatography–mass spectrometry (31). Beta-glucuronidase/sulfatase was added to 0.1 mL of plasma. The aglycones of the isoflavones and their metabolites were recovered by diethyl ether extraction. The diethyl ether extract of the sample was dried under nitrogen flow and redissolved in acetonitrile. The ionizing method was electrospray using negative ions; multiple reaction monitoring was used for mass analysis.

To assure quality control (QC), the precision of laboratory measurement was assessed before and after each assay using a pooled blood sample from healthy volunteers. Based on 20 replicated measurements of the QC sample at a mean concentration of 122.1 ng/mL for genistein, 88.0 ng/mL for daidzein, 10.6 ng/mL for glycitein, and 39.6 ng/mL for equol, the coefficients of variation were 3.0% or less for intraday variation and 3.9% or less for interday variation. Cases and matched controls were assayed in the same batch. Detection limits were less than 1.0 ng/mL for all isoflavones. All samples were analyzed at a single laboratory (SRL, Tokyo, Japan) while blinded to case–control status.

### Statistical analysis

Baseline characteristics between cases and controls were evaluated by the Mantel–Haenszel procedure with

matched-set strata (32). For genistein and daidzein, study participants were classified into quintiles according to plasma concentration. For glycitein and equol, the lowest category comprised study participants with amounts below the detection limit (<1.0 ng/mL), and those with detectable concentrations were divided into quartiles. Total isoflavones was defined as the sum of genistein, daidzein, glycitein, and equol concentrations and was classified by quintile of plasma concentration. Glycitein and equol concentrations below the detection limit were regarded as zero in the calculation of total isoflavones. Cutoff points for plasma isoflavone concentration were based on the control distribution.

We used a conditional logistic regression model to estimate odds ratios (OR) and 95% CIs of lung cancer risk by category of plasma isoflavones and to adjust for potentially confounding variables. Dummy variables were created for the categories of plasma isoflavone concentration, and the lowest category was used as the reference category. We calculated *P* values for the analysis of linear trends by assigning ordinal values for categories of plasma isoflavone concentration and entering the number as a continuous term in the regression model. All reported *P* values are 2-tailed. All statistical analyses were performed using SAS statistical software, version 9.1 (SAS Institute Inc; ref. 33).

Multivariate-adjusted ORs were adjusted for family history of lung cancer (yes or no), pack-years of smoking among current smokers (1–19 or  $\geq 20$  pack-years, as defined by multiplying the years of smoking by the average number of cigarettes per day and dividing by 20), passive smoke exposure at work ( $\leq 1$ –3 days/month, 1–4 days/week, or almost daily), past or current use of exogenous female hormones (yes or no), and fruit and vegetable intake (continuous variable). All analyses were repeated after excluding participants who received a diagnosis of lung cancer within 3 years of blood collection ( $n = 20$ ).

### Results

The characteristics of cases and controls are shown in Table 1. The prevalence of never smokers among both cases and control was 92.9% ( $n = 117$  and  $n = 234$ , respectively). We found no significant differences in the characteristics of cases and controls. Table 2 shows plasma isoflavone concentrations in cases and controls. The median plasma concentrations of genistein, daidzein, glycitein, equol, and total isoflavones in cases were all slightly lower than those in controls; however, the differences were not statistically significant.

Table 3 shows the associations between plasma isoflavone concentrations and risk of lung cancer. After adjustment for potential confounders, there was a U-shaped association between plasma isoflavone concentrations and lung cancer risk. However, after exclusion of the 20 lung cancer cases diagnosed in the first

**Table 1.** Baseline characteristics of cases and controls

Characteristic	Cases (n = 126)	Controls (n = 252)	P <sup>a</sup>
Age, mean (SD), y	57.3 (7.4)	57.0 (7.3)	-
Family history of lung cancer, n (%)	4 (3.2)	3 (1.2)	0.18
Never smokers, n (%)	117 (92.9)	234 (92.9)	-
Current smokers, n (%)	8 (0.1)	16 (0.1)	-
1–19 pack years, n (%) <sup>b</sup>	5 (31.3)	5 (62.5)	0.15
Passive smoke exposure, almost daily, n (%)	24 (19.5)	46 (18.5)	0.41
Nondrinkers, n (%)	98 (78.4)	209 (82.9)	0.33
Postmenopausal status, n (%)	101 (82.8)	200 (82.0)	0.39
Age at menarche, mean (SD), y <sup>c</sup>	15.8 (2.0)	15.9 (2.1)	0.34
Age at menopause, mean (SD), y <sup>c</sup>	48.7 (4.5)	49.3 (4.0)	0.56
Past or current use of exogenous female hormones, n (%)	19 (17.1)	26 (11.8)	0.21
Dietary intake <sup>d</sup>			
Total energy, mean (SE), kcal/d	1278 (37.5)	1249 (26.5)	0.49
Vegetables, mean (SE), g/d	118 (6.6)	119 (4.6)	0.98
Fruit, mean (SE), g/d	102 (8.7)	106 (6.2)	0.70
Fish, mean (SE), g/d	44 (2.6)	49 (18)	0.14

<sup>a</sup>P value on Mantel-Haenszel test with matched-set strata.

<sup>b</sup>Among current smoking women.

<sup>c</sup>Among postmenopausal women.

<sup>d</sup>Adjusted for cohort.

3 years after blood collection, we found an inverse association between plasma genistein concentration and lung cancer risk. After adjustment for family history of lung cancer, pack-years of smoking among current smokers, passive smoke exposure at work, past or current use of exogenous female hormones, and fruit and vegetable intake, the multivariate-adjusted ORs (95% CIs) of lung cancer across increasing quintiles of plasma genistein, with the lowest quintile as reference, were 1.00, 0.27 (0.10, 0.75), 0.21 (0.08, 0.59), 0.24 (0.08, 0.71), and 0.31 (0.12, 0.86) (*P* for trend = 0.085). For

daidzein, glycitein, and total isoflavones, the ORs of lung cancer were also below unity, but were not statistically significant. We found no association between plasma equol concentration and lung cancer risk, even after lung cancer cases in the first 3 years after blood collection were excluded.

For the purpose of sensitivity analysis, we included additional variables in the model, namely, menopausal status (premenopausal or postmenopausal), ages at menarche (<16 or ≥16 years) and menopause (≤50 or >50 years) among postmenopausal women, and

**Table 2.** Plasma isoflavone concentrations in cases and controls

Isoflavone	Cases (n = 126)		Controls (n = 252)		P <sup>a</sup>
	Median, ng/mL	IQR	Median, ng/mL	IQR	
Genistein	72.0	(25.4–163.1)	72.4	(29.8–127.0)	0.84
Daidzein	29.3	(9.9–66.3)	31.8	(11.6–61.4)	0.82
Glycitein <sup>b</sup>	1.8	(0–4.1)	2.1	(0–4.1)	0.34
Equol <sup>b</sup>	2.8	(0–20.2)	3.5	(0–15.4)	0.78
Total isoflavones <sup>c</sup>	124.7	(42.2–267.1)	126.4	(51.8–214.6)	0.82

Abbreviation: IQR, interquartile range.

<sup>a</sup>P value on Mantel-Haenszel test with matched-set strata.

<sup>b</sup>Values below the detection limit (<1 ng/ml) were regarded as zero.

<sup>c</sup>Total isoflavones is the sum of genistein, daidzein, glycitein, and equol concentrations.

**Table 3.** ORs and 95% CIs of lung cancer, by plasma isoflavone concentration<sup>a</sup>

Plasma concentration	Quintile of Plasma Isoflavone Concentration <sup>b</sup>					P for trend
	Q1 (lowest)	Q2	Q3	Q4	Q5 (highest)	
<b>Genistein, ng/mL</b>	<24.8	24.8–52.3	52.4–88.7	88.8–151.2	>151.2	
No. of cases	34	21	19	17	35	
No. of controls	50	51	50	51	50	
OR1 (95% CI) <sup>c</sup>	1.00 (Reference)	0.51 (0.25, 1.07)	0.47 (0.23, 0.99)	0.43 (0.20, 0.93)	0.88 (0.45, 1.74)	0.915
OR2 (95% CI) <sup>d</sup>	1.00 (Reference)	0.40 (0.17, 0.94)	0.36 (0.15, 0.86)	0.36 (0.14, 0.93)	0.68 (0.30, 1.53)	0.700
OR3 (95% CI) <sup>e</sup>	1.00 (Reference)	0.27 (0.10, 0.75)	0.21 (0.08, 0.59)	0.24 (0.08, 0.71)	0.31 (0.12, 0.86)	0.085
<b>Daidzein, ng/mL</b>	<8.3	8.3–21.7	21.8–40.7	40.8–72.2	>72.2	
No. of cases	31	24	22	19	30	
No. of controls	50	51	50	51	50	
OR1 (95% CI) <sup>c</sup>	1.00 (Reference)	0.71 (0.35, 1.40)	0.68 (0.34, 1.34)	0.57 (0.27, 1.18)	0.94 (0.47, 1.86)	0.709
OR2 (95% CI) <sup>d</sup>	1.00 (Reference)	0.81 (0.37, 1.76)	0.84 (0.39, 1.82)	0.56 (0.23, 1.36)	1.03 (0.46, 2.29)	0.874
OR3 (95% CI) <sup>e</sup>	1.00 (Reference)	0.79 (0.34, 1.86)	0.56 (0.23, 1.36)	0.35 (0.13, 0.97)	0.73 (0.29, 1.82)	0.258
<b>Glycitein, ng/mL</b>	<1.0	1.0–1.9	2.0–3.0	3.1–5.4	>5.4	
No. of cases	48	15	22	22	19	
No. of controls	82	42	41	45	42	
OR1 (95% CI) <sup>c</sup>	1.00 (Reference)	0.59 (0.29, 1.20)	0.90 (0.48, 1.67)	0.80 (0.42, 1.51)	0.74 (0.36, 1.49)	0.513
OR2 (95% CI) <sup>d</sup>	1.00 (Reference)	0.42 (0.19, 0.95)	0.94 (0.47, 1.88)	0.79 (0.35, 1.79)	0.72 (0.32, 1.64)	0.601
OR3 (95% CI) <sup>e</sup>	1.00 (Reference)	0.42 (0.18, 1.03)	0.77 (0.36, 1.63)	0.44 (0.17, 1.19)	0.52 (0.21, 1.31)	0.147
<b>Equol, ng/mL</b>	<1.0	1.0–4.3	4.4–12.1	12.2–26.7	>26.8	
No. of cases	53	15	14	23	21	
No. of controls	99	38	39	38	38	
OR1 (95% CI) <sup>c</sup>	1.00 (Reference)	0.73 (0.36, 1.48)	0.67 (0.33, 1.37)	1.13 (0.61, 2.11)	1.03 (0.53, 2.00)	0.796
OR2 (95% CI) <sup>d</sup>	1.00 (Reference)	0.73 (0.32, 1.66)	0.82 (0.35, 1.94)	0.94 (0.44, 2.01)	1.08 (0.51, 2.31)	0.845
OR3 (95% CI) <sup>e</sup>	1.00 (Reference)	0.86 (0.35, 2.12)	0.78 (0.32, 1.92)	0.97 (0.43, 2.20)	1.07 (0.47, 2.44)	0.889
<b>Total isoflavones, ng/mL</b>	<42.1	42.1–86.0	86.1–148.1	148.2–257.1	>257.1	
No. of cases	30	20	21	20	35	
No. of controls	50	51	50	51	38	
OR1 (95% CI) <sup>c</sup>	1.00 (Reference)	0.57 (0.26, 1.25)	0.63 (0.31, 1.30)	0.56 (0.25, 1.25)	1.06 (0.52, 2.17)	0.590
OR2 (95% CI) <sup>d</sup>	1.00 (Reference)	0.59 (0.24, 1.49)	0.61 (0.26, 1.41)	0.58 (0.22, 1.57)	0.95 (0.41, 2.20)	0.729
OR3 (95% CI) <sup>e</sup>	1.00 (Reference)	0.46 (0.16, 1.34)	0.43 (0.16, 1.12)	0.41 (0.13, 1.29)	0.55 (0.20, 1.49)	0.442

<sup>a</sup>A conditional logistic regression model was used to estimate ORs and 95% CIs.

<sup>b</sup>For genistein and daidzein, study participants were classified into quintiles according to plasma concentration. For glycitein and equol, the lowest category (Q1) comprised study participants with concentrations below the detection limit (<1.0 ng/mL); those with detectable concentrations were divided into quartiles.

<sup>c</sup>Matched variables were age, public health center area, geographic area (city, or town and village), date on which blood was collected, time of day of blood collection, duration of fasting at blood collection, and smoking status.

<sup>d</sup>OR2 was adjusted for family history of lung cancer (yes or no), pack-years of smoking among current smokers (1–19 or >20 pack-years, defined by multiplying the years of smoking by the average number of cigarettes per day and dividing by 20), passive smoke exposure at work (<1–3 days/month, 1–4 days/week, or almost daily), past or current use of exogenous female hormones (yes or no), and fruit and vegetable intake (continuous variable).

<sup>e</sup>OR3 was adjusted for the same variables as OR2, after exclusion of lung cancer cases diagnosed in the first 3 years after blood collection.

fish intake. The results were similar (data not shown). The findings were also similar when the analysis was limited to never smokers: after exclusion of lung cancer cases in the first 3 years, the multivariate-

adjusted OR (95% CI) for the highest quintile of genistein concentration versus the lowest quintile was 0.36 (0.13–0.98; *P* for trend = 0.151), when the analysis was restricted to women who provided a fasting



blood sample (i.e., 6 or more hours after a meal), and when only participants with lung adenocarcinoma or NSCLC (adenocarcinoma, squamous cell carcinoma, or large cell carcinoma) were defined as cases (data not shown).

## Discussion

In this nested case-control study within a large-scale, population-based, prospective study of Japanese women, we found that plasma concentrations of genistein, but not daidzein, glycitein, equol, or total isoflavones, were associated with a significant decrease in lung cancer risk after exclusion of lung cancer cases diagnosed within 3 years of blood collection. At the time of blood collection, participants who later developed early lung cancer might have had preclinical lung cancer, which could have changed their dietary behavior. Also, if participants with preclinical lung cancer were more likely due to ill health to have health checkups than the apparently healthy population at the baseline, they would be more likely to be cases. If indeed this occurred, any association would be distorted. We consider that the results obtained after excluding these early lung cancer cases suggest a preventive effect of genistein on lung cancer incidence. To our knowledge, this is the first study to investigate the association between plasma isoflavone concentrations and lung cancer risk.

We did not find a dose-response relationship between plasma genistein concentration and lung cancer risk, as lung cancer risk remained constant across the second through the fifth quintiles of plasma genistein concentration. Although we cannot characterize the shape of the exposure-disease relation because of the limited number of cases, the results suggest that a low genistein concentration is important in lung carcinogenesis. However, further study of a larger number of lung cancer cases is needed to confirm this hypothesis.

We observed an inverse association only for genistein. If isoflavones have an effect via estrogen-dependent mechanisms, this inverse association with genistein is plausible, as it has been reported to have greater estrogenic activity (34–36) than daidzein. Reports have shown that equol has even higher estrogenic activity than genistein (34, 36); however, the median plasma concentration of genistein in controls was 2.3 to 34.5 times that of other isoflavones, including equol (Table 2), which may explain why we failed to detect an association with isoflavones other than genistein.

In addition to the estrogen receptor-mediated mechanism, we speculate that a mechanism mediated by the epidermal growth factor receptor (EGFR) may be involved. The EGFR mediates signals related to increased cell proliferation and inhibition of apoptosis (37). While mutations in the *EGFR* gene activate the EGFR pathway (38), NSCLC with mutated *EGFR*

is highly responsive to gefinitib, an EGFR protein-tyrosine kinase (PTK) inhibitor (39). Interestingly, genistein is reported to be a PTK inhibitor, based on the fact that it inhibited EGFR PTK activity *in vitro* (40). Genistein inhibited growth of NSCLC cell lines, particularly one with mutated *EGFR* (16). Furthermore, a case-control study in Japan found that soy food intake was inversely associated with *EGFR*-mutated NSCLC only (41). Although information was not available on the *EGFR* status of lung cancer in our study, the present participants had characteristics similar to those associated with the *EGFR* mutation, that is, never-smoking status, East Asian ethnicity, and female sex (42). Genistein might exert its preventive effect on lung cancer through the EGFR-mediated mechanism.

Only 2 prospective studies have examined the association between isoflavone intake and lung cancer risk in Asian countries, where isoflavone intake is higher than in Western countries. We previously reported an association between isoflavone intake and lung cancer risk, using data from a 5-year follow-up questionnaire in our cohort (20). In that study, we found a nonsignificant inverse association between isoflavone intake (determined by using genistein intake) and lung cancer risk in women (hazard ratio for the highest vs. lowest quartile of intake: 0.83; 95% CI: 0.54, 1.29; *P* for trend = 0.409). In the Singapore Chinese Health Study, Seow and colleagues reported an inverse association between isoflavone intake and overall risk of lung cancer in nonsmoking women: the multivariate-adjusted hazard ratio for lung cancer incidence in the highest versus the lowest quartile of isoflavone intake was 0.59 (95% CI: 0.38, 0.91) (19). These findings conform to those of the current study.

The limitations of this study warrant mention. First, we used a single measurement of plasma isoflavone concentration, which may be subject to day-to-day and diurnal variation. However, in a validation study using a subsample of the cohort, high reproducibility of genistein intake was observed: the correlation coefficients for FFQ estimates separated by 1 year and 5 years were 0.72 (43) and 0.61 (28), respectively. Furthermore, our validation study yielded satisfactorily high correlation coefficients for genistein estimates from dietary records (DR) measured repeatedly for a year, a fasting serum sample, and a single FFQ (DR vs. serum: 0.33; DR vs. FFQ: 0.59; ref. 43). As isoflavone intake was likely to have been stable for a long period in this population, we consider it unlikely that day-to-day variation in plasma isoflavone concentrations substantially distorted the association between plasma isoflavone concentration and lung cancer risk. Because of the half-life of genistein and daidzein in blood (7.7 to 9.5 hours; ref. 44), plasma concentrations of isoflavones vary with regard to fasting time. To minimize attenuation in risk estimation due to diurnal variation, fasting time was matched in cases and controls. A second

limitation of the study was that, due to the relatively small number of lung cancer cases among ever smokers ( $n = 9$ ), it was not possible to conduct stratified analyses by smoking status. Third, the current nested case-control study was conducted among health check-up participants, a subsample of the entire cohort that had different background characteristics from nonparticipants in health check-ups (45). Female participants in health check-ups had a favorable lifestyle profile. As compared with nonparticipants, they smoked and drank less, but tended to eat fruit and green vegetables more often and to participate more in sports and physical exercise in their leisure time. Thus, the associations between plasma isoflavone concentrations and lung cancer risk could differ from those of the entire cohort. However, because there was a similar inverse association between lung cancer and isoflavone in both the current participants and the entire cohort (20), the findings of the current study are not likely to be substantially biased as compared with those of the entire cohort.

Our study has several strengths. First, we directly measured plasma isoflavone concentrations, which reflect absorption and metabolism. Second, collecting blood samples before a diagnosis of lung cancer enabled us to infer a protective effect of genistein on lung cancer risk. Third, the quality of information measured at baseline was comparable in cases and controls, because both were selected from the same cohort.

In conclusion, plasma genistein concentration was associated with a decreased risk of lung cancer in Japanese women.

## Appendix

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## Disclosure of Potential Conflicts of Interests

No potential conflicts of interest were disclosed.

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