Plasma Isoflavone Concentrations Are Not Associated with Gastric Cancer Risk among Japanese Men and Women1,2

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Abstract
The incidence of gastric cancer throughout the world is ~2–3 times higher in men than in women. Previous research suggested that isoflavones, which are structurally similar to 17β-estradiol, may prevent gastric cancer. Based on a large, population-based, prospective study, we recently reported a null association between dietary isoflavone intake and gastric cancer. However, epidemiologic studies using blood concentrations of isoflavones might better reflect the effect of isoflavones on gastric cancer carcinogenesis than dietary assessment. We therefore conducted a nested case-control study within the Japan Public Health Center-Based Prospective Study. Participants were followed-up from 1990 to 2004. Among 36,745 participants who answered the baseline questionnaire and provided blood samples, 483 gastric cancer cases matched to 483 controls were used in the analysis. ORs and 95% CIs were estimated with a conditional logistic regression model. The overall distribution of plasma isoflavone concentrations was not associated with the development of gastric cancer. Compared with groups with the lowest plasma concentrations (reference groups), the groups with the highest daidzein and genistein concentrations had adjusted ORs and 95% CIs of 1.11 (0.74–1.66; P trend = 0.6) and 0.96 (0.64–1.44; P trend = 0.9), respectively. The results did not change when analysis was based on sex, subsite, or histological type. We found no association of plasma isoflavone concentrations with gastric cancer risk. Our data support the previously observed null association between isoflavone intake and gastric cancer risk. J. Nutr. 143: 1293–1298, 2013.

Introduction
The incidence of gastric cancer is ~2–3 times higher in men than in women (1). This sex difference is consistent across international populations with different prevalences of environmental risk factors such as Helicobacter pylori infection and tobacco smoking and different dietary patterns (2,3). A possible explanation involves biological differences related to sex hormones such as estrogen (2).

Isoflavones, which are structurally similar to 17β-estradiol, have a particular affinity for the β-estrogen receptor (4) and may have the potential to prevent gastric cancer. However, we previously reported no association between dietary isoflavone intake and gastric cancer risk on the basis of data from a 5-y follow-up questionnaire given to participants in the population-based Japan Public Health Center-Based Prospective Study (JPHC Study)5 (5).

Although FFQs can measure usual dietary habits (assuming that study participants do not change their dietary habits for long periods of time), such questionnaires are vulnerable to information bias such as memory decay, differential recall, and misclassification bias. In addition, the concentration of isoflavones in blood reflects individual differences in absorption and metabolism, in which intestinal microflora have an important role (6). Therefore, epidemiologic studies using blood concentrations of isoflavones might better reflect the effect of isoflavones on gastric cancer carcinogenesis than dietary assessment. However, only one small nested case-control study on isoflavone concentrations in blood samples has been reported (7).

Here, in a nested case-control study within a large, population-based, prospective study, we investigated the effect of plasma isoflavone concentrations on subsequent gastric cancer within a Japanese population that had substantially varied intakes of isoflavones (8).

Materials and Methods
Study population
The JPHC Study is an ongoing cohort study of cancer, cardiovascular disease, and other lifestyle-related diseases. The first group (Cohort I) of
the study was started in 1990 and the second group (Cohort II) in 1993 (9). The study included 140,420 participants (68,722 men and 71,698 women), who were defined as inhabitants in the study areas [27 cities, towns, and villages served by 11 public health centers (PHCs)] and 40–59 y old (Cohort I) or 40–69 y old (Cohort II) at baseline. For the present analysis, we excluded 2 PHC areas (Tokyo and Osaka), because data on cancer incidence were not available in Tokyo and the study population was defined differently in Tokyo and Osaka. We thus defined 123,576 participants (61,009 men and 62,567 women) for the present study. The JPHC Study was approved by the institutional review board of the National Cancer Center, Tokyo, Japan.

Baseline survey
In 1990 for Cohort I and 1993–1994 for Cohort II, participants were asked to reply to a lifestyle questionnaire that included sociodemographic characteristics, medical history, smoking and drinking habits, and diet. The FFQ included in the baseline questionnaire was previously described in detail (10). A total of 99,808 (81%) participants, 47,525 men and 52,283 women, responded to the questionnaire.

We excluded participants who self-reported cancer at baseline (n = 2136), those who were not Japanese (n = 18), and those who were later discovered to have moved away at baseline (n = 11). This left 97,644 eligible participants (46,803 men and 50,841 women), among whom 36,745 participants (38%; 13,467 men and 23,278 women) each donated 10-mL blood samples at health checkups conducted by the PHC in each area. As is customary, participants were asked to avoid consuming a meal after 17:00 h on the day before the examination. The time of either the last meal or the last drink of water or tea was recorded. The plasma and buffy layer were divided into four 1.0-mL tubes (3 tubes for plasma and 1 for the buffy layer), which were stored at −80°C. Blood was collected from 1990 to 1992 in Cohort I and from 1993 to 1995 in Cohort II.

Follow-up and identification of gastric cancer
Death and relocation. We observed study participants until 31 December 2004. The changes in residency status, including death, were identified annually through the residential registry in each area. To confirm causes of death, we used mortality data from the Ministry of Health, Labor and Welfare. Among 36,745 study participants, 4.5% moved outside the study area, 6.1% died, and 0.3% were lost to follow-up during the study period.

Cancer registry for the JPHC Study. Data on newly diagnosed cases of cancer were collected from 2 sources: active patient notification from the major hospitals in each study area and data linkage with population-based registries. Death certificate information was used as a supplementary information source. In our cancer registry system, the proportion of cases of gastric cancer for which information was available from death certificates only was 1.04%. This level of information quality was considered satisfactory for the present study.

Identification of gastric cancer and selection of controls. Cases of gastric cancer were extracted from the cancer registry for the JPHC Study on the basis of site [International Classification of Diseases for Oncology, 3rd edition (ICD-O-3) codes C160–169] (11). Up until the end of the study period, 512 new gastric cancer cases were identified. Until quite recently in Japan, the upper one-third of the stomach was called the “cardia” on the basis of the guidelines for gastric cancer classification (12). Because distinguishing the cardia, which is located mainly in the esophagogastric junction, from the upper one-third of the stomach seemed difficult, we combined tumors at these sites into one classification (12). Because distinguishing the cardia, which is located mainly in the esophagogastric junction, from the upper one-third of the stomach seemed difficult, we combined tumors at these sites into one classification (12).

Laboratory assay
Plasma concentrations of isoflavones were analyzed by HPLC with a coulometric array detector in accordance with the modified methods of Gamache and Acworth (16). Concentrations of daidzein and genistein were determined by linear regression of the peak height for the corresponding standards and adjusted according to the recovery rate of the internal plasma standard. The calculated regression coefficients for peak height and isoflavone concentration revealed a linearity range of 0–1.0 µg/mL and the correlation coefficient exceeded 0.996 for both daidzein and genistein. Voltammetric response for the standard solution displayed CVs of 10.7 and 8.7% for intraday daidzein and genistein concentration variations, respectively. Recovery rates of daidzein and genistein in plasma samples were 84.8 and 82.1%, respectively. Detection limits were 2.70 µg/L for daidzein and 2.21 µg/L for genistein.

Laboratory personnel were unaware of the case-control status when performing the analyses.

Statistical analysis
The comparison of baseline characteristics between cases and controls was evaluated by χ2 test or 1-way ANOVA. Matched ORs and 95% CIs were calculated to indicate the relationship between isoflavone concentrations and gastric cancer risk. Multiple conditional logistic regression analyses were conducted to control for potential confounding factors, such as cigarette smoking, alcohol consumption, intake of salted fish preserves, salt intake, BMI, family history of gastric cancer, and H. pylori infection.

Results
In baseline characteristics (Table 1), cases had a lower BMI and more H. pylori infection than controls. Other variables did not significantly differ between cases and controls.
We calculated the matched ORs and 95% CIs for developing gastric cancer in relation to plasma concentrations of isoflavones for all participants and separately for men and women (Table 2). We found no significant association between gastric cancer risk and the plasma concentration of either daidzein or genistein. By comparing the groups with the lowest plasma daidzein and genistein concentrations (reference groups) with the groups with the highest concentrations, we calculated adjusted ORs of 1.11 (95% CI: 0.74, 1.66; P-trend = 0.60) and 0.96 (95% CI: 0.64, 1.44; P-trend = 0.90) for all participants, 1.04 (95% CI: 0.64, 1.70; P-trend = 0.97) and 0.88 (95% CI: 0.54, 1.44; P-trend = 0.70) for men, and 1.00 (95% CI: 0.46, 2.15, P-trend = 0.9) and 1.09 (95% CI: 0.48, 2.47; P-trend = 0.80) for women, respectively. These results did not change when we estimated the ORs after excluding participants diagnosed as having gastric cancer within 3 y of baseline. In addition, dietary intakes of total energy, vegetables, fruits, fish, and green tea did not interact with any of the above results (for all interactions, P > 0.10). There was no significant association between total plasma isoflavones (sum of daidzein and genistein) concentrations and the risk of gastric cancer (data not shown).

When the cancers were stratified by histological type, smaller ORs were observed for undifferentiated cancer than for differentiated cancer, but the association did not reach significance. By comparing the groups with the highest plasma daidzein and genistein concentrations with the groups with the lowest concentrations (reference groups), we obtained adjusted OR values of 0.68 (95% CI: 0.30, 1.57; P-trend = 0.40) and 0.52 (95% CI: 0.19, 1.40; P-trend = 0.10) for undifferentiated cancer, respectively (Table 3).

When we based the analysis on distal gastric cancer, we obtained results similar to those for all gastric cancers; comparison between the lowest (reference) groups and the second, third, and fourth quartiles resulted in adjusted OR values of 1.11 (95% CI: 0.71, 1.74), 1.12 (95% CI: 0.71, 1.79), and 1.38 (95% CI: 0.85, 2.24) (P-trend = 0.20), respectively, for daidzein and 1.37 (95% CI: 0.86, 2.18), 1.40 (95% CI: 0.89, 2.22), and 1.21 (95% CI: 0.74, 1.97) (P-trend = 0.40), respectively, for genistein. There was no association for cancer in the upper one-third of the stomach (data not shown). These associations did not differ by sex (data not shown).

Our present results did not substantially change when we excluded participants who used exogenous female hormones or who were premenopausal among women (data not shown).

### TABLE 1 Baseline characteristics of participants in a nested case-control study (JPHC Study)

<table>
<thead>
<tr>
<th></th>
<th>Cases (n = 483)</th>
<th>Controls (n = 483)</th>
<th>P-difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>57.6 ± 7.2</td>
<td>57.6 ± 7.2</td>
<td>—</td>
</tr>
<tr>
<td>Women, %</td>
<td>32.9</td>
<td>32.9</td>
<td>—</td>
</tr>
<tr>
<td>Current smoker, %</td>
<td>35.8</td>
<td>30.6</td>
<td>0.3</td>
</tr>
<tr>
<td>Alcohol drinking ≥1 d/wk, %</td>
<td>51.1</td>
<td>50.9</td>
<td>0.5</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23.0 ± 2.9</td>
<td>23.4 ± 2.8</td>
<td>0.04</td>
</tr>
<tr>
<td>Family history of gastric cancer, %</td>
<td>11.4</td>
<td>8.1</td>
<td>0.08</td>
</tr>
<tr>
<td>Salt intake, g/d</td>
<td>5.2 ± 2.6</td>
<td>5.1 ± 2.3</td>
<td>0.4</td>
</tr>
<tr>
<td>Salted fish preserves intake ≥1 d/wk, %</td>
<td>31.5</td>
<td>32.1</td>
<td>0.8</td>
</tr>
<tr>
<td>H. pylori infection, %</td>
<td>98.8</td>
<td>89.7</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

1 Values are means ± SDs or %. CagA, cytotoxin-associated gene A; JPHC Study, Japan Public Health Center-Based Prospective Study.
2 P2 test or 1-way ANOVA.
3 Including H. pylori IgG positive or CagA positive.

Furthermore, the results did not change when we restricted cases and controls to participants with an *H. pylori* infection (data not shown).

### Discussion

In this nested case-control study within a large prospective study of the Japanese population, we found that plasma concentrations of daidzein and genistein were not significantly associated with the risk of gastric cancer in either men or women. The
results did not substantially change when we analyzed for gastric cancer by subsite or histological type.

We previously reported an association between plasma isoflavone concentrations and breast, prostate, and lung cancer risks from nested case-control studies within the JPHC Study (17–19), and for each cancer, we found results similar to those from studies using an FFQ (8,20,21). We previously observed that isoflavone intake was not associated with gastric cancer risk (5). As for other cancers, our observations in the present study are in line with those of our previous investigation, which was based on a cohort design in the same JPHC Study (5).

To our knowledge, only one nested case-control study (131 cases, 393 matched controls) has reported an inverse association between blood concentrations of isoflavones and gastric cancer risk (7). However, these results were based on a small series and the data were not analyzed by anatomic or histological site. In addition, cases and controls were selected from the same cancer registry system during the study period was satisfactory. In conclusion, we found no suggestion of an association between plasma isoflavone concentrations and gastric cancer. However, the presence of estrogen receptors in normal stomach and gastric cancer tissues and their clinical importance, including the prognostic effect, have not been clearly demonstrated. In the present study, our results suggest that isoflavone concentrations have no substantial influence on gastric cancer development. On this basis, we do not think that the sex discrepancy in gastric cancer incidence can be explained by isoflavones, which have structures similar to that of 17β-estradiol. Another possible explanation for the null association between plasma isoflavone concentrations and gastric cancer risk in the present study is that the influence of established causes of gastric cancer, such as cigarette smoking (27), remained even after adjustment.

Several limitations of the study warrant mention. First, plasma isoflavone concentrations were measured only once for each individual. Although the consumption of soy foods is a personal dietary preference, the intake levels of most individuals are assumed to be relatively stable over time in Japan, as suggested by our validation study, in which we showed that repeated measurements of genistein intake by means of a FFQ were highly reproducible (correlation coefficient = 0.72 for a 1-y interval and 0.61 for a 5-y interval) (28,29). By comparison, plasma isoflavone concentrations may reflect short-term rather than long-term intake; isoflavones have short half-lives in blood (e.g., 7.7–9.5 h) (30) and plasma isoflavone concentrations are particularly affected by time elapsed since the last meal. To minimize the attenuation of risk estimates due to short-term intake, fasting time was matched in cases and controls. In addition, our study participants were restricted to those who participated in the baseline health checkup survey. We previously reported that health checkup participants had different background characteristics from nonparticipants and favorable lifestyle profiles (31). Thus, the associations between plasma isoflavone concentrations and gastric cancer risk could differ from those of the entire cohort and any generalization of our results should be done with caution.

The strengths of this study include its relatively large sample size and almost complete follow-up, because the quality of our cancer registry system during the study period was satisfactory. In addition, cases and controls were selected from the same cohort and the loss to follow-up was negligible, thereby avoiding selection bias. Moreover, the prospective study design and the estimation of ORs after exclusion of participants diagnosed as having gastric cancer within 3 y of baseline ensured that blood samples were collected before gastric cancer diagnosis, reducing the probability of reverse causality. Furthermore, we directly measured plasma isoflavone concentrations, which reflect absorption and metabolism.

In conclusion, we found no suggestion of an association of plasma isoflavone concentrations with the risk of gastric cancer.

Acknowledgments
The authors’ responsibilities were as follows: S.T. (principal investigator), M. Inoue conducted the study and managed the cancer data collection; A.H. analyzed and interpreted the data and prepared the manuscript; T.M. assayed to measure the concentration of isoflavones; and S.S., M. Iwasaki, N.S., T.S., and T.Y. helped to conduct the study. All authors read and approved the final manuscript.
Literature Cited


Appendix

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